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# Ensuring that UK cereals used in malting, milling and animal feed achieve food and feed safety standards

by

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# 1. ABSTRACT

This project is the latest in a series looking at the occurrence of key contaminants in UK-grown cereals to ensure compliance with legal and guideline limits for food and animal feedstuffs. The project covered wheat, barley and oats from the 2009, 2010 and 2011 harvests intended for use in the milling, malting and animal feed industries. Samples of each type of grain were collected immediately after harvest and after storage of up to six months. Relevant contaminants were identified through regular “horizon scanning” of official publications and scientific and agricultural literature and a sampling programme agreed by a steering committee comprising representatives of the relevant Trade Associations, HGCA and scientists from the contract laboratories. The contaminants selected were mycotoxins (Fusarium toxins, Ochratoxin A and ergot alkaloids), pesticides, including some growth regulators and desiccants, polycyclic aromatic hydrocarbons, and heavy metals.

The overwhelming majority of samples complied with legal and guideline limits. The storage mycotoxin, Ochratoxin A, although quite common in most sample types, was generally detected only at low concentrations, suggesting that mould growth and toxin synthesis are being adequately controlled by suitable storage conditions. Fusarium mycotoxins, produced during growth in the field, showed significant seasonal variations, though the trend of increasing prevalence observed in preceding years has not been sustained and to some extent has declined over the three years surveyed. This can probably be ascribed to a combination of climate conditions and agronomic practices.

Associated toxins, such as ergot alkaloids and masked mycotoxins, for which there is little historical data, were found in some cereal samples but only at levels that imply contamination of UK cereals is minimal.

Levels of heavy metals and pesticides were all within legal limits and did not vary substantially from season to season.

# 2. SUMMARY

## 2.1. Introduction/Background and aims

The aim of this project was to investigate the occurrence of key contaminants in UK-grown wheat, barley and oats and their co-products to demonstrate safety for use for milling, malting and animal feed, as well as the extent of compliance with legal and guideline limits. Throughout the project a "horizon scanning" exercise was carried out, looking at legislation, publications from official bodies such as the UK Food Standards Agency, the European Food Safety Authority (EFSA), and the World Health Organisation's Joint Expert Committee on Food Additives (JECFA), as well as the scientific, agricultural and medical press, in order to identify emerging issues and trends. The contaminants investigated were selected based on this literature survey, in consultation with a steering committee consisting of representatives of the relevant Trade Associations (the Agricultural Industries Confederation (AIC), the National Association of British and Irish Millers (nabim) and the Maltsters' Association of Great Britain (MAGB)), the HGCA and scientists from the contract laboratory involved in the project. Samples of milling and feed wheat, malting and feed barley, feed oats, wheatfeed and oatfeed were collected from harvests 2009, 2010 and 2011, either immediately after harvest, or after a period of storage. Contaminants sought included:

- Mycotoxins – Ochratoxin A, Fusarium toxins
- Pesticides – storage insecticides, growth regulators and desiccants
- Metals/metalloids – aluminium, arsenic, cadmium, copper, lead, mercury

## 2.2. Materials and methods

A steering committee drawn from the relevant trade associations (AIC, MAGB and nabim) together with representatives from HGCA and Campden BRI, oversaw the sampling and analysis. This committee met in August each year and decided on the analytes and the number of analyses to be carried out on samples from that year's harvest based on results from previous years and risk factors such as the prevailing weather conditions. Sampling was managed by the trade associations and covered all the main flour mills, maltings and feed processing plants in the UK. Samples included commercial milling wheat, malting barley, feed wheat, wheatfeed, feed oats and oatfeed. Malted barleys produced from some of the malting barley samples were also sampled. Two main tranches of samples were collected each year:

(a) immediately after harvest (September)
(b) after 6 months storage (March)

An additional tranche of milling wheat samples was collected in January of each year. The paired malting barley/malt samples were collected as soon as malting was completed, typically between October and February following harvest.
All samples were despatched to Campden BRI for analysis. Freshly harvested samples were analysed for Fusarium toxins, heavy metals, plant growth regulators and glyphosate. Stored samples were analysed for Ochratoxin A, storage pesticides and in some instances Fusarium toxins. Selected samples, either from these sample sets or specifically sampled, were analysed for PAHs, ergot alkaloids and masked mycotoxins.
Heavy metals were analysed by ICP-MS (inductively-coupled plasma mass spectrometry). All other analyses were carried out using gas or liquid chromatography coupled to mass spectrometry or other specific detectors. All methods were fully validated and most were accredited to the international standard ISO17025.

## 2.3. Results

*Mycotoxins*
The results of the mycotoxin analyses showed that the overwhelming majority of samples tested were compliant with legal and guideline limits, indicating that UK-grown cereals provide a safe source of raw materials for the milling, malting and animal feed industries.
Ochratoxin A (OTA) is the principal mycotoxin found in stored cereals such as wheat and barley. It is formed because of infection by the mould *Penicillium verrucosum* and by *Aspergillus* species, both of which are widespread contaminants of cereals in temperate climates. They invade grain mainly during storage and can grow rapidly given suitable conditions of temperature and moisture. In the UK, *P. verrucosum* is the most common source of OTA in barley, wheat and oats.
OTA analysis was carried out on milling wheat samples taken from grain stores several months after harvest. The incidence of contamination was low: less than 10% over the three years and the average level was typically 5% of the EU maximum of 5 µg/kg. Occasional samples were close to the EU maximum and in two cases exceeded the limit; in these instances the mills were advised immediately and action taken by the miller. Extended storage did not lead to higher incidence or level of Ochratoxin A; in fact, samples taken in March had slightly lower levels than those taken in January.
Some of the samples taken at mills originated from outside the United Kingdom. There was some evidence that these samples were more likely to contain Ochratoxin A: both the incidence of contamination and the mean levels over the three-year period were higher than for domestically grown wheat. However, the mean level remained relatively low and 76% of samples contained no detectable Ochratoxin A.
Malting barley samples showed a slightly higher incidence of contamination than the wheat samples though mean levels were similar – approximately 5% of the EU maximum. All samples were well below the EU maximum level. When these barleys were used to produce malts there

was a slight increase in mean values in each of the three years. However, this increase was only significant in one year (2010) and was probably skewed by the incidence of two samples with levels above the EU limit for processed cereals. In both instances, the samples were re-sampled and re-analysed: in one case, the repeat result was low and the original result ascribed to a “hot spot”; in the other instance, the batch was removed from the food chain.
There was no correlation between Ochratoxin A levels in individual pairs of barley and malt. The malting process includes stages in which Ochratoxin A is removed and in which, potentially, Ochratoxin A can be formed if conditions are not adequately controlled. However, the largest factor in explaining the disparity between some of the barleys and malts is the difficulty in obtaining truly representative samples from large bulks of grain, despite the use of EC recommended sampling procedures. The data generally indicate that storage of malting barley over several months is well controlled and does not lead to significant increases in Ochratoxin A levels in grain processed into malt.
Feed cereals contained significantly higher levels of Ochratoxin A than those destined for food use; the majority of samples of wheatfeed and oatfeed containing detectable residues. However, none exceeded guideline levels for Ochratoxin A in complementary and complete feedingstuffs and mean values were below 5 µg/kg.

The principal mycotoxins formed during growth of wheat, barley and oats are the trichothecenes produced by various Fusarium species associated with Fusarium head blight. Each species produces one or more of the trichothecenes; deoxynivalenol (DON), the most commonly found toxin in wheat and barley, is produced predominantly by *F. culmorum* and *F. graminearum*. Other important trichothecenes include T-2 and HT-2 toxins, produced predominantly by *F. sporotrichioides* and *F. langsethiae*. T-2 and HT-2 have been widespread in raw oats for many years and more recently have been found in barley and wheat.
Freshly harvested grain samples from deliveries to mills, maltings and processing plants were analysed for a range of trichothecenes. DON was by far the most common of the trichothecenes detected in wheat and barley derived samples. Incidence and mean levels in wheat declined over the three harvest seasons from 2009 to 2011. Levels in malting barley did not show a similar obvious decline but were generally lower than in wheat. The decline follows three years (2006 to 2008) during which levels rose and suggests year-to-year variation rather than a long-term upward trend. Levels in feed grain were higher than for the corresponding food grain but all samples were within EC guideline levels for feedingstuffs. As with the food grains levels were higher in wheat than barley and showed a general decline from 2009 onwards.
Processing of the malting barleys into malt had little effect on DON levels overall. As with OTA there was little correlation between individual barley and malt pairs and in occasional instances the level in malt exceeded that of the parent barley. Again, it is possible that this was due to *de novo*

synthesis of DON during the malting process but the more likely explanation is the difficulty in obtaining comparable homogeneous samples from the barley and malt.

After DON, the most significant Fusarium toxins are T-2 and HT-2 (these are generally treated as a pair when considering incidence and regulatory levels). They were rarely detected in wheat samples, in line with historic patterns. Malting barley was more prone to contamination but even here, incidence and levels were very low. Data from this series of projects and other published studies has shown an increase in incidence of T-2 and HT-2 contamination in UK cereals from around 2004 onwards: 2010 represents perhaps the worst year to date but even here, the mean level of the two toxins was only 15 µg/kg. There are as yet no maximum levels set in the EU for T-2 and HT-2 but an EC draft recommendation in early 2012 posited a level of 100–200 µg/kg as appropriate for barley.

As with DON, the correlation between T-2 and HT-2 in barley and malt pairs was poor but there was clear evidence that processing into malt reduced levels substantially.

DON and T-2/HT-2 are produced by different Fusarium species and competition between species would be expected to give rise to differences in the relative incidence of these toxins in barley; this was reflected in poor correlation between the occurrences of two types of trichothecenes, both within and between the three harvest years. The few incidences of high levels of deoxynivalenol and T-2/HT-2 were mutually exclusive.

T-2 and HT-2 were not found in any feed wheat or wheatfeed samples over the three harvests and levels in feed barley were only slightly higher than in malting barley. In line with earlier published data, there was widespread contamination of feed oats and near universal contamination of oatfeed. Levels were higher in the oatfeed samples as would be expected from their higher content of husk. However, mean and maximum levels over the three years were slightly lower than for the previous three years (2006-2008). An EC draft recommendation proposes an action level of 1000-1500 µg/kg for unprocessed oats intended for human consumption; the majority of raw oat samples would fall below these levels.

Nivalenol was the only other trichothecene detected on a regular basis (no limits have been set for nivalenol in cereals but the European Commission has requested an opinion from EFSA on it as a possible prelude to monitoring or legislation). It was only intermittently found in milling wheat: incidence in malting barley was higher but levels were generally very low. Levels in processed malt were consistently lower than in the parent barleys. This indicates that nivalenol is largely removed during the malting process.

The results for the wheat and barley based samples in the cereal feedingstuffs were similar to the food samples though with higher levels in the feed barleys. Nivalenol was found in the majority of oats and oatfeed samples.

Generally, nivalenol co-occurred with DON but in some cases, there were relatively high levels of NIV when DON was either low or absent. They are both produced by *F. culmorum* and *F. Graminearum*, though by different chemotypes, and even here, NIV is produced at low levels by

DON chemotypes. NIV is also produced by *F. Poae,* which occurs under different conditions to *F. culmorum* and *F. Graminearum* and could explain the occurrence of NIV in the absence of DON. The other trichothecenes sought, 3-acetyl-DON, 15-acetyl-DON, diacetoxyscirpenol, fusarenone-X and neosolaniol, were rarely detected in any samples. There were occasional instances of 15-acetyl-DON in samples with very high levels of DON and isolated instances of diacetoxyscirpenol and neosolaniol in oats or oatfeed samples heavily contaminated with other toxins.

Zearalenone is another mycotoxin produced predominantly by *F. culmorum* and *F. graminearum*. It differs somewhat from the trichothecenes in being predominantly produced late in the crop-growing season, close to harvest.

Zearalenone was analysed in all freshly harvested samples of milling wheat and malting barley from each harvest. Levels were relatively high in 2009 but negligible in 2010 and 2011. In previous studies, incidence and levels in both wheat and barley have generally been low. High levels have previously been seen in 2004 and 2008: along with 2009, these can all be linked to wet conditions immediately prior to harvest when the grain is particularly susceptible to infection and production of the toxin.

A largely similar pattern was seen with the feed wheat and feed barley samples, though there was an isolated case of a very high level in a feed barley sample in 2011. This sample apart, all samples were well within either EU limits or guideline levels and even the high barley would only have exceeded guideline levels for particular feedingstuffs intended for pigs. Levels of zearalenone in oats and oatfeed were low, even in 2009 when high levels were seen in wheat.

From the results above, it is evident that for the vast majority of UK cereals mycotoxin levels are well below legislative limits or guideline limits, an indication that control measures and agronomic practices are largely effective in minimising toxin levels in raw grain.

In recent years, some attention has been focused on “masked” mycotoxins. These are compounds where the mycotoxin is conjugated or bound to another molecule such as a sugar or protein. In this form, they escape detection by conventional analytical methods but could be liberated to the free form during processing into cereal products or subsequent consumption of the cereal. Methods have recently been developed for several of these species and deoxynivalenol-3-glucoside (DON-3-Glu) is the major masked analogue of the trichothecenes reported to date. Analysis of selected barley, malt and oat samples here indicated that DON-3-Glu is only found when the free form is present in significant quantities and accounts for only a small percentage of the total deoxynivalenol present. It would thus seem that in raw and malted grain the masked form contributes only a small part of the deoxynivalenol content. Evidence was also found of the presence of the corresponding glycosides of T-2 and HT-2 toxins in oatfeed, though again probably only as a small proportion of the free toxins.

*Pesticide residues*

Stored whole grains were tested for a range of insecticides currently or recently approved for use on stored cereal grain or in cereal stores in the UK. Barley and oat samples were additionally analysed for a limited number of field fungicides commonly used on cereals. Milling wheat and feed grains were sampled six to eight months after harvest and malting barleys three to eight months after harvest. Only a few pesticides were detected in any of the samples and in virtually all cases the levels were very low, typically only a few per cent of the MRL. Approximately, 80% of food grains contained no detectable residues and pirimiphos-methyl was by far the most common residue detected in the remainder; no sample exceeded 0.2 mg/kg, against an MRL of 5 mg/kg. Chlorpyrifos-methyl and malathion were detected in a small percentage of wheat samples (8% and 2% respectively). Malathion is not currently approved for use in the UK but one of the two samples where it was found was imported and the very low level would imply that it was treated some time prior to import. The second positive sample was only just above the limit of detection. Neither sample exceeded the current MRL.

Glyphosate is a widely used herbicide but is also authorised for use as a desiccant on cereals, where it may be used immediately before harvesting. The MRL is relatively high (20 mg/kg) and it is one of the residues most frequently reported in official surveys of cereals in the UK. Selected samples of barley and wheat from the 2011 harvest were analysed for glyphosate. Negligible amounts were found in malting barley and though a majority of other barley and wheat samples contained glyphosate the levels were low with only a couple of samples exceeding 10% of the EU MRL. The 2011 harvest was relatively wet, particularly in Scotland, hence usage of glyphosate might be expected to be higher than in drier years. However, there was no clear evidence of higher levels in cereal samples grown in Scotland.

The growth regulator chlormequat is very widely used on cereals, either alone or in combination with mepiquat, to restrict stem elongation and reduce the risk of lodging (which can cut yield and increase the likelihood of mould growth and mycotoxin contamination). It has been cited as one of the most common residues detected on cereals in several EU member states, including the UK. Selected malting barley and milling wheat samples from the 2011 harvest were tested for both chlormequat and mepiquat. Chlormequat was detected in the majority of samples (41% of barleys and 80% of wheats) but actual concentrations were low - mean values were well below the EU MRL for chlormequat and no samples exceeded this limit. Mepiquat was much less common and again all samples were below the EU MRL.

*Heavy metals*

Limits are set in the EU for lead, cadmium, arsenic and mercury in cereals for food and feed use. Previous studies have shown UK cereals to be compliant with these limits but there is little recent published data and consequently samples from the 2011 harvest were analysed for cadmium, lead, aluminium, arsenic and mercury.

In 2009, EFSA set a reduced tolerable weekly intake (TWI) for cadmium of 2.5µg/kg body weight and the European Commission has subsequently proposed reductions in the maximum levels allowed in certain foodstuffs. Cadmium levels found in milling wheat and malting barley were all within current EU limits but a small percentage of samples were close to or above the reduced limits being discussed at the time of writing (0.1 mg/kg for wheat; 0.075 mg/kg for barley). A 95$^{th}$ percentile value of 0.097 mg/kg for wheat implies that potentially a significant proportion of the harvest could exceed the proposed new limit.

All samples of food and feed grain were well below current EU limits for lead in cereal foods and feedingstuffs. The levels were similar to those reported in previous surveys HGCA surveys. Levels of arsenic were similarly well below legal limits and in line with previous surveys. Mercury was not detected in any sample.

Samples were also analysed for aluminium: no limits have been set for aluminium in cereals, but there are few data available on levels in cereals. The levels found were within the ranges reported in previous surveys in the past 20 years.

*Ergot alkaloids*

Ergot (*Claviceps purpurea*) is an important disease of cereals, which can lead to extensive financial losses to growers due to the toxicity of ergot present in the grain. Ergot levels vary from year to year, and are influenced by weather at flowering. Ergot is also the name given to the black fungal bodies or sclerotia that replace the grain in the ear and can easily be seen on visual inspection of the grain. There are no legal limits for ergot set in the EU but in the UK the cereals sector has a limit for ergot of 0.001% ergot by weight for feed grain and a zero tolerance for all other grain.

Controls based on sclerotia have significant limitations; determination of the contamination rate is often inaccurate, the composition and toxicity of the sclerotia are variable and it is impossible to detect (and therefore to remove) sclerotia in processed feedingstuffs. It has been suggested that the current limits on sclerotia should be replaced by chemical analysis of the alkaloids produced by ergot. In early 2012, the EC recommended monitoring of ergot alkaloids in feed and food.

Analysis for ergot alkaloids was carried out exclusively on grain deliveries (wheat, barley and rye) that had been rejected at intake following routine checks for the presence of ergot sclerotia. Samples were taken at the flour mill or maltings site and analysed for the six alkaloids and epimers recommended for monitoring by the European Commission. The broad aim was to establish the level of alkaloid contamination of the whole grain and if possible, the extent to which alkaloids were transferred from sclerotia to uninfected grain. For some of the samples it was possible to analyse the grain before and after removal of visible sclerotia; in others the whole sample including sclerotia was analysed. It was not possible to isolate sufficient sclerotia to analyse them directly. A broad range of results were obtained. In some cases no alkaloids were detected, even in samples with sclerotia present. Where alkaloids were detected levels were generally lower in samples after

removal of sclerotia but the pattern was not consistent, either in terms of the total alkaloids found or even in the individual alkaloids present. This probably reflects the inherent heterogeneity of the grain samples and the difficulties in ensuring complete removal of sclerotial material.
All of the six key alkaloids were found, though the combinations found on individual samples were quite varied. The principal alkaloids found were ergotamine, ergosine and ergocristine, in each case usually accompanied by lower levels of the corresponding epimers. Overall, the results provide some evidence that ergot sclerotia leave “footprints” of alkaloids on grain although the level of these alkaloids appears to be quite low.

## 2.4. Discussion/Conclusions and implications

The data established by this project imply that the bulk of UK-grown cereals comply with EU and UK legislation and recommendations for the contaminants covered by the surveillance.
**Mycotoxins:** The storage mycotoxin Ochratoxin A was detected regularly, but the incidence in food grains (milling wheat and malting barley) was relatively low, in the range of 10-30%, and there was no consistent pattern of incidence. Incidence in compounded samples (wheatfeed and oatfeed) was significantly higher, suggested that contamination with the causative mould *P verrucosum* is widespread but at a low level, and that toxin synthesis in food grains is being successfully kept in check by storage conditions. The occasional samples that exceeded legal limits were generally much lower when bulks were re-sampled, suggesting that the well-recognised difficulties with obtaining representative samples remain a problem.
The situation with trichothecenes was very different from that of OA. Concentrations of these toxins varied from year to year. Over the short term, concentrations followed variations in climatic conditions. DON was the commonest trichothecene in barley and wheat, whilst T-2 and HT-2 toxins predominated in oats. In barley, DON and T-2/HT-2 toxins are generally mutually exclusive. This is probably due to competition between Fusarium species producing the toxins and has implications for control measures; agronomic practices intended to minimise DON are well developed and used, those for T-2/HT-2 are less understood.
**Pesticides:** Although many samples contained detectable residues of agrochemicals, concentrations were very low, and were invariably well below legal MRLs. The residue detected most frequently was the growth regulator chlormequat, which was found in a large percentage of samples tested. The desiccant glyphosate was also detected quite frequently; samples were only tested in one year and it was not possible to say whether the incidence correlated with wet conditions at harvest. The only other pesticide detected with any frequency was the storage insecticide pirimiphos-methyl and even this was generally only found at trace levels, even in compounded feed samples. Overall, the low concentrations detected for all pesticides relative to legal limits implied that pesticides in UK-grown cereals are not a concern.
**Heavy metals:** concentrations of metals were generally low in the samples tested and mostly well below legal limits. The ranges of concentrations found were in agreement with other published

reports. A possible reduction in the legal limits for cadmium in cereals might lead to a greater risk of a small percentage of samples exceeding the new limits. Overall, it is unlikely that heavy metals in cereals present a health hazard.

**Emerging issues:** masked mycotoxins (mycotoxins that escape detection in conventional analysis because they are bound to other residues) were identified at the start of the project as an emerging issue, which could have an impact on the market acceptability or future legislation for grain. The data presented here, on DON and T-2/HT-2, suggest that there is no major concern but the increasing number of publications in this area indicates that further studies are needed.

# 3. TECHNICAL DETAIL

## 3.1. Introduction

The primary objective of the project was to ensure that UK grown cereals destined for malting, flour milling and animal feed are safe for human consumption. This was achieved by producing robust and cumulative analytical data on the incidence of key contaminants in representative samples of UK-produced cereals. These data can be used by HGCA and cereal producers to assure customers of the wholesomeness of UK-grown cereals and cereal products, and to inform discussions on proposed legislation.

In addition, an objective was to identify any emerging issues or legislation that could affect the safety of cereal-based foods or their acceptability in key markets and to compile relevant data that could help inform discussion, for example, on new legislative limits. This was achieved by the scanning of relevant literature, databases and legislation and maintenance of databases of information.

The project follows on from earlier HGCA projects *(Baxter, 2003, 2006a, b, Salmon, 2006, Baxter et al., 2009),* which accumulated data for a number of contaminants over several years. Most of these contaminants remain relevant; these, together with other contaminants included in this project, are described below.

### 3.1.1. Storage mycotoxins

Ochratoxin A (OTA) is the principal mycotoxin found in stored cereals such as wheat and barley. It is formed because of infection by *Penicillium verrucosum* and by *Aspergillus* species. Both of these moulds are widespread contaminants of cereals in temperate climates, particularly in Europe, but rarely colonise the growing crop. They invade grain mainly during storage and can grow rapidly given suitable conditions of temperature and moisture. *P. verrucosum* is the most common source of OTA in barley, wheat and oats in the UK and Western Europe.

The European Commission (EC, 2006) has set limits for OTA in unprocessed cereals and processed cereal products. These are listed in Table 1, along with guideline levels (EC 2006) for various feed materials.

**Table 1: Legal limits (LL) and guideline levels (GL) for Ochratoxin A in food and feed cereals in the EU**

| Matrix | Ochratoxin A (µg/kg) | Status |
|---|---|---|
| Unprocessed cereals | 5 | LL *(EC, 2006c)* |
| Processed cereals/products | 3 | LL *(EC, 2006c)* |
| Feed materials | 250 | GL *(EC, 2006b)* |
| Complementary & complete feedingstuffs for pigs | 50 | GL *(EC, 2006b)* |
| Complementary & complete feedingstuffs for poultry | 100 | GL *(EC, 2006b)* |

Mould infections are frequently discontinuous in stored grain, often due to localised damp areas within a bulk (“hot spots”). Sampling protocols based on multiple incremental samples are used to acquire representative samples but even these leave scope for substantial variation in analytical results. The European Commission has laid down a protocol for sampling cereals for the official control of mycotoxins (EC 2006). This specifies 100 incremental samples and an aggregate sample of 10kg, so is generally impractical for routine use, although it has been used for some of the samples collected within this project.

### 3.1.2. Field mycotoxins

The principal mycotoxins formed during growth of wheat, barley and oats are the trichothecenes produced by various Fusarium species associated with Fusarium head blight. Deoxynivalenol (DON), the most commonly found toxin in wheat and barley, is produced predominantly by *F. culmorum* and *F. graminearum*.

The European Commission (EC 2006) has set limits for DON in unprocessed cereals and processed cereal products. These are listed in Table 2, along with guideline levels (EC 2006) for various feed materials. The lowest guidance levels have been set for pigs owing to their higher sensitivity to Fusarium mycotoxins.

**Table 2: Legal limits and guideline levels for deoxynivalenol (DON) in food and feed cereals in the EU**

| Matrix | DON (µg/kg) | Status |
|---|---|---|
| Unprocessed cereals for food (except durum wheat, oats and maize) | 1250 | LL *(EC, 2006c)* |
| Unprocessed durum wheat and oats | 1750 | LL *(EC, 2006c)* |
| Feed cereals and cereal byproducts | 8000 | GL *(EC, 2006b)* |
| Complete and complementary feedingstuffs (except for those listed below) | 5000 | GL *(EC, 2006b)* |
| Complete and complementary feedingstuffs for pigs | 900 | GL *(EC, 2006b)* |
| Complete and complementary feedingstuffs for calves | 2000 | GL *(EC, 2006b)* |

Zearalenone is another mycotoxin produced predominantly by *F. culmorum* and *F. graminearum*. It differs from the trichothecenes in being predominantly produced late in the crop-growing season, close to harvest (Matthaus *et al*., 2004).

The European Commission (EC) has set legislative limits for zearalenone in cereal grains and cereal-based products intended for human consumption (Table 3) (EC, 2006b). Guideline levels for animal feed materials have also been set.

**Table 3: EU limits and guideline levels for Zearalenone in cereals and animal feed**

| Matrix | Zearalenone (mg/kg) | Status |
|---|---|---|
| Unprocessed cereals other than maize | 0.10 | LL *(EC, 2006c)* |
| Cereals and cereal products with the exception of maize by-products | 2.00 | GL *(EC, 2006b)* |
| Complementary and complete feedingstuffs for piglets and gilts (young sows) | 0.10 | GL *(EC, 2006b)* |
| Complementary and complete feedingstuffs for sows and fattening pigs | 0.25 | GL *(EC, 2006b)* |
| Complementary and complete feedingstuffs for calves, dairy cattle, sheep (including lamb) and goats (including kids) | 0.50 | GL *(EC, 2006b)* |

Although DON is the predominant trichothecene mycotoxin in grain, some of the other trichothecenes have greater toxicity; hence, it is important that they are also monitored. Nivalenol (NIV) is produced by the same Fusarium species as DON, though probably by different isolates, and by *F. Poae.* EFSA are expected to publish an opinion on nivalenol in 2013.
T-2 and HT-2 are Type A trichothecenes, which are generally more toxic than Type B trichothecenes such as DON. They are thought to be produced predominantly by *F. sporotrichioides* and *F. langsethiae*. T-2 and HT-2 have been widespread in raw oats for many years and more recently have been found in barley and wheat. Legal limits for these two toxins have been under discussion for a number of years. The European Food Safety Authority (EFSA) has published a scientific opinion (EFSA, 2011) on the risks for animal and public health related to the presence of T-2 and HT-2 toxins in food and feed. This established a TDI of 100 ng/kg b.w. for the sum of T-2 and HT-2 and concluded that at current human dietary intakes they do not present a concern to health. However, discussions on legal limits continue.
Other trichothecenes found in cereals include 3-acetyldeoxynivalenol and 15-acetyldeoxynivalenol, (metabolites of deoxynivalenol), neosolaniol (commonly found on oats) and diacetoxyscirpenol (on which the EC asked EFSA for a scientific opinion in 2012).

### 3.1.3. Masked mycotoxins

All the mycotoxins described above occur in free form. They can also be found as conjugates, either through substitution with moieties such as acetyl or sulphate groups or through linkage to other molecules such as sugars or amino acids. These chemical modifications take place as part of the detoxification of the mycotoxin by plants. However, these reactions may be reversible and could allow the free toxin to be regenerated by hydrolysis either during subsequent processing or when consumed by humans or livestock. In addition, chemical analysis for the toxins will not detect these “masked” forms with a consequent potential for under reporting of the dietary intake of individual toxins.

Two acetylated forms of deoxynivalenol, 3-acetyldeoxynivalenol and 15-acetyl deoxynivalenol, have long been recognised and are found only when deoxynivalenol is present in large amounts. Much fewer data are available for other masked mycotoxins such as glucosides. Deoxynivalenol-3-glucoside has been reported in wheat contaminated with *F. graminearum* (Berthiller *et al*., 2005). Methods for its detection with high performance liquid chromatography - mass spectrometry (LC-MS/MS) have been described (Berthiller *et al.,* 2005). Typically, it accounts for around 10-20% of the total deoxynivalenol present in grain, though an increased proportion has been reported during processing of barley into malt (Lancova *et al*., 2008) and in other surveys of wheat and maize. The same authors also suggested the proportion increased further when the malt was processed into beer (Kostelanska *et al*., 2009). The effects of milling and baking of wheat have also been studied (Kostelanska *et al*., 2011).The formation, determination and significance of various other masked and conjugated mycotoxins have been reviewed (Berthiller *et al.,* 2009).

More recently, the existence of masked forms of the trichothecenes T-2 and HT-2 has been reported. Busman *et al* (2011) characterised 3-O-glucosides of T-2 and HT-2 produced in wheat and oats inoculated with *F. Sporotrichioides.* Lattanzio *et al* (2012) found the two 3-O-glucosides and the 4-O-glucoside of HT-2 in naturally contaminated wheat and oats. They were not able to quantify the amounts of the glucosides but estimated that they contributed up to 27% of the total T-2 or HT-2 level.

### 3.1.4. Pesticide residues

A broad range of herbicides, fungicides and insecticides are used in cereal production. In preceding monitoring projects analysis has focussed on insecticides either used in grain stores, as treatments for the fabric of the store or sprayed on to the grain itself. A number of organophosphate insecticides have been used over the years: these have largely been phased out but a few remain in use, particularly in countries exporting grain to the UK.

The growth regulator chlormequat is very widely used on cereals, either alone or in combination with mepiquat, to restrict stem elongation and reduce the risk of lodging (which can cut yield and increase the likelihood of mould growth and mycotoxins contamination). It has been cited as one of the most common residues detected on cereals by several EU member states including the UK *(EC 2005a)*.

The herbicide glyphosate is commonly used in cereal production as a desiccant close to harvest time where it promotes grain ripening and assists drying down of the crop and efficiency of harvesting.

### 3.1.5. Heavy metals

Cadmium is a naturally occurring metallic element found in the earth's crust. It has a number of industrial uses, and may be present as a contaminant in materials such as metals, cements and fertilisers. The main exogenous sources in soil are use of sewage sludge and other animal manures, fertilisers and aerial deposition. Steady decreases in all three of these have been recorded over the past 30 years.
All foods contain low levels of cadmium, with the highest concentrations being found in shellfish and offal. Some types of cereals, particularly hard wheats used for breadmaking are considered more at risk of taking up cadmium from the soil, thus bread is often a major source of cadmium in the diet.

In 2009, EFSA set a reduced tolerable weekly intake (TWI) of 2.5µg/kg body weight *(EFSA, 2009).* This was reaffirmed in 2011*(EFSA, 2011)*, despite a decision by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2010 to set a monthly intake level of 25 ug/kg bw. The European Commission has subsequently proposed reductions in the maximum levels of cadmium in certain foodstuffs, including barley and wheat.

In April 2010, dietary lead was reviewed by EFSA *(EFSA, 2010).* The Panel concluded that there was no evidence of a threshold for adverse effects on neurodevelopment and it therefore withdrew the existing Provisional Tolerable Weekly Intake (PTWI) of 25 µg/kg body weight and did not set a revised figure. Instead they calculated the benchmark levels of intake equivalent to blood levels of lead considered to pose no more than a 1% increase in the main adverse health effects (BMDL). EFSA also noted that the current estimated exposure levels might exceed benchmark levels for adverse effects on neurodevelopment for some susceptible population groups.

Cereals do not readily take up lead; consequently, levels in grain are not related to the levels in soils except in heavily contaminated areas. The most likely sources of contamination are from atmospheric deposition due to traffic emissions, which have fallen substantially in Europe since the introduction of lead-free fuel, and industrial pollution. It was though appropriate to establish if this decline in usage was reflected in levels in grain. Limits for lead and cadmium in cereals are set by Contaminants Regulation 1881/2006 (*EC, 2006c*) for grain for food use and by Directive 2002/32 *(EC, 2002)* for use in animal feed. Limits are also set for arsenic and mercury and, under pesticides legislation, for copper (*EC, 2005b)*.

### 3.1.6. Ergot alkaloids

Ergot (*Claviceps purpurea*) is an important disease of cereals, which can lead to extensive financial losses to growers due to the toxicity of ergot present in the grain. Ergot levels vary from year to year, and are influenced by weather at flowering, which affects both the host and the

pathogen. Ergot is also the name given to the black fungal bodies or sclerotia that replace the grain in the ear. Ergots are hard, purplish-black, dense tuber-like bodies, 2 - 22mm long, which can be easily seen on visual inspection of the grain. There are no national legal limits for ergot in EU member states, but there may be commercial limitations in some countries. For example, in the UK UKASTA standards for ergot are 0.001% ergot by weight for feed grain and a zero tolerance for all other grain *(HGCA, 2002)*.

EFSA published a background document on ergot in 2009 *(EFSA, 2009).* Most published European surveys focussed on rye, but wheat was included in some cases.

Controls based on sclerotia have significant limitations; determination of the contamination rate is often inaccurate, the composition and toxicity of the sclerotia are variable and it is impossible to detect (and therefore to remove) sclerotia in processed feedingstuffs. It has therefore been suggested that the current limits on sclerotia should be replaced by chemical analysis of the alkaloids *(EFSA 2005).* In early 2012 the EC recommended monitoring of ergot alkaloids in feed and food with a focus on the six predominantly present ergot alkaloids, i.e. ergometrine, ergotamine, ergosine, ergocristine, ergocryptine and ergocornine and their related –inines.

An EFSA Opinion on ergot alkaloids is expected in 2013.

## 3.2. Materials and methods

### 3.2.1. Samples

Samples of (1) commercial milling wheat, feed wheat, wheatfeed; (2) malting barley and malts prepared from those barleys, and (3) feed barley, feed oats and oatfeed were taken by the companies and sent to Campden BRI for analysis. Sampling and analysis was overseen by a steering committee drawn from the relevant trade associations (AIC, MAGB and nabim) together with representatives from HGCA and Campden BRI. This committee met in August each year and decided on the analytes to be tested for in samples from the coming harvest based on results from previous years and risk factors such as the prevailing weather conditions. The number of samples from each flour mill, maltings or processing plant was decided by the appropriate trade association and was intended to give a broad geographical spread representative as far as possible of the UK market. Two main tranches were collected each year:

- At delivery immediately after harvest (September): these were usually analysed for Fusarium toxins. Selected samples were also analysed for heavy metals, plant growth regulators and glyphosate
- From grain stores after 6 months storage (March): these were analysed for Ochratoxin A and storage pesticides

In addition, paired samples of malting barley and malt were collected between November and March; the exact time depended on when the first batches of malt were produced from the new season’s harvest. These samples (10 kg each) were collected using the recommended sampling

protocols for official surveillance *(EC, 2006)* and were analysed for Fusarium toxins, Ochratoxin A; the barley samples were additionally analysed for pesticide residues.

An additional tranche of milling wheat was collected in January and analysed for Ochratoxin A only. Not all samples in each tranche were tested for all analytes specified; the number was agreed by the steering group, based on the risk of that analyte occurring in that sample type. A summary of the samples and analyses is shown in Tables 4 to 6 below.

**Table 4: Samples collected – 2009 harvest**

| Cereal | Date collected | Samples | Analytes |
|---|---|---|---|
| Malting barley | Sep-09 | 39 | Trichothecenes & Zearalenone |
| Feed barley | Sep-09 | 12 | Trichothecenes & Zearalenone |
| Milling wheat | Sep-09 | 45 | Trichothecenes & Zearalenone |
| Feed wheat | Sep-09 | 10 | Trichothecenes & Zearalenone |
| Wheatfeed | Sep-09 | 20 | Trichothecenes & Zearalenone |
| Feed oats | Sep-09 | 11 | Trichothecenes & Zearalenone |
| Oatfeed | Sep-09 | 7 | Trichothecenes & Zearalenone |
| Malting barley | Nov 09 - Mar 10 | 18 | Trichothecenes, Zearalenone, Ochratoxin A & pesticides |
| Malted barley | Nov 09 - Mar 10 | 18 | Trichothecenes, Zearalenone & Ochratoxin A |
| Feed barley | Mar-10 | 29 | Ochratoxin A |
| Milling wheat | Jan-10 | 25 | Ochratoxin A |
| Milling wheat | Mar-10 | 25 | Ochratoxin A & pesticides |
| Feed wheat | Mar-10 | 39 | Ochratoxin A |
| Wheatfeed | Mar-10 | 9 | Ochratoxin A |
| Feed oats | Mar-10 | 13 | Ochratoxin A |
| Oatfeed | Mar-10 | 10 | Ochratoxin A |

**Table 5: Samples collected – 2010 harvest**

| Cereal | Date collected | Samples | Analytes |
|---|---|---|---|
| Malting barley | Sep-10 | 40 | Trichothecenes & Zearalenone |
| Feed barley | Sep-10 | 10 | Trichothecenes & Zearalenone |
| Milling wheat | Sep-10 | 42 | Trichothecenes & Zearalenone |
| Feed wheat | Sep-10 | 10 | Trichothecenes & Zearalenone |
| Wheatfeed | Sep-10 | 17 | Trichothecenes & Zearalenone |
| Feed oats | Sep-10 | 11 | Trichothecenes & Zearalenone |
| Oatfeed | Sep-10 | 8 | Trichothecenes & Zearalenone |
| Malting barley | Nov 10 - Mar 11 | 20 | Trichothecenes, Zearalenone, Ochratoxin A & pesticides |
| Malted barley | Nov 10 - Mar 11 | 20 | Trichothecenes, Zearalenone & Ochratoxin A |
| Feed barley | Mar-11 | 23 | Ochratoxin A |
| Milling wheat | Jan-11 | 34 | Ochratoxin A |
| Milling wheat | Mar-11 | 26 | Ochratoxin A & pesticides |
| Feed wheat | Mar-11 | 6 | Ochratoxin A |
| Wheatfeed | Mar-11 | 35 | Ochratoxin A |
| Feed oats | Mar-11 | 12 | Ochratoxin A |
| Oatfeed | Mar-11 | 6 | Ochratoxin A |

**Table 6: Samples collected – 2011 harvest**

| Cereal | Date collected | Samples | Analytes |
|---|---|---|---|
| Malting barley | Sep-11 | 33 | Trichothecenes, zearalenone, glyphosate, plant growth regulators & heavy metals |
| Feed barley | Sep-11 | 11 | Trichothecenes, zearalenone, glyphosate & heavy metals |
| Milling wheat | Sep-11 | 47 | Trichothecenes, zearalenone, glyphosate, plant growth regulators & heavy metals |
| Feed wheat | Sep-11 | 10 | Trichothecenes, zearalenone, glyphosate & heavy metals |
| Wheatfeed | Sep-11 | 18 | Trichothecenes & zearalenone |
| Feed oats | Sep-11 | 11 | Trichothecenes, zearalenone, & heavy metals |
| Oatfeed | Sep-11 | 11 | Trichothecenes & zearalenone |
| Malting barley | Nov 11 - Mar 12 | 18 | Trichothecenes, zearalenone, Ochratoxin A & pesticides |
| Malted barley | Nov 11 - Mar 12 | 18 | Trichothecenes, zearalenone & Ochratoxin A |
| Feed barley | Mar-12 | 21 | Ochratoxin A & pesticides |
| Milling wheat | Jan-12 | 45 | Ochratoxin A |
| Milling wheat | Mar-12 | 51 | Ochratoxin A & pesticides |
| Feed wheat | Mar-12 | 30 | Ochratoxin A & pesticides |
| Wheatfeed | Mar-12 | 10 | Ochratoxin A & pesticides |
| Feed oats | Mar-12 | 10 | Ochratoxin A & pesticides |
| Oatfeed | Mar-12 | 9 | Ochratoxin A & pesticides |

Sampling for ergot alkaloid analysis was carried out separately. Samples of milling wheat, rye and malting barley were taken from grain loads rejected at intake due to the visual presence of ergot sclerotia on the surface of the load. Approximately 1 kg of grain was drawn from the load: no attempt was made to quantify sclerotial bodies within the sample.

### 3.2.2. "Horizon scanning" for emerging issues

Scientific literature and government publications in the UK, the EU and other countries representing major customers or cereal suppliers (such as Canada, Australia and Japan) were scanned regularly in order to identify emerging issues. The information gained was used during the project to inform decisions on which analytes to test for in the various matrices.

### 3.2.3. Analysis of mycotoxins

Mycotoxins in barley (malting and feed) and oats were analysed at the Nutfield site, while those in wheat (milling and feed) were analysed at the Campden site.

#### *Ochratoxin A*

Ochratoxin A was analysed by in-house procedures. After extraction with acetonitrile/water (Nutfield site) or phosphate buffered methanol/water (Campden site) specific immunoaffinity columns were used for the clean-up stage. Detection and quantification were carried out by HPLC with fluorescence detection. The limit of quantification was 0.1 µg/kg. Both sites are accredited to ISO17025:2005 for this analysis.

#### *Fusarium toxins: trichothecenes and zearalenone*

Trichothecenes were analysed by three in-house procedures. Barley and oat samples were analysed by a GC-MS procedure based on a published method (Patel *et al.,* 1996). Samples were ground to a fine powder, trichothecenes (deoxynivalenol, 3- and 15- acetyl-DON, nivalenol, neosolaniol, diacetoxyscirpenol, fusarenone-X, T-2 toxin and HT-2 toxin) extracted using acetonitrile/water, then partially purified using trichothecene clean-up columns, derivatised and analysed by GC-MS. The limit of quantification for each trichothecene was 5 µg/kg.
For paired barley and malted barley samples a separate, more sensitive method was used for T-2 and HT-2 toxins. Samples were ground to a fine powder, trichothecenes extracted using methanol/water, and then purified using specific immunoaffinity columns, separated and quantified by liquid chromatography-mass spectrometry. The limit of quantification for each trichothecene in this procedure was 1µg/kg. The method is accredited to ISO17025:2005.
Wheat samples were analysed by a LC-MS/MS procedure. Samples were ground to a fine powder and trichothecenes extracted using acetonitrile/water, then partially purified using trichothecene

clean-up columns, then separated and quantified by liquid chromatography-mass spectrometry. The limit of quantification for each trichothecene in this procedure was 10µg/kg. The method is accredited to ISO17025:2005.
Zearalenone was analysed by in-house procedures. Barley and oat samples were analysed by a HPLC-FD method. Samples were ground to a fine powder then after extraction with acetonitrile/water, specific immunoaffinity columns were used for the clean-up stage. Detection and quantification were achieved by HPLC with fluorescence detection. Wheat samples were analysed by a LC-MS/MS procedure. Samples were ground to a fine powder then after extraction with acetonitrile/water, the extracts were filtered then the zearalenone separated and quantified by liquid chromatography-mass spectrometry. The limit of quantification for both methods was 2 µg/kg. Both sites are accredited to ISO17025:2005 for this analysis.

### 3.2.4. Analysis of pesticide residues

*Storage pesticides*
Cereal storage pesticides were analysed by in-house methods. After extraction with acetone/methanol the extract was purified by gel permeation chromatography. The fraction containing pesticide residues was recovered, concentrated and injected into a gas chromatograph-mass spectrometer to separate and quantify residues. The limit of quantification was 0.01 mg/kg for each residue tested. Both sites are accredited to ISO17025:2005 for this analysis.

**Table 7: Storage insecticides and selected field fungicides included in the pesticide screening**

| Type | Active ingredient | EU MRL (mg/kg) |
|---|---|---|
| | bifenthrin | 0.5 |
| | chlorpyrifos | barley 0.2; oats & wheat 0.05* |
| | chlorpyrifos-methyl | 3 |
| post harvest insecticides | cypermethrin | 2 |
| | deltamethrin | 2 |
| | diazinon | 0.02* |
| | dichlorvos | 0.01* |
| | etrimfos | 0.01* |
| | fenitrothion | 0.05* |
| | fenvalerate | barley & oats 0.05; wheat 0.02* |
| | lindane | 0.01* |
| | malathion | 8 |
| | methacrifos | 0.05* |
| | permethrin | 0.05* |
| | pirimiphos-methyl | 5 |
| Synergist | piperonyl butoxide | None |
| field fungicides | azoxystrobin | barley & oats 0.5; wheat 0.3 |
| (Barley and oat samples only) | cyprodinil | barley 3; oats 2; wheat 0.5 |
| | kresoxim-methyl | 0.05* |
| | trifloxystrobin | barley 0.3; oats 0.02*; wheat 0.05 |

***** Limit of detection applies

*Glyphosate*

Glyphosate was analysed by solvent extraction followed by solid phase clean up. Detection and quantification was by LC-MS/MS *(Granby et al., 2003).* The limit of quantification was 0.05 mg/kg.

*Growth regulators*

Chlormequat and mepiquat were analysed by solvent extraction followed by separation, detection and quantification by LC-MS/MS. The method was based on a published procedure *(Vahl et al., 1998)*. The limit of quantification was 0.01 mg/kg.

### 3.2.5. Analysis of heavy metals

All metal analysis was carried out at the Campden site. Samples of grain were digested in a microwave digestion oven at elevated pressure in the presence of nitric acid. The diluted digest was introduced into an inductively-coupled plasma mass spectrometer (ICP-MS). The individual isotopes were determined by their response relative to that obtained from standard solutions. The limits of quantification were 0.001mg/kg for cadmium and 0.01 mg/kg for lead, arsenic, mercury and aluminium. The method is accredited to ISO17025:2005.

### 3.2.6. Analysis of ergot alkaloids

Ergot alkaloids (ergometrine, ergosine, ergotamine, ergocornine, ergocryptine, ergocristine and their corresponding epimers) were analysed by an in-house method developed. Samples were extracted into acetonitrile/ammonium carbonate buffer, cleaned up by dispersive solid phase extraction and analysed by liquid chromatography with tandem mass spectrometry.

### 3.2.7. Analysis of "masked" mycotoxins

Deoxynivalenol-3-glucoside was analysed using an in-house method. Samples were ground to a fine powder, extracted using acetonitrile/water and the extracts partially purified by passing through charcoal/alumina clean-up columns. The eluents were concentrated and analysed by LC-MS/MS operating in atmospheric pressure chemical ionisation mode and using multiple reaction monitoring. The method also quantified deoxynivalenol and the limit of quantification for both analytes was 5 µg/kg.

Glucosides of T-2 and HT-2 were analysed using an in-house method. Samples were ground to a fine powder, extracted using acetonitrile/water and the extracts partially purified by passing through charcoal/alumina clean-up columns. The eluents were concentrated and analysed by LC-MS/MS. The MS was operated in positive electrospray mode with ammonium as a modifier ion. Product ion scans were used to detect a range of predicted fragmentations of potential metabolites of T-2 and HT-2. The MRM transitions used to screen samples for T-2 and HT-2 glycosides and their parent molecules are listed in Table 31.

## 3.3. Results

### 3.3.1. Storage mycotoxins

*Ochratoxin A (OA)*

Milling wheat samples taken from grain stores in January and March following the 2009, 2010 and 2011 harvests were tested for Ochratoxin A. Results are shown in Table 9 (unless otherwise stated, throughout this report results below the limit of quantification have been set at half the limit of quantification for the purposes of calculating mean, median and percentile values).

**Table 9: Ochratoxin A in stored milling wheat**

| Harvest year | Sampling month | Incidence | Mean | Median | 95$^{th}$ Percentile | Maximum |
|---|---|---|---|---|---|---|
| | | % | µg/kg | µg/kg | µg/kg | µg/kg |
| 2009 | January 2010 | 8 | 0.24 | 0.05 | 0.25 | 4.5 |
| 2009 | March 2010 | 8 | 0.24 | 0.05 | 0.09 | 4.8 |
| 2010 | January 2011 | 15 | 0.59 | 0.05 | 0.54 | 11.7 |
| 2010 | March 2011 | 17 | 0.18 | 0.05 | 0.17 | 3.3 |
| 2011 | January 2012 | 9 | 0.16 | 0.05 | 0.28 | 3.6 |
| 2011 | March 2012 | 4 | 0.09 | 0.05 | 0.05 | 2.2 |

The incidence of contamination was low; generally less than 10% and mean levels were close to the limit of quantification in most years. Occasional samples were close to the EU maximum of 5 µg/kg and in two cases exceeded the limit; in these instances the mills were advised immediately and action taken by the miller. Extended storage did not lead to higher incidence or level of Ochratoxin A; in fact, samples taken in March had slightly lower levels than those taken in January. Approximately 17% of the samples taken at mills originated from outside the United Kingdom. There was some evidence that these samples were more likely to contain Ochratoxin A: both the incidence of contamination and the mean levels over the three year period were higher than for domestically grown wheat (Table 10). However, the mean level remained relatively low and 76% of samples contained no detectable Ochratoxin A.

**Table 10: Ochratoxin A in domestic and imported milling wheat**

| | Incidence | Mean | Median | 95$^{th}$ Percentile | Maximum |
|---|---|---|---|---|---|
| | % | µg/kg | µg/kg | µg/kg | µg/kg |
| Imported | 24 | 0.43 | 0.05 | 2.5 | 5.9 |
| UK | 6 | 0.19 | 0.05 | 0.10 | 11.7 |
| Overall | 9 | 0.24 | 0.05 | 0.26 | 11.7 |

Malting barley samples showed a slightly higher incidence of contamination than the wheat samples though mean levels were similar (Table 11). All samples were well below the EU maximum level. When these barleys were then used to produce malts there was a slight increase in mean values in each of the three years. However, this increase was only significant in one year, 2010, and was probably skewed by the incidence of two samples with levels above the EU limit for processed cereals. In both instances, the samples were re-sampled and re-analysed: in one case the repeat result was low and the original result ascribed to a “hot spot”; in the other case, the batch was removed from the food chain.

**Table 11: Ochratoxin A in stored barley and malt**

| Harvest year | Sample | Incidence % | Mean | Median | 95$^{th}$ Percentile | Maximum |
|---|---|---|---|---|---|---|
| | | | µg/kg | µg/kg | µg/kg | µg/kg |
| 2009 | Barley | 21 | 0.14 | 0.05 | 0.53 | 0.80 |
| 2009 | Malt | 21 | 0.32 | 0.05 | 1.56 | 2.10 |
| 2010 | Barley | 35 | 0.28 | 0.05 | 1.43 | 1.90 |
| 2010 | Malt | 30 | 0.79 | 0.05 | 5.93 | 6.40 |
| 2011 | Barley | 11 | 0.07 | 0.05 | 0.22 | 0.30 |
| 2011 | Malt | 22 | 0.19 | 0.05 | 0.97 | 1.40 |

There was no correlation between Ochratoxin A levels in individual pairs of barley and malt (Figure 1); there were instances of high levels in the barley but low level in the malt and vice versa. The malting process includes steeping stages in which it has been shown that Ochratoxin A can be removed. It has also been shown that *de novo* formation of Ochratoxin A can take place during malting if conditions are not adequately controlled. However, a more likely explanation for the disparity between some of the barleys and malts is the difficulty in obtaining truly representative samples from large bulks of grain. Overall, the mean level in malt was below 0.5 µg/kg, well below the EU limit.

The barley and malt samples were taken immediately before and after malting respectively between October and July. When Ochratoxin A levels were plotted against approximate sampling dates for barley and malt (Figures 2 and 3) there was evidence of an increase in Ochratoxin A levels in both barley and malt with extended storage of the barley. However, the increase was slight and the correlation poor and would indicate that storage of malting barley over several months is well controlled and does not lead to significant increases in Ochratoxin A levels.

**Figure 1: Ochratoxin A in barley and malt pairs**

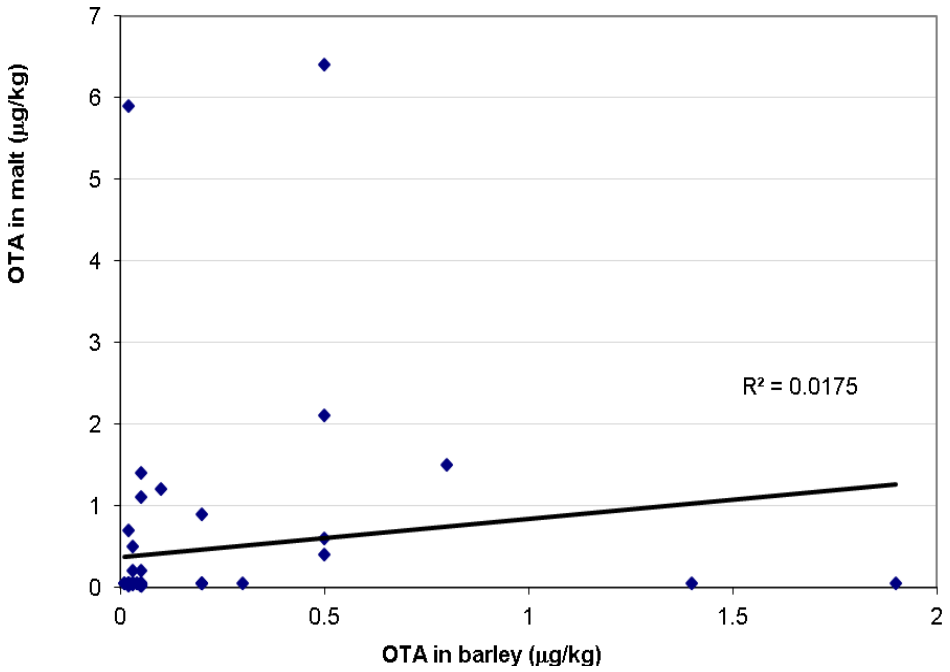


**Figure 2: Ochratoxin A in barley in extended storage**

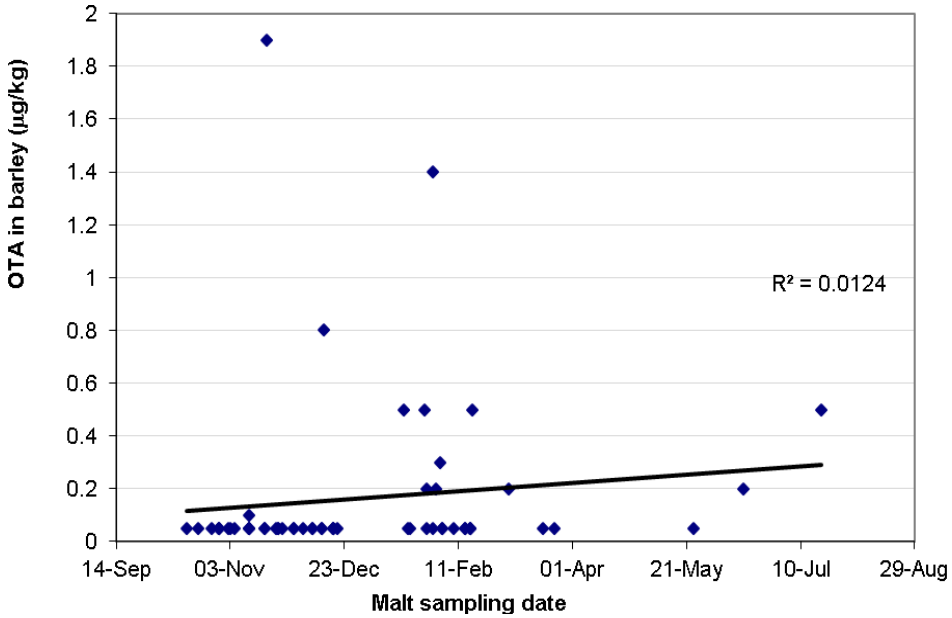
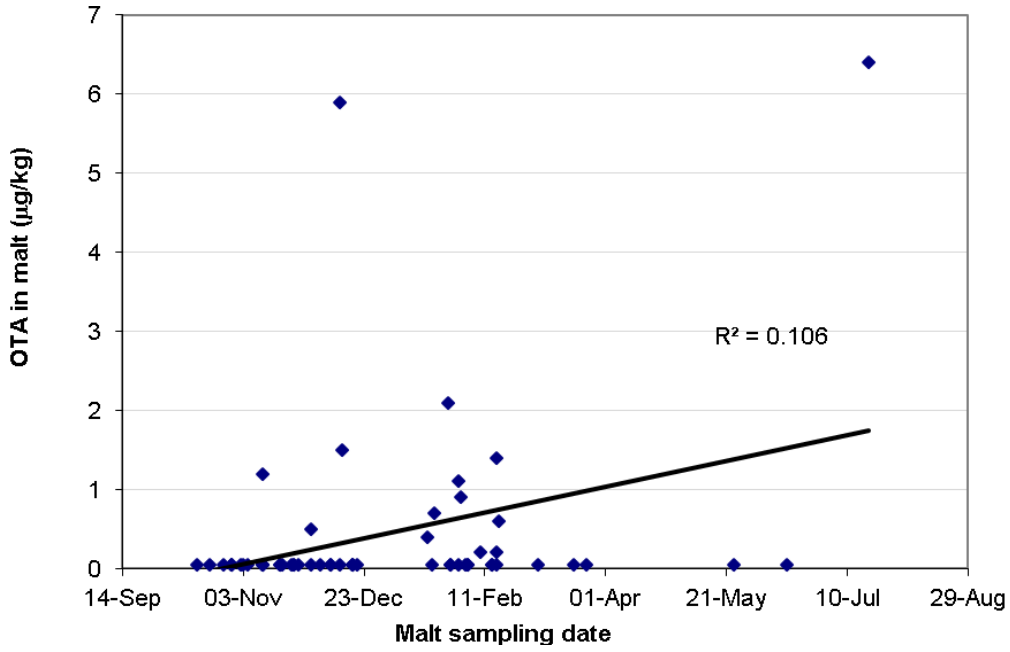



**Figure 3: Ochratoxin A in malt produced from barley in extended storage**



The incidence of Ochratoxin A was significantly higher in feed cereals than in those destined for food use, with the majority of samples of wheatfeed and oatfeed containing detectable residues (Table 12). Actual concentrations were generally moderate, with mean values in any category not exceeding 4 µg/kg but the range of concentrations was wider than for food cereals, with several samples containing more than 10 µg/kg. The highest concentration (55 µg/kg) was found in a sample of feed wheat. None of the samples exceeded the lowest guideline level for Ochratoxin A in any complementary and complete feedingstuffs.

**Table 12: Ochratoxin A in stored feed cereals**

| Sample | Harvest year | Incidence % | Mean | Median | 95$^{th}$ Percentile | Maximum |
|---|---|---|---|---|---|---|
| | | | µg/kg | µg/kg | µg/kg | µg/kg |
| Feed wheat | 2009 | 18 | 1.20 | 0.05 | 0.93 | 4.3 |
| | 2010 | 20 | 0.48 | 0.05 | 1.20 | 11.4 |
| | 2011 | 28 | 2.00 | 0.05 | 2.90 | 55.0 |
| Wheatfeed | 2009 | 67 | 0.49 | 0.40 | 1.40 | 1.9 |
| | 2010 | 83 | 3.40 | 0.75 | 9.90 | 10.4 |
| | 2011 | 25 | 0.83 | 0.65 | 1.80 | 1.9 |
| Feed barley | 2009 | 28 | 1.50 | 0.05 | 3.80 | 32.7 |
| | 2010 | 26 | 0.26 | 0.05 | 1.20 | 1.6 |
| | 2011 | 19 | 0.14 | 0.05 | 0.40 | 1.1 |
| Feed oats | 2009 | 8 | 0.66 | 0.05 | 3.20 | 8.0 |
| | 2010 | 50 | 2.40 | 0.13 | 7.20 | 7.2 |
| | 2011 | 0 | 0.05 | 0.05 | 0.05 | <0.1 |
| Oatfeed | 2009 | 100 | 1.60 | 1.30 | 3.80 | 4.7 |
| | 2010 | 83 | 1.40 | 1.00 | 3.00 | 3.1 |
| | 2011 | 100 | 0.82 | 0.20 | 2.30 | 2.8 |

### 3.3.2. Field mycotoxins

*Deoxynivalenol*

Freshly harvested grain samples from deliveries to mills, maltings and processing plants were analysed for a range of trichothecenes. Deoxynivalenol (DON) was by far the most common of the trichothecenes detected in wheat and barley derived samples. Results for milling wheat and malting barley are shown in Tables 13 and 14. Incidence and mean levels in wheat declined over the three harvest seasons. Levels in malting barley did not show a similar obvious decline but were generally lower than in wheat; this difference between wheat and barley has been observed in previous comparable analyses (Baxter *et al*., 2009). The decline follows three years during which levels rose (2006 to 2008; Figure 4) and suggests a pattern of annual variation rather than a long term upward trend. This pattern is illustrated in Figure 5, which shows comparable data for malting barley from 1999 onwards.

**Table 13: Deoxynivalenol in freshly harvested milling wheat**

| Harvest year | Incidence % | Mean µg/kg | Median µg/kg | 95th Percentile µg/kg | Maximum µg/kg |
|---|---|---|---|---|---|
| 2009 | 91 | 138 | 94 | 390 | 511 |
| 2010 | 48 | 25 | 5 | 115 | 138 |
| 2011 | 32 | 13 | 5 | 48 | 87 |

**Table 14: Deoxynivalenol in freshly harvested malting barley**

| Harvest year | Incidence % | Mean µg/kg | Median µg/kg | 95th Percentile µg/kg | Maximum µg/kg |
|---|---|---|---|---|---|
| 2009 | 28 | 14 | 5 | 53 | 59 |
| 2010 | 35 | 13 | 5 | 43 | 58 |
| 2011 | 43 | 15 | 5 | 34 | 95 |

**Figure 4: DON in food grains 2006-11**

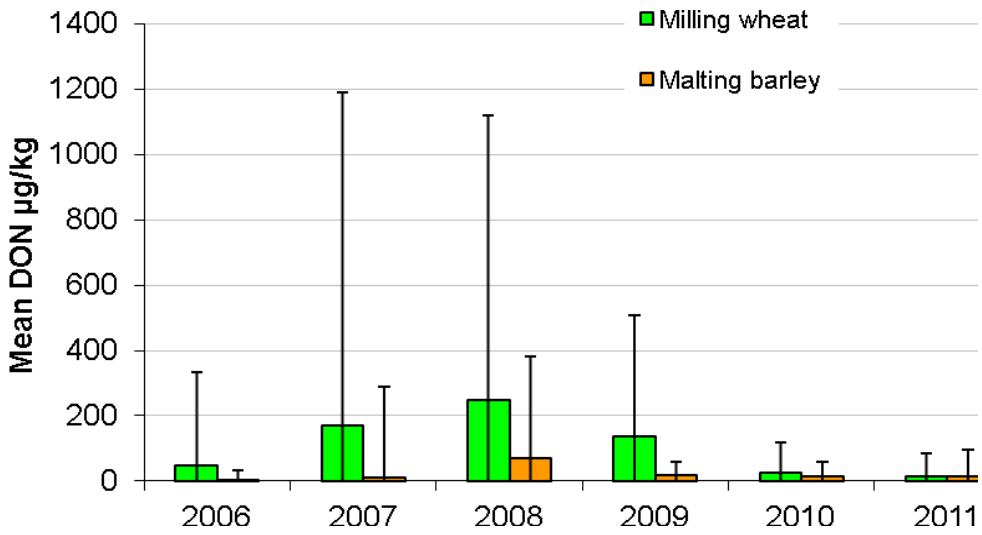
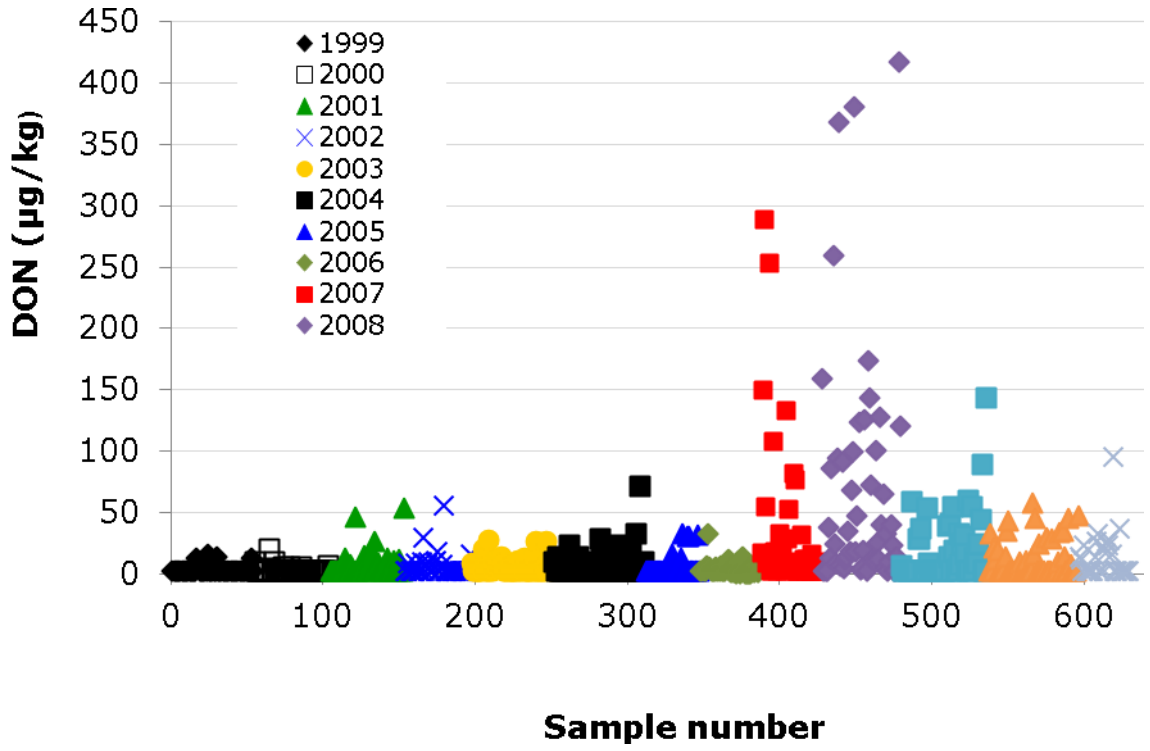


**Figure 5: DON in malting barley 1999 - 2011**

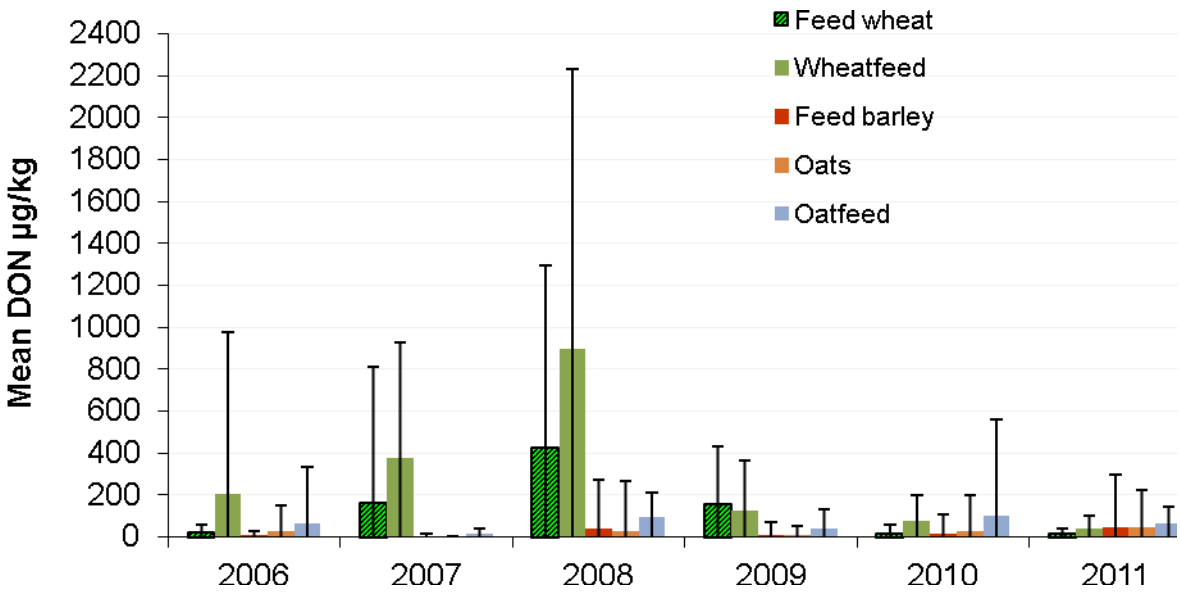



Results for feed grains are shown in Table 15. Incidence and mean levels were higher than that for the corresponding food grain but all samples were within EC guideline levels for feedingstuffs. As with the food grains both incidence and toxin concentrations were higher in wheat than barley and levels were highest from the 2009 harvest. Data from 2006 onwards (Figure 6) show the same pattern as for the food grain, with grain from recent harvests showing relatively low levels of DON.

**Table 15: Deoxynivalenol in cereal feedingstuffs**

| Sample | Harvest year | Incidence % | Mean µg/kg | Median µg/kg | 95th Percentile µg/kg | Maximum µg/kg |
|---|---|---|---|---|---|---|
| Feed wheat | 2009 | 80 | 153 | 95 | 375 | 434 |
| | 2010 | 44 | 16 | 5 | 35 | 39 |
| | 2011 | 50 | 14 | 8 | 37 | 40 |
| Wheatfeed | 2009 | 90 | 127 | 93 | 304 | 389 |
| | 2010 | 89 | 77 | 59 | 191 | 199 |
| | 2011 | 95 | 44 | 36 | 91 | 102 |
| Feed barley | 2009 | 18 | 11 | 5 | 39 | 54 |
| | 2010 | 10 | 15 | 5 | 62 | 105 |
| | 2011 | 55 | 48 | 12 | 180 | 299 |
| Feed oats | 2009 | 22 | 11 | 5 | 42 | 55 |
| | 2010 | 27 | 28 | 5 | 121 | 200 |
| | 2011 | 36 | 46 | 5 | 203 | 222 |
| Oatfeed | 2009 | 20 | 11 | 5 | 38 | 47 |
| | 2010 | 50 | 99 | 11 | 402 | 562 |
| | 2011 | 89 | 67 | 46 | 133 | 146 |

**Figure 6: DON in feed 2006 - 2011**



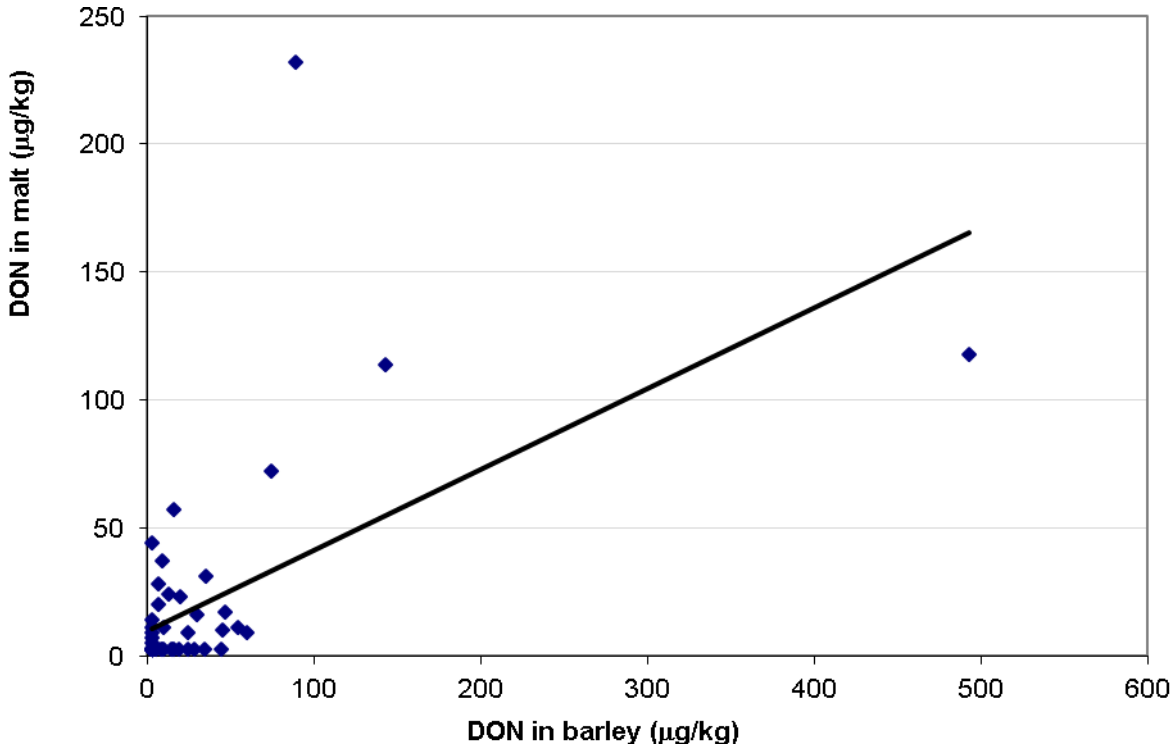

Whiskers denote maximum values.

A second set of malting barleys was collected each year, together with a sample of the malt produced from each barley. Results for these paired samples are shown in Table 16 and Figure 7. Mean levels in the malts were slightly lower than in the barleys overall over the three years but there was little correlation between individual barley and malt pairs. In occasional instances the level in malt exceeded that of the parent barley. It is possible that this was due to *de novo* synthesis of DON during the malting process but a more likely explanation is the difficulty in obtaining comparable homogeneous samples from the barley and malt.

**Table 16: Deoxynivalenol in malting barley and malt**

| Harvest year | Sample | Incidence % | Mean µg/kg | Median µg/kg | 95$^{th}$ Percentile µg/kg | Maximum µg/kg |
|---|---|---|---|---|---|---|
| 2009 | Barley | 68 | 28 | 13 | 94 | 143 |
| | Malt | 45 | 14 | 5 | 45 | 47 |
| 2010 | Barley | 44 | 38 | 3 | 137 | 493 |
| | Malt | 58 | 25 | 9 | 126 | 232 |
| 2011 | Barley | 25 | 9 | 5 | 28 | 31 |
| | Malt | 44 | 22 | 3 | 79 | 118 |

**Figure 7: Correlation between barley and malt for DON**




*T-2 and HT-2 toxins*

After deoxynivalenol the most significant Fusarium toxins are T-2 and HT-2. These are generally treated as a pair when considering incidence and regulatory levels hence all results are presented as the sum of the two toxins. Results for the freshly harvested milling wheat and malting barley are shown in Tables 17 and 18. T-2 and HT-2 were rarely detected in wheat samples, in line with historic patterns. Malting barley was more prone to contamination but even here incidence and levels were very low. Long term data from 1999 to 2011 (Figure 8) shows an increase in incidence of T-2 and HT-2 contamination from 2004 onwards: the 2010 harvest probably represents the worst year to date but even here the mean level of the sum of the two toxins was only 15 µg/kg. There are as yet no maximum levels set in the EU for T-2 and HT-2.

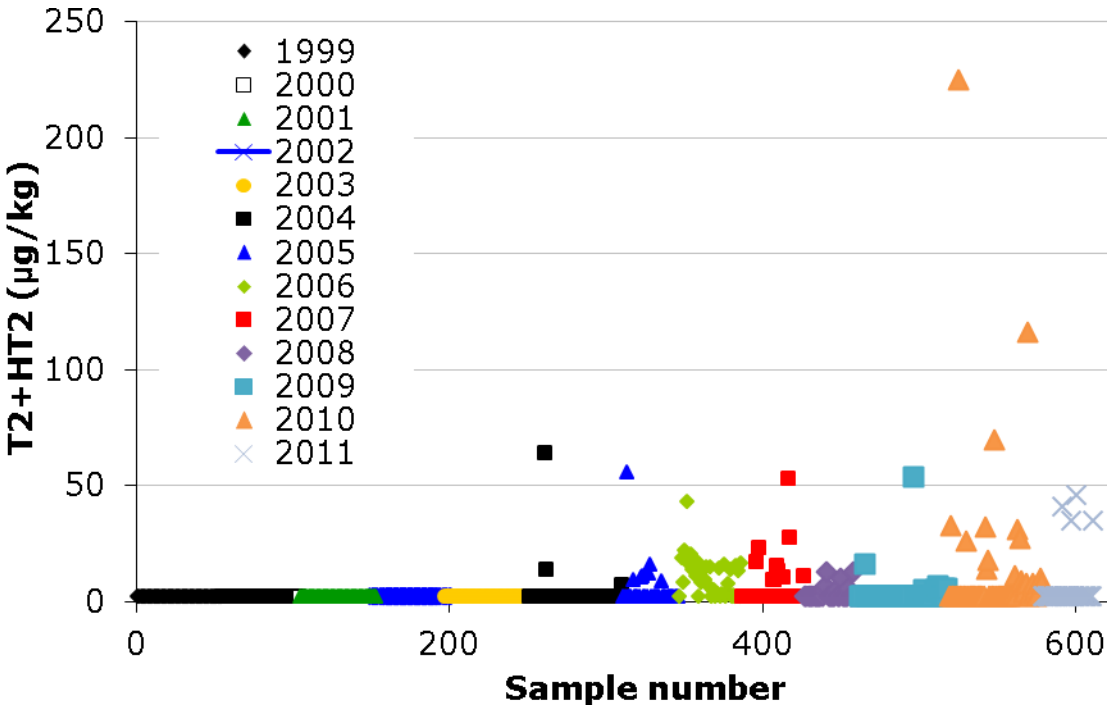
**Table 17: T-2 + HT-2 in freshly harvested milling wheat**

| Harvest year | Incidence % | Mean µg/kg | Median µg/kg | 95th Percentile µg/kg | Maximum µg/kg |
|---|---|---|---|---|---|
| 2009 | 0 | 5 | 5 | 5 | 5 |
| 2010 | 2 | 5 | 5 | 5 | 19 |
| 2011 | 0 | 5 | 5 | 5 | 5 |

**Table 18: T-2 + HT-2 in freshly harvested malting barley**

| Harvest year | Incidence % | Mean µg/kg | Median µg/kg | 95th Percentile µg/kg | Maximum µg/kg |
|---|---|---|---|---|---|
| 2009 | 5 | 7 | 5 | 6 | 54 |
| 2010 | 18 | 15 | 5 | 35 | 225 |
| 2011 | 12 | 9 | 5 | 37 | 46 |

**Figure 8: T-2 + HT-2 in malting barley 1999 - 2011**



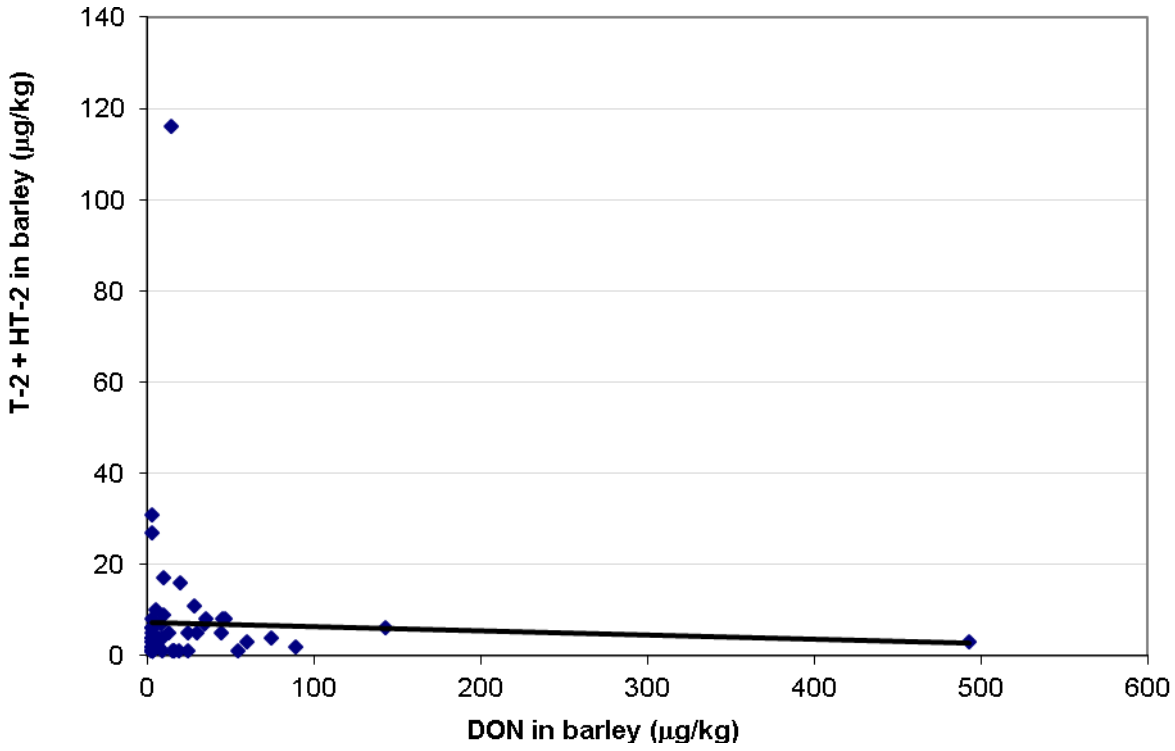

The paired barley and malt samples were analysed by a LC-MS/MS method with greater sensitivity (a quantification limit of 1 µg/kg rather than 10 µg/kg). This led to a greater incidence of contamination (Table 19) but also to lower mean values in the barleys. As with deoxynivalenol the correlation between barley and malt pairs was poor but there was clear evidence that levels were generally lower in malt than the corresponding barley.

**Table 19: T-2 + HT-2 in malting barley and malt**

| Harvest year | Sample | Incidence % | Mean µg/kg | Median µg/kg | 95th Percentile µg/kg | Maximum µg/kg |
|---|---|---|---|---|---|---|
| 2009 | Barley | 74 | 3.4 | 3.0 | 6.1 | 7.0 |
| | Malt | 0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 2010 | Barley | 44 | 3.5 | 1.0 | 16 | 17 |
| | Malt | 0 | 1.0 | 1.0 | 1.0 | 1.0 |
| 2011 | Barley | 20 | 1.5 | 1.0 | 3.1 | 5.0 |
| | Malt | 11 | 1.1 | 1.0 | 2.0 | 2.0 |

Deoxynivalenol and T-2/HT-2 are produced by different Fusarium species. Competition between species would be expected to give rise to differences in the relative incidence of these toxins in barley. Figure 9 shows this to be the case: the few incidences of high levels of deoxynivalenol and T-2/HT-2 were mutually exclusive.

**Figure 9: Correlation between DON and T-2 + HT-2 in malting barley**

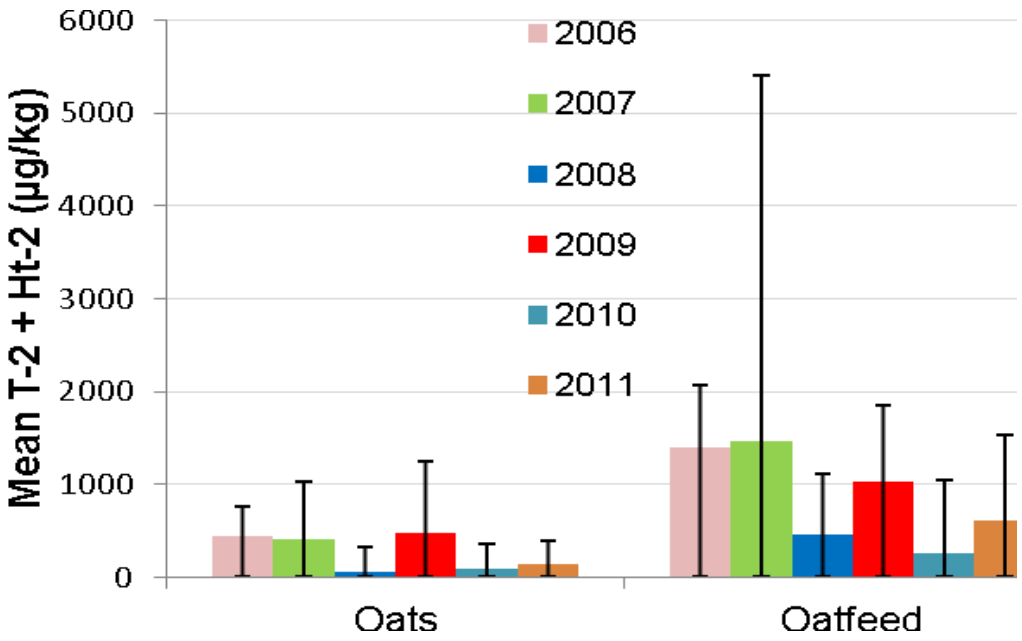



The incidences of T-2 and HT-2 in feed wheat and feed barley matched those of the food grain (Table 20). In line with earlier published data there was widespread contamination of feed oats and near universal contamination of oatfeed. However, mean and maximum levels over the three years were slightly lower than for the previous three years (Figure 10). No EC limits or guideline levels have yet been set for T-2 and HT-2 but the EC draft recommendation proposes an action level of 1000-1500 µg/kg for unprocessed oats intended for human consumption. The majority of raw oat samples would fall below these levels. The mean level of T-2+HT-2 over the three years was 230 µg/kg with a 95$^{th}$ percentile of 808 µg/kg. These values are very close to those reported in the 2011 EFSA Opinion (EFSA, 2011) for over 1400 unprocessed oat samples (236 µg/kg and 981 µg/kg respectively). Levels were higher in the oatfeed samples as would be expected from their higher content of husk.

**Table 20: T-2 + HT-2 in cereal feedingstuffs**

| Sample | Harvest year | Incidence % | Mean µg/kg | Median µg/kg | 95th Percentile µg/kg | Maximum µg/kg |
|---|---|---|---|---|---|---|
| Feed wheat | 2009 | 0 | 5 | 5 | 5 | 5 |
| | 2010 | 0 | 5 | 5 | 5 | 5 |
| | 2011 | 0 | 5 | 5 | 5 | 5 |
| Wheatfeed | 2009 | 0 | 5 | 5 | 5 | 5 |
| | 2010 | 0 | 5 | 5 | 5 | 5 |
| | 2011 | 0 | 5 | 5 | 5 | 5 |
| Feed barley | 2009 | 9 | 6 | 5 | 11 | 17 |
| | 2010 | 30 | 60 | 5 | 298 | 488 |
| | 2011 | 27 | 15 | 5 | 56 | 66 |
| Feed oats | 2009 | 100 | 529 | 329 | 1086 | 1240 |
| | 2010 | 64 | 81 | 28 | 303 | 362 |
| | 2011 | 91 | 141 | 93 | 368 | 386 |
| Oatfeed | 2009 | 100 | 962 | 1135 | 1746 | 1846 |
| | 2010 | 100 | 493 | 480 | 1034 | 1049 |
| | 2011 | 89 | 606 | 582 | 1395 | 1539 |

**gure 10: T-2 + HT-2 in oats and oatfeed 2006 - 2011**




*Nivalenol*

No limits have been set for nivalenol in cereals but the European Commission has requested an opinion from EFSA on it. It has commonly been measured and reported alongside deoxynivalenol. It was only intermittently found in milling wheat (Table 21): incidence in both malting barley sets

was higher but levels were generally very low (Tables 22 and 23). Levels in malt were consistently lower than in the parent barleys, both overall and in individual pairs. This indicates that nivalenol is largely removed during the malting process.

**Table 21: Nivalenol in freshly harvested milling wheat**

| Harvest year | Incidence % | Mean µg/kg | Median µg/kg | 95th Percentile µg/kg | Maximum µg/kg |
|---|---|---|---|---|---|
| 2009 | 13 | 8 | 5 | 23 | 46 |
| 2010 | 0 | 5 | 5 | 5 | 5 |
| 2011 | 4 | 5 | 5 | 5 | 17 |

**Table 22: Nivalenol in freshly harvested malting barley**

| Harvest year | Incidence % | Mean µg/kg | Median µg/kg | 95th Percentile µg/kg | Maximum µg/kg |
|---|---|---|---|---|---|
| 2009 | 59 | 19 | 8 | 72 | 86 |
| 2010 | 43 | 12 | 5 | 53 | 69 |
| 2011 | 61 | 48 | 25 | 242 | 314 |

**Table 23: Nivalenol in malting barley and malt**

| Harvest year | Sample | Incidence % | Mean µg/kg | Median µg/kg | 95th Percentile µg/kg | Maximum µg/kg |
|---|---|---|---|---|---|---|
| 2009 | Barley | 63 | 15 | 14 | 33 | 45 |
| | Malt | 5 | 5 | 5 | 5 | 10 |
| 2010 | Barley | 25 | 7 | 5 | 14 | 23 |
| | Malt | 0 | 5 | 5 | 5 | 5 |
| 2011 | Barley | 28 | 21 | 5 | 63 | 247 |
| | Malt | 17 | 11 | 5 | 43 | 71 |

The results for the wheat and barley based samples in the cereal feedingstuffs (Table 24) were similar to the food samples though with higher levels in the feed barleys. Nivalenol was found in the majority of oats and oatfeed samples.

**Table 24: Nivalenol in cereal feedingstuffs**

| Sample | Harvest year | Incidence % | Mean µg/kg | Median µg/kg | 95th Percentile µg/kg | Maximum µg/kg |
|---|---|---|---|---|---|---|
| Feed wheat | 2009 | 20 | 7 | 5 | 15 | 16 |
| | 2010 | 0 | 5 | 5 | 5 | 5 |
| | 2011 | 10 | 28 | 5 | 131 | 234 |
| Wheatfeed | 2009 | 15 | 6 | 5 | 12 | 15 |
| | 2010 | 0 | 5 | 5 | 5 | 5 |
| | 2011 | 6 | 6 | 5 | 7 | 19 |
| Feed barley | 2009 | 42 | 23 | 5 | 79 | 120 |
| | 2010 | 50 | 28 | 15 | 86 | 120 |
| | 2011 | 64 | 320 | 21 | 1309 | 1600 |
| Feed oats | 2009 | 100 | 284 | 236 | 678 | 922 |
| | 2010 | 82 | 94 | 11 | 351 | 411 |
| | 2011 | 91 | 111 | 65 | 321 | 438 |
| Oatfeed | 2009 | 86 | 211 | 222 | 374 | 384 |
| | 2010 | 88 | 171 | 93 | 480 | 538 |
| | 2011 | 100 | 219 | 185 | 565 | 697 |

In contrast to DON and T-2/HT-2 the relationship between DON and NIV is more complex. They are both produced by *F. culmorum* and *F. graminearum*, though by different chemotypes, and even here NIV is produced at low levels by DON chemotypes. However, NIV is also produced by *F. Poae,* which occurs under different conditions to *F. culmorum* and *F. Graminearum.* Scatter plots for the milling wheat and malting barley samples (Figures 11 and 12) indicate that the occurrence of NIV fits this relationship: in most instances NIV co-occurs with DON but in some cases there are relatively high levels of NIV when DON is either low or absent. These latter may be due to infections with *F poae.*

**Figure 11: Correlation between DON and NIV in milling wheat**

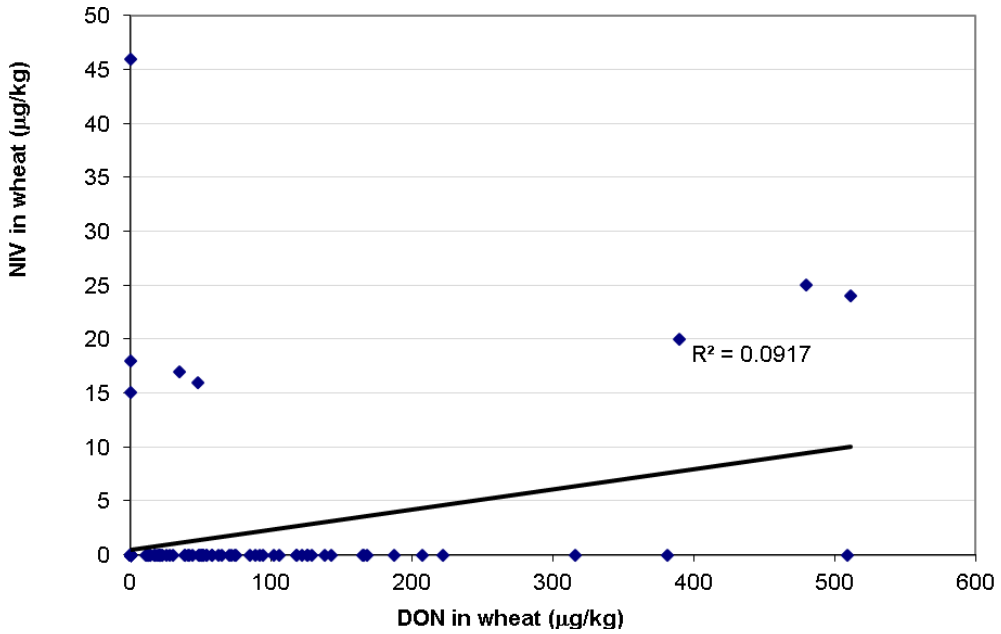
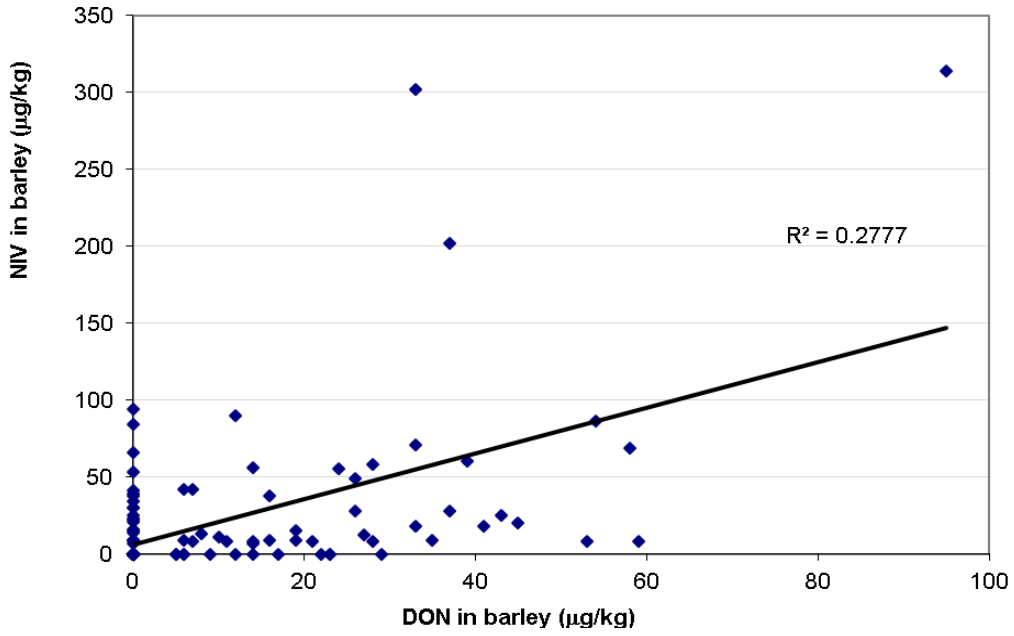
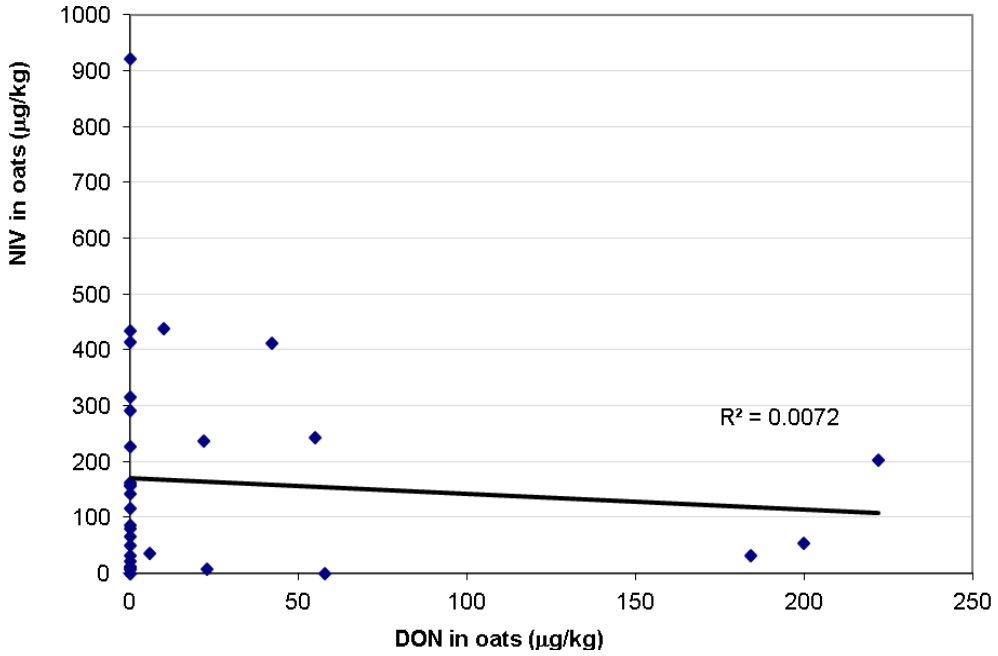



**Figure 12: Correlation between DON and NIV in malting barley**



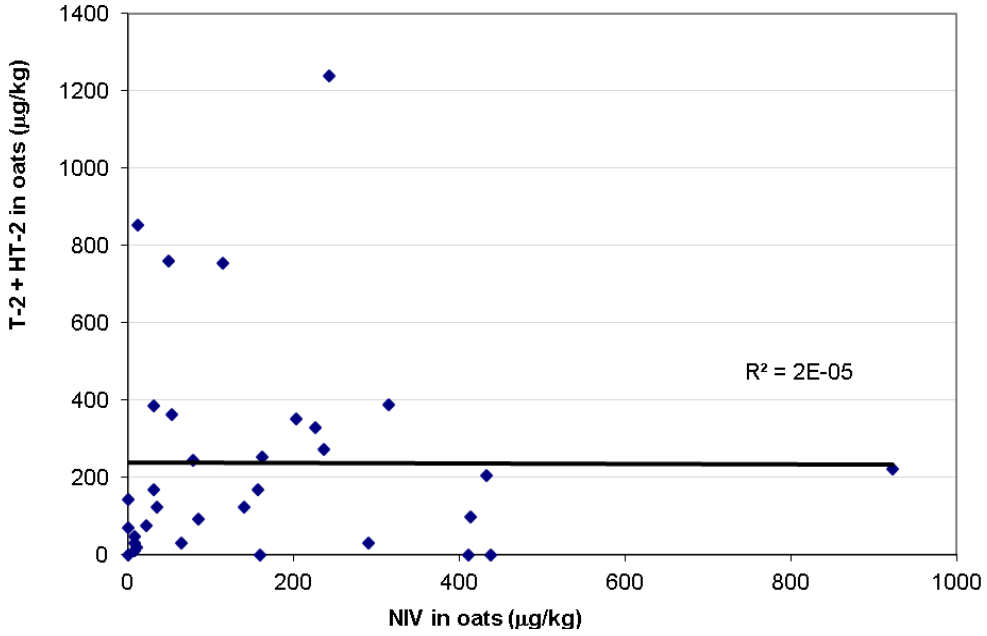

A similar relationship is seen in the feed oats samples, though here NIV is much more prevalent than DON is (Figure 13).

**Figure 13: Correlation between DON and NIV in feed oats**




The correlation between NIV and T-2 + HT-2 was similarly poor, in line with the different Fusarium species producing the toxins (Figure 14).

**Figure 14: Correlation between NIV and T-2 + HT-2 in feed oats**




*Other trichothecenes*

The other trichothecenes sought, 3-acetyl-DON, 15-acetyl-DON, diacetoxyscirpenol, fusarenone-X and neosolaniol, were rarely detected in any samples. There were occasional instances of 15-

acetyl-DON in samples with very high levels of DON and isolated instances of diacetoxyscirpenol and neosolaniol in oats or oatfeed samples heavily contaminated with other toxins.

*Field mycotoxins: Fusarium toxin zearalenone (ZON)*

Zearalenone was analysed in all freshly harvested samples of milling wheat and malting barley from each harvest (Tables 25 and 26). Levels were relatively high in 2009 but negligible in 2010 and 2011. In previous studies, incidence and levels in both wheat and barley have generally been low. Occasional harvests have shown high levels in wheat: 2004, 2008 (Edwards, 2011; Baxter *et al*., 2009; Salmon, 2006) and now 2009. These can all be linked to wet conditions immediately prior to harvest when the grain is particularly susceptible to infection and production of the toxin. A previous report (Baxter *et al.,* 2009) has suggested that the malting process reduces levels of zearalenone but the levels in the paired barley and malt samples here (Table 27) were too low to draw any meaningful conclusion.

A largely similar pattern was seen with the feed wheat and barley samples though there was an isolated case of a very high level in a feed barley sample in 2011 (Table 28). This sample apart, all samples were well within either EU limits or guideline limits and even the high barley would only have exceeded guideline limits for particular feedingstuffs intended for pigs. Levels of zearalenone in oats and oatfeed were low, even in 2009 when high levels were seen in wheat.

**Table 25: Zearalenone in freshly harvested milling wheat**

| Harvest year | Incidence % | Mean µg/kg | Median µg/kg | 95$^{th}$ Percentile µg/kg | Maximum µg/kg |
|---|---|---|---|---|---|
| 2009 | 72 | 11 | 7 | 40 | 84 |
| 2010 | 21 | 2 | 1 | 3 | 12 |
| 2011 | 4 | 1 | 1 | 1 | 4 |

**Table 26: Zearalenone in freshly harvested malting barley**

| Harvest year | Incidence % | Mean µg/kg | Median µg/kg | 95$^{th}$ Percentile µg/kg | Maximum µg/kg |
|---|---|---|---|---|---|
| 2009 | 23 | 2 | 1 | 4 | 16 |
| 2010 | 3 | 1 | 1 | 1 | 3 |
| 2011 | 3 | 1 | 1 | 1 | 7 |

**Table 27: Zearalenone in malting barley and malt**

| Harvest year | Sample | Incidence % | Mean µg/kg | Median µg/kg | 95$^{th}$ Percentile µg/kg | Maximum µg/kg |
|---|---|---|---|---|---|---|
| 2009 | Barley | 21 | 3 | 1 | 10 | 18 |
| | Malt | 5 | 1 | 1 | 1 | 7 |
| 2010 | Barley | 11 | 3 | 1 | 10 | 41 |
| | Malt | 16 | 3 | 1 | 6 | 33 |
| 2011 | Barley | 10 | 1 | 1 | 2 | 2 |
| | Malt | 17 | 2 | 1 | 4 | 13 |

**Table 28: Zearalenone in cereal feedingstuffs**

| Sample | Harvest year | Incidence % | Mean µg/kg | Median µg/kg | 95th Percentile µg/kg | Maximum µg/kg |
|---|---|---|---|---|---|---|
| Feed wheat | 2009 | 60 | 20 | 17 | 51 | 58 |
| | 2010 | 33 | 6 | 1 | 11 | 35 |
| | 2011 | 20 | 1 | 1 | 3 | 3 |
| Wheatfeed | 2009 | 90 | 37 | 26 | 109 | 128 |
| | 2010 | 28 | 14 | 1 | 89 | 97 |
| | 2011 | 22 | 1 | 1 | 6 | 6 |
| Feed barley | 2009 | 9 | 1 | 1 | 1 | 3 |
| | 2010 | 20 | 2 | 1 | 7 | 11 |
| | 2011 | 9 | 37 | 1 | 199 | 397 |
| Feed oats | 2009 | 33 | 3 | 1 | 9 | 10 |
| | 2010 | 18 | 1 | 1 | 3 | 3 |
| | 2011 | 0 | 1 | 1 | 1 | 1 |
| Oatfeed | 2009 | 60 | 9 | 9 | 19 | 21 |
| | 2010 | 75 | 9 | 10 | 18 | 21 |
| | 2011 | 33 | 3 | 1 | 12 | 17 |

### 3.3.3. Masked mycotoxins

The results imply that only a small percentage of the DON is present in bound form in any of the samples. Previous reports have indicated that the proportion of masked DON can rise during processing of cereals but the data here are insufficient to draw any conclusions.

A total of 11 malting barleys from the 2009 harvest, 5 barley malts, 1 wheat malt and 2 samples of oatfeed were analysed for deoxynivalenol-3-glucoside (DON-3-Glu; Table 29). The samples were chosen to cover as wide a range of DON concentrations as possible and included three of the barley/malt pairs (in which the malt was prepared from that barley). For each sample the amount of DON-3-Glu as a proportion of the total (free and bound) was calculated.

**Table 29: DON-3-Glucoside (D3G) in barley, malt and oatfeed**

| Sample | DON µg/kg | D3G µg/kg (as DON) | D3G as a % of DON |
|---|---|---|---|
| Barley | 145 | 37 | 20 |
| Barley | 1192 | 84 | 7 |
| Barley | 342 | 52 | 13 |
| Barley | 8 | 4 | 35 |
| Barley | 20 | 4 | 17 |
| Barley | 43 | 7 | 14 |
| Barley | 58 | 14 | 19 |
| Barley | 43 | 8 | 15 |
| Malt | 135 | 80 | 37 |
| Malt | 11 | 8 | 42 |
| Wheat malt | 1482 | 106 | 7 |
| Oatfeed | 209 | 9 | 4 |
| Oatfeed | 109 | 8 | 7 |
| | | Paired barley / malt samples | |
| Barley | 54 | 16 | 22 |
| Malt | 185 | 123 | 40 |
| Barley | <2 | <2 | Not applicable |
| Malt | <2 | <2 | Not applicable |
| Barley | 82 | 9 | 10 |
| Malt | 40 | 9 | 19 |

Although D3G was present in most of the samples in this set, concentrations were always less than 50% of the DON concentration. For most samples, D3G was less than 20% of the DON concentration.

These results are consistent with those reported for wheat (Berthiller, 2005, 2009). However the differences between these results and those published by the Lancova group (Lancova *et al*., 2008), as well as the variations within the small sample set, confirm that there is still much to learn about factors affecting the formation and destruction of D3G and other glycosylated mycotoxins.

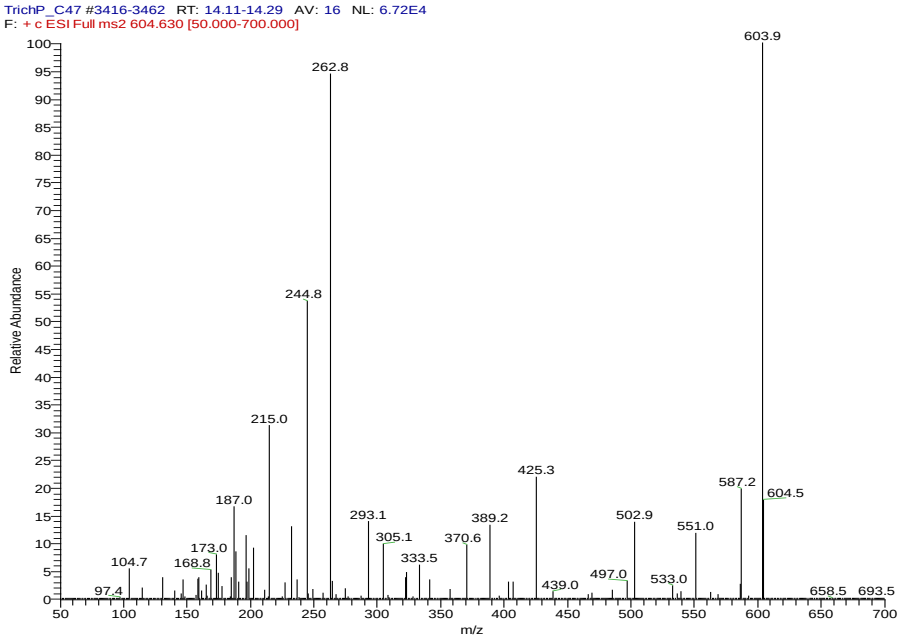
DON-3-Glu is the major masked analogue of the trichothecenes reported to date. The corresponding glycosides of T-2 and HT-2 toxins have recently been reported in both naturally contaminated and artificially inoculated wheat and in oats. Reference standards for these masked forms are not yet available and thus quantification is not possible and confirmation of their presence in cereals depends on mass spectrometric data.
Two samples of oatfeed with high levels of T-2 and HT-2 were analysed by LC-MS/MS to establish if glycosides or other metabolites were present. Product ion scans were used to screen for a range of predicted metabolites or adducts of T-2 and HT-2 (Table 30).

**Table 30: Calculated m/z values for $NH_4^+$ adducts of predicted metabolites of HT2 and T2**

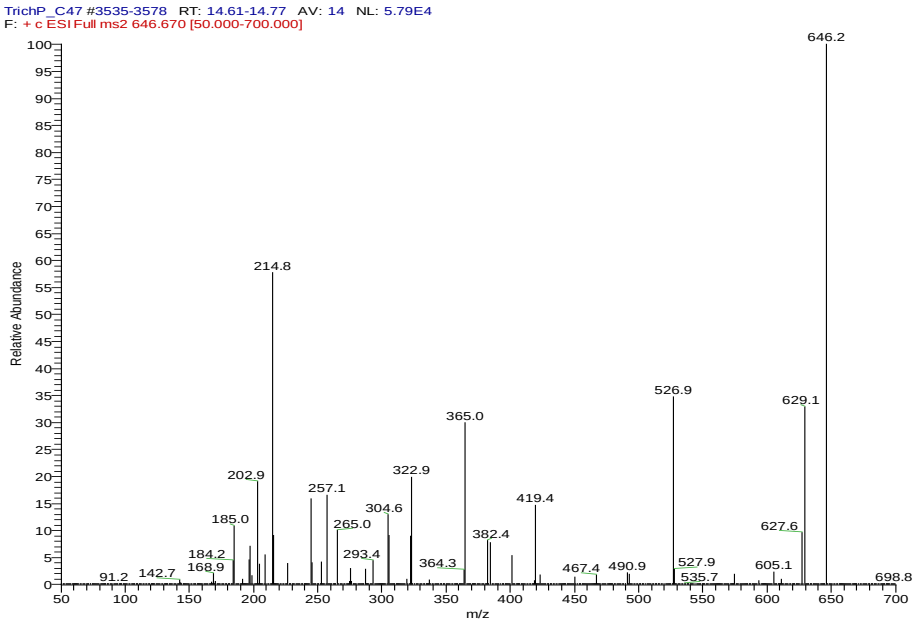
| Metabolite | Difference in mass from parent compound | MW of **T-2** Metabolite | $NH4^+$ adduct observed for **T-2** form | MW of **HT-2** Metabolite | $NH4^+$ adduct observed for **HT-2** form |
|---|---|---|---|---|---|
| Glucoside (Glc) | 162 | 628.52 | 646.52 | 586.48 | 604.48 |
| Hydroxy | 17 | 483.00 | 501.52 | 441.50 | 459.48 |
| Acetyl | 42 | 508.52 | 526.52 | 466.48 | 484.48 |
| Sulphate | 80 | 546.52 | 564.52 | 504.48 | 522.48 |
| Cysteine | 119 | 585.52 | 603.52 | 543.48 | 561.48 |
| Glutathione | 305 | 771.52 | 789.52 | 729.48 | 747.48 |
| GlcGlc | 324 | 790.52 | 808.52 | 748.48 | 766.48 |

Most of the ions chosen did not produce any clear signals for the predicted analytes. For a positive indication a discrete peak with MS/MS spectrum consistent with losses from the predicted analyte of interest was required. Three components were tentatively identified by this method: HT-2 Glucoside, T-2 Glucoside and T-2 Sulphate. The MS/MS spectra are shown in Figure 15.

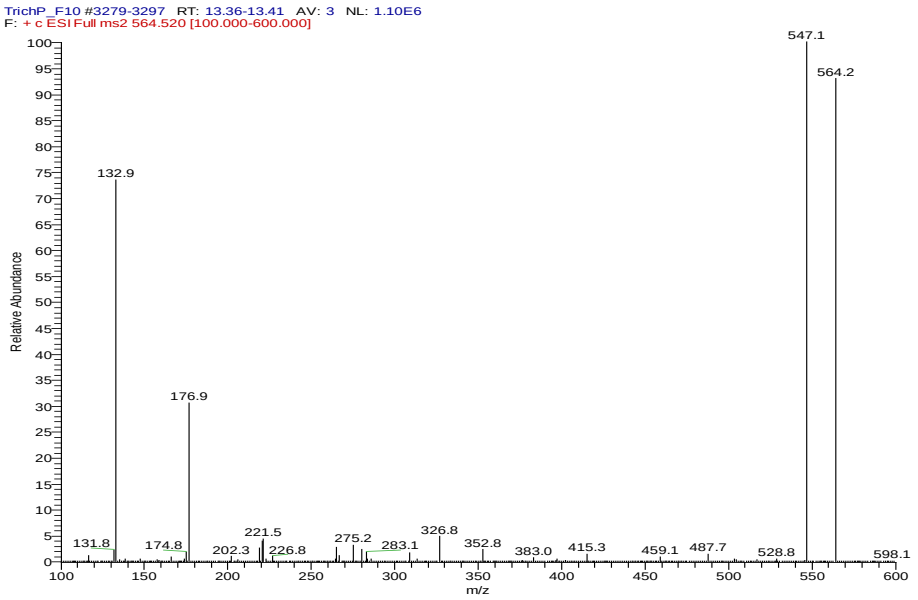
**Figure 15: MS/MS Spectra of Masked Mycotoxins Identified by Product Scans**




MS/MS spectrum of m/z 604.63 corresponding to HT-2Glc $[M+NH_4]^+$.




MS/MS spectrum of m/z 646.67 corresponding to T-2Glc $[M+NH_4]^+$.




MS/MS spectrum of m/z 564.2 corresponding to T-2-Sulphate $[M+NH_4]^+$.

It should be stressed that it is not possible to identify unambiguously an unknown component using low resolution data without a known standard to confirm the retention time and fragmentation pattern that is produced. In the case of the suggested T-2-Sulphate, the MS/MS spectrum did show three clear fragment ions at m/z 547, 177 and 133 all of which can have structures assigned that could be formed from the predicted structure of T-2-Sulphate. However, the MS/MS spectrum did not show isotopes around the pseudo-molecular ion so it was not possible to confirm either the presence of sulphur or the exact number of carbon atoms present in the molecule. Thus the identification must be taken as very tentative: further analysis of a known standard or high resolution MS data would be required to unambiguously characterise the molecule. Chromatographic peaks were found with MS/MS spectra consistent with recently published data (Lattanzio *et al*., 2012) for the T-2 and HT-2 glycosides. The data fitted with substitution at the C-3

position: however, the HT-2 toxin has an additional free hydroxyl functionality at the C-4 position, making that position an alternative location for the glucoside. It was not possible to confirm which form was present though a comparison with DON would suggest that substitution at the C-3 position is more likely.

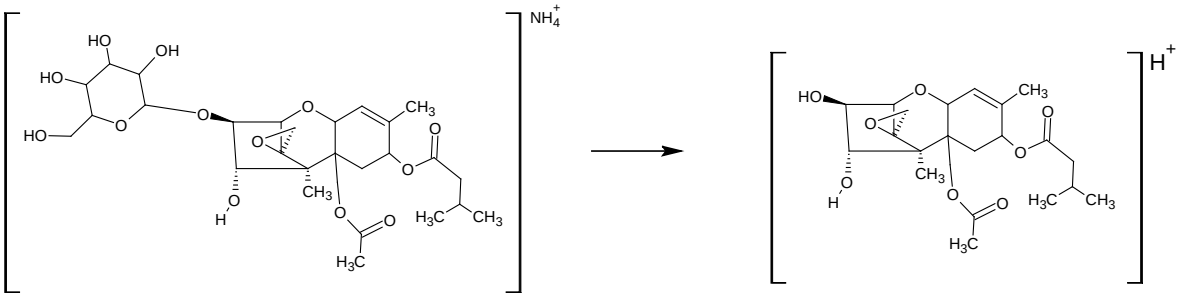
Once the retention time and spectra of the two glycosides had been established, a set of transitions was chosen to screen a selection of oatfeed and barley samples with known levels of T-2 and HT-2 (Table 31). The fragmentation pathways for the glycoside transitions are shown in Figure 16.

**Table 31: MRM transitions chosen for analysis of metabolites in the cereal screen.**

| | Precursor Ion | Product Ions | Retention Time (minutes) |
|---|---|---|---|
| T-2-Glc (MW=628.7) | 646.7 $(M+NH4)^+$ | 467.5 | 13.2 |
| HT-2-Glc (MW=586.6) | 604.6 $(M+NH4)^+$ | 425.5 | 11.0 |
| T-2 (MW=466.3) | 484.3 $(M+NH4)^+$ | 185; 219 | 13.0 |
| HT2 (MW=424.5) | 442.2 $(M+NH4)^+$ | 215; 263 | 8.0 |

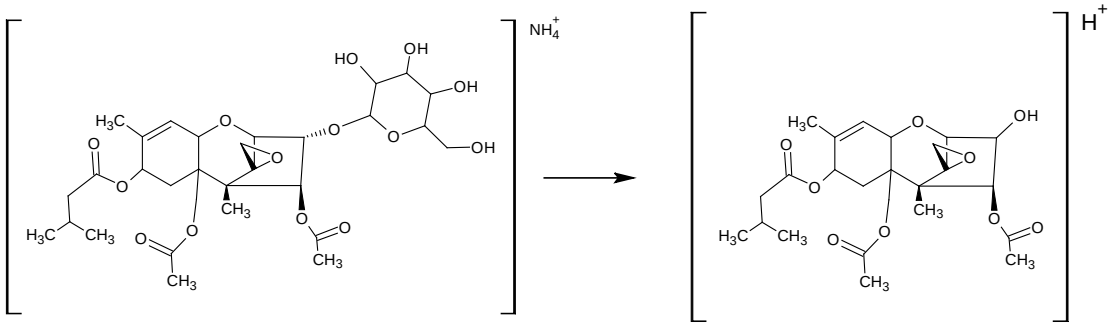
**Figure 16: Fragmentation Pathways for Transitions Characteristic of Masked Forms of HT-2 and T-2**

$HT_2Glc$



M/z 604.63 M/z 425.5

$T_2Glc$



M/z 646.7 M/z 467.5

13 barley and oatfeed samples with low, medium and high levels of T-2 and HT-2 were analysed. Though quantification of the glycosides was not possible the relative proportions could be estimated from peak intensities. No glycosides were detected in any of the barley samples, even

when low levels of T-2 and HT-2 were present. Both glycosides were detected in contaminated oatfeed samples, with the proportions roughly correlating with levels of T-2 and HT-2 (Table 32).

**Table 32: T-2 and HT-2 glycosides in barley and oatfeed samples**

| Sample | T-2 µg/kg | HT-2 µg/kg | T-2 glucoside Peak Intensity | HT-2 glucoside Peak Intensity |
|---|---|---|---|---|
| Barley A | <5 | <5 | 0 | 0 |
| Barley B | <5 | <5 | 0 | 0 |
| Barley C | <5 | <5 | 0 | 0 |
| Barley D | <5 | <5 | 0 | 0 |
| Barley E | 10 | 31 | 0 | 0 |
| Barley F | 5 | 32 | 0 | 0 |
| Barley G | 10 | 36 | 0 | 0 |
| Barley H | 5 | 30 | 0 | 0 |
| Oatfeed A | 28 | 85 | x | x |
| Oatfeed B | 127 | 434 | x x | x x x |
| Oatfeed C | 234 | 609 | x x | x x x |
| Oatfeed D | 294 | 834 | x x | x x x x |
| Oatfeed E | 287 | 809 | x x | x x x |

x; x x; etc. Denote relative peak intensity

### 3.3.4. Pesticide residues

*Storage insecticides and selected field fungicides*

Stored whole grains were tested for a range of insecticides currently or recently approved for use on stored cereal grain or in cereal stores in the UK. Barley and oat samples were additionally analysed for a limited number of field fungicides commonly used on cereals. The residues sought are listed in the Materials and Methods section (Table 7). Milling wheat and feed grains were sampled six to eight months after harvest and malting barleys three to eight months after harvest. Only a few pesticides were detected in any of the samples and in virtually all cases the levels were very low, typically only a few *per cent* of the MRL (Tables 33 to 35). Approximately 80% of food grains contained no detectable residues and pirimiphos-methyl was by far the most common residue detected in the remainder; no sample exceeded 0.2 mg/kg, against an MRL of 5 mg/kg. Chlorpyrifos-methyl and malathion were detected in a small percentage of wheat samples. Malathion is not currently approved for use in the UK but one of the two samples where it was found was imported and the very low level would imply that it was treated some time prior to import. The second positive sample was only just above the limit of detection. Neither sample exceeded the current MRL.

**Table 33: Pesticide residues in stored milling wheats, 2009 – 2011 harvests**

| Pesticide | LOD | % > LOD | Mean of all samples* | Mean of positive samples | Maximum | EU MRL |
|---|---|---|---|---|---|---|
| | mg/kg | | mg/kg | mg/kg | mg/kg | mg/kg |
| pirimiphos-methyl | 0.01 | 6 | <0.01 | 0.07 | 0.19 | 5 |
| chlorpyrifos-methyl | 0.01 | 8 | <0.01 | 0.03 | 0.08 | 3 |
| malathion | 0.01 | 2 | <0.01 | 0.09 | 0.16 | 8 |

* Mean is calculated by assuming that all samples below the limit of detection contained half that limit.

**Table 34: Pesticide residues in stored malting barleys, 2009 – 2011 harvests**

| Pesticide | LOD | % > LOD | Mean of all samples* | Mean of positive samples | Maximum | EU MRL |
|---|---|---|---|---|---|---|
| | mg/kg | | mg/kg | mg/kg | mg/kg | mg/kg |
| pirimiphos-methyl | 0.01 | 19 | 0.02 | 0.07 | 0.19 | 5 |
| cyprodinil | 0.01 | 2 | <0.01 | 0.08 | 0.08 | 3 |

* Mean is calculated by assuming that all samples below the limit of detection contained half that limit.

Piperonyl butoxide, a synergist used with pyrethroid insecticides, was detected in a small percentage of samples each year. This reflects use of the pyrethroid deltamethrin, which is authorised for use on stored cereals and in cereal stores. However, residues of deltamethrin were only detected in oatfeed samples and feed barley.

**Table 35: Pesticide residues in stored feed cereals, 2009 – 2011 harvests**

| Cereal | Chemical | LOD | % > LOD | Mean of all samples* | Mean of positive samples | Maximum |
|---|---|---|---|---|---|---|
| | | mg/kg | | mg/kg | mg/kg | mg/kg |
| Feed wheat | pirimiphos-methyl | 0.01 | 9 | <0.01 | 0.44 | 0.85 |
| Wheatfeed | pirimiphos-methyl | 0.01 | 25 | 0.01 | 0.05 | 0.07 |
| | chlorpyrifos-methyl | 0.01 | 25 | 0.05 | 0.08 | 0.10 |
| Feed barley | pirimiphos-methyl | 0.01 | 36 | 0.01 | 0.03 | 0.08 |
| | deltamethrin | 0.01 | 9 | <0.01 | 0.03 | 0.03 |
| | cyprodinil | 0.01 | 9 | <0.01 | 0.12 | 0.12 |
| Feed oats | pirimiphos-methyl | 0.01 | 71 | 2.10 | 3.00 | 8.40 |
| Oatfeed | pirimiphos-methyl | 0.01 | 78 | 0.02 | 0.30 | 0.67 |
| | deltamethrin | 0.01 | 11 | <0.01 | 0.03 | 0.03 |

* Mean is calculated by assuming that all samples below the limit of detection contained half that limit.

*Glyphosate*

Glyphosate is a herbicide that is also authorised for use as a desiccant on cereals, where it may be used immediately before harvesting. The MRL is relatively high – 20 mg/kg – and it is one of the residues most frequently reported in official surveys of cereals in the UK *(EC, 2005a).* Selected samples of barley and wheat from the 2011 harvest were analysed for glyphosate. Results are shown in Table 36. Negligible amounts were found in malting barley and though a majority of other samples contained glyphosate the levels were low with only a couple of samples exceeding 10% of the EU MRL.

**Table 36: Glyphosate in selected cereals (freshly harvested)**

| | Incidence % | Mean | Range |
|---|---|---|---|
| | % | mg/kg | mg/kg |
| Malting barley | 14 | 0.03 | < 0.05 - 0.09 |
| Milling wheat | 55 | 0.23 | < 0.05 - 1.60 |
| Feed barley | 82 | 1.20 | < 0.05 - 3.70 |
| Feed wheat | 60 | 0.11 | < 0.05 - 0.40 |

* Mean is calculated by assuming that all samples below the limit of detection contained half that limit.

The 2011 harvest was relatively wet, particularly in Scotland; hence, usage of glyphosate might be expected to be higher than in drier years. However, there was no clear evidence of higher levels in cereal samples grown in Scotland.

*Plant growth regulators*

Selected malting barley and milling wheat samples were tested for both chlormequat and mepiquat in 2011 (Table 37). Chlormequat was detected in the majority of samples (41% of barleys and 80% of wheats) with higher levels in the wheats. Although the incidence of residues was high, actual concentrations were low. Mean values were well below the EU MRL for chlormequat as was the highest level found (1.14 mg/kg in a sample of milling wheat). The incidence and range of concentrations were broadly similar to those previously found in wheat *(Griffiths and Mason, 2003; Baxter et al., 2009)* and barley (Baxter *et al.*, 2009). Mepiquat was much less common, again in line with previous data.

**Table 37: Chlormequat and mepiquat in selected cereals (freshly harvested)**

| | Chlormequat (mg/kg) | | |
|---|---|---|---|
| | Incidence % | Mean | Range |
| Malting barley | 41 | 0.08 | <0.01 - 0.56 |
| Milling wheat | 80 | 0.43 | <0.01 - 1.14 |

| | Mepiquat mg/kg | | |
|---|---|---|---|
| | Incidence % | Mean | Range |
| Malting barley | 9 | 0.01 | <0.01 - 0.08 |
| Milling wheat | 0 | <0.03 | All < 0.03 |

* Mean is calculated by assuming that all samples below the limit of detection contained half that limit.

## 3.3.5. Heavy metals

Selected freshly harvested samples from the 2011 harvest were analysed for cadmium, lead, aluminium, arsenic and mercury. Results are summarised in Tables 38 to 41.

Cadmium levels in milling wheat and malting barley were all within the current EU limits but a small percentage of samples were close to or above the reduced limits (0.1 mg/kg for wheat; 0.075 for barley) being discussed at the time of writing. A 95$^{th}$ percentile value of 0.097 mg/kg for wheat implies that potentially a significant proportion of the harvest could exceed the proposed new limit. Results were similar to those for samples from previous HGCA surveys *(Baxter, 2002, 2006; Baxter et al., 2009; Salmon, 2006)*.

**Table 38: Cadmium in selected freshly harvested grain samples**

| | Mean | Range | 95$^{th}$ percentile | EU limit | Proposed EU limit |
|---|---|---|---|---|---|
| | mg/kg | mg/kg | | mg/kg | mg/kg |
| Malting barley | 0.020 | 0.002 - 0.081 | 0.040 | 0.1 | 0.075 |
| Milling wheat | 0.043 | 0.008 - 0.108 | 0.097 | 0.2 | 0.10 |
| Feed barley | 0.025 | 0.002 - 0.051 | 0.048 | 1.0 | – |
| Feed oats | 0.014 | 0.005 - 0.038 | 0.331 | 1.0 | – |
| Feed wheat | 0.036 | 0.015 - 0.067 | 0.066 | 1.0 | – |

All samples were well below current EU limits for lead in cereal foods and feedingstuffs. Mean values and range of values were similar to those reported in the previous surveys cited above.

**Table 39: Lead in selected freshly harvested grain samples**

| | Mean | Range | EU limit |
|---|---|---|---|
| | mg/kg | mg/kg | mg/kg |
| Malting barley | 0.014 | <0.01 - 0.03 | 0.2 |
| Milling wheat | 0.012 | <0.01 - 0.06 | 0.2 |
| Feed barley | 0.031 | <0.01 - 0.07 | 10.0 |
| Feed oats | 0.040 | <0.01 - 0.24 | 10.0 |
| Feed wheat | 0.010 | <0.01 - 0.02 | 10.0 |

Levels of arsenic were similarly well below legal limits and in line with previous surveys. Mercury was not detected in any samples (limit of detection was 0.01 mg/kg).

**Table 40: Arsenic in selected freshly harvested grain samples**

| | Mean | Range | EU limit |
|---|---|---|---|
| | mg/kg | mg/kg | mg/kg |
| Malting barley | 0.013 | <0.01 - 0.02 | 1 |
| Milling wheat | 0.015 | <0.01 - 0.03 | 1 |
| Feed barley | 0.015 | 0.01 - 0.03 | 2 |
| Feed oats | 0.039 | <0.01 - 0.22 | 2 |
| Feed wheat | 0.010 | 0.01 - 0.02 | 2 |

No limits have been set for aluminium in cereals, but the levels found in malting barley were within the range reported by the Ministry of Agriculture, Fisheries and Food (MAFF) for barley in their multi-element survey of food in the UK *(MAFF, 1994).* The mean value (3.2 mg/kg) was lower than in the MAFF survey (6.4 mg/kg) and a previous HGCA survey (Baxter *et al*., 2009) (4.1 mg/kg). The mean level in wheat of 2.7 mg/kg was similar to that in the MAFF survey (2.4 mg/kg). The mean values for the feed barley, feed oats and feed wheat samples were skewed for a small number of very high values: the median values imply levels are generally similar to the food grains.

**Table 41: Aluminium in selected freshly harvested grain samples**

| | Mean | Range | Median |
|---|---|---|---|
| | mg/kg | mg/kg | mg/kg |
| Malting barley | 3.2 | 0.70 – 6.4 | 3.1 |
| Milling wheat | 2.7 | 0.50 – 6.4 | 2.2 |
| Feed barley | 15.8 | <0.01 – 97 | 3.5 |
| Feed oats | 29.7 | 0.13 – 300.0 | 2.3 |
| Feed wheat | 4.5 | 0.90 – 22.6 | 2.2 |

### 3.3.6. Ergot alkaloids

Analysis for ergot alkaloids was carried out exclusively on grain deliveries that had been rejected at intake following routine checks for the presence of ergot sclerotia. Samples were taken at the flour mill or maltings site and analysed for the six alkaloids and epimers recommended for monitoring by the European Commission.

Nine wheat samples from the 2009 harvest were analysed initially. Eight were tested as entire ground samples; no attempt was made to establish if the sub-samples contained sclerotia. One visibly heavily contaminated sample of organic wheat was analysed both whole and after sieving to remove the sclerotia. 7 of the 8 wheat samples had detectable alkaloids though only 2 contained individual alkaloids significantly above 10 µg/kg. The highest total alkaloid content was 339 µg/kg in a sample containing 8 of the 12 alkaloids. Ergosine, ergotamine and ergocristine were the most commonly found alkaloids. The organic wheat sample contained high levels of alkaloids though the alkaloids and levels found differed before and after sieving. The levels were higher after sieving (1550 µg/kg vs. 229 µg/kg), a conflict with the assumption that removal of the sclerotia would lower levels in the body of the grain and probably due to heterogeneity of the sample.

18 wheat, 7 barley and 5 rye samples from the 2010 harvest were analysed. In an attempt to establish if alkaloids were being transferred from sclerotia to otherwise clean, grain two sub-samples were taken: one was analysed directly and the second sieved to remove sclerotia prior to analysis. It was not possible to isolate sufficient sclerotia to analyse them directly. 22 of the samples contained no detectable alkaloids when analysed whole and hence the sieved samples were not analysed. Of the remaining samples, 3 wheat, 3 barley and 2 rye samples contained alkaloids in one or both of the sub-samples. Results for these samples are shown in Tables 42 to 44. It was difficult to discern consistent patterns of distribution of alkaloids between whole and sieved samples. The two rye samples were heavily contaminated and both showed substantial reductions after sieving as did one of the wheat samples; the reduction was less clear cut for the other two wheat samples. The barley samples in contrast had no alkaloids prior to sieving but detectable levels of several alkaloids after sieving. Given the low levels in the wheat and barley samples it is not possible to say what impact sieving had on the levels of alkaloids.

**Table 42: Ergot alkaloids in contaminated wheat before and after sieving**

| | Sample Reference | | | | | |
|---|---|---|---|---|---|---|
| Alkaloid (µg/kg) | Wheat A - whole | Wheat A - sieved | Wheat B - whole | Wheat B - sieved | Wheat C - whole | Wheat C - sieved |
| Ergometrine | <10 | <10 | <10 | <10 | <10 | <10 |
| Ergometrinine | <10 | <10 | <10 | <10 | <10 | <10 |
| Ergosine | <10 | <10 | 22 | <10 | 39 | <10 |
| Ergosinine | <10 | <10 | <10 | <10 | 10 | <10 |
| Ergotamine | <10 | <10 | <10 | <10 | 36 | <10 |
| Ergotaminine | <10 | <10 | <10 | <10 | 10 | <10 |
| Ergocornine | <10 | <10 | <10 | <10 | <10 | <10 |
| Ergocorninine | <10 | <10 | <10 | <10 | <10 | <10 |
| Ergocryptine | <10 | <10 | <10 | <10 | <10 | <10 |
| Ergocryptinine | <10 | <10 | <10 | <10 | <10 | <10 |
| Ergocristine | 32 | 10 | 13 | <10 | 45 | <10 |
| Ergocristinine | <10 | <10 | <10 | <10 | <10 | <10 |

**Table 43: Ergot alkaloids in contaminated barley before and after sieving**

| | Sample Reference | | | | | |
|---|---|---|---|---|---|---|
| Alkaloid (µg/kg) | Barley A - whole | Barley A - sieved | Barley B - whole | Barley B - sieved | Barley C - whole | Barley C - sieved |
| Ergometrine | <10 | <10 | <10 | <10 | <10 | <10 |
| Ergometrinine | <10 | <10 | <10 | <10 | <10 | <10 |
| Ergosine | <10 | 38 | <10 | <10 | <10 | 28 |
| Ergosinine | <10 | 10 | <10 | <10 | <10 | 62 |
| Ergotamine | <10 | 15 | <10 | <10 | <10 | <10 |
| Ergotaminine | <10 | <10 | <10 | <10 | <10 | <10 |
| Ergocornine | <10 | <10 | <10 | <10 | <10 | <10 |
| Ergocorninine | <10 | <10 | <10 | <10 | <10 | <10 |
| Ergocryptine | <10 | <10 | <10 | <10 | <10 | <10 |
| Ergocryptinine | <10 | <10 | <10 | <10 | <10 | <10 |
| Ergocristine | <10 | 30 | <10 | 27 | <10 | 30 |
| Ergocristinine | <10 | 10 | <10 | <10 | <10 | 25 |

**Table 44: Ergot alkaloids in contaminated rye before and after sieving**

| | Sample Reference | | | |
|---|---|---|---|---|
| Alkaloid (µg/kg) | Rye A - whole | Rye A - sieved | Rye B - whole | Rye B - sieved |
| Ergometrine | 13 | <10 | <10 | <10 |
| Ergometrinine | <10 | <10 | <10 | <10 |
| Ergosine | 191 | 27 | 88 | <10 |
| Ergosinine | 68 | 10 | 21 | <10 |
| Ergotamine | 43 | <10 | <10 | <10 |
| Ergotaminine | 13 | <10 | <10 | <10 |
| Ergocornine | 73 | <10 | 93 | <10 |
| Ergocorninine | 44 | <10 | 27 | <10 |
| Ergocryptine | 134 | 135 | 426 | <10 |
| Ergocryptinine | 126 | 40 | 21 | <10 |
| Ergocristine | 88 | <10 | <10 | <10 |
| Ergocristinine | 45 | <10 | <10 | <10 |

A further 14 malting barley samples from the 2011 harvest were analysed. Again, these were from grain deliveries rejected due to visible sclerotia and as with the 2009 samples these were analysed without any attempt to remove sclerotia. Eight of the samples contained no detectable alkaloids; results for the remainder showed only two samples contained individual alkaloids at significantly more than 10 µg/kg (Table 45).

**Table 45: Ergot alkaloids in contaminated malting barley**

| | Sample Reference | | | | | |
|---|---|---|---|---|---|---|
| Alkaloid (µg/kg) | Barley A | Barley B | Barley C | Barley D | Barley E | Barley F |
| Ergometrine | 50 | <10 | <10 | <10 | 11 | <10 |
| Ergometrinine | <10 | <10 | <10 | <10 | <10 | <10 |
| Ergosine | 385 | 20 | 10 | <10 | <10 | 17 |
| Ergosinine | 105 | <10 | 10 | <10 | <10 | 10 |
| Ergotamine | 1200 | <10 | <10 | <10 | <10 | <10 |
| Ergotaminine | 120 | <10 | <10 | <10 | <10 | <10 |
| Ergocornine | 70 | <10 | <10 | <10 | <10 | 56 |
| Ergocorninine | 25 | <10 | <10 | <10 | <10 | 18 |
| Ergocryptine | 40 | <10 | <10 | <10 | <10 | 35 |
| Ergocryptinine | 15 | <10 | <10 | <10 | <10 | 10 |
| Ergocristine | 43 | <10 | 33 | 25 | <10 | <10 |
| Ergocristinine | <10 | <10 | 15 | <10 | <10 | <10 |

Ergot is known to occur as ‘races’ dependent on the plant hosts on which the disease develops. There is evidence from other published data that the production of different alkaloids is not just

dependent on host but is influenced by a range of both biotic and abiotic factors. Even from this limited study, it appears that all of the alkaloids (with the possible exception of ergometrine) may be produced on any plant host. However, the mix of these appears to be quite variable. The principal alkaloids found were ergotamine, ergosine and ergocristine, in each case usually accompanied by lower levels of the corresponding epimers. Overall the results provide some evidence that ergot sclerotia leave 'footprints' on grain although the level of these alkaloids appears to be quite low.

## 3.4. Discussion

Overall, the data established by this project suggest that the bulk of UK-grown cereals comply with EU and UK legislation and recommendations with regard to the presence of contaminants.
**Mycotoxins:** Ochratoxin A was detected regularly, in the range of 15-35% of food grains (milling wheat and malting barley) but concentrations were generally low, suggesting that toxin synthesis in food grains is being successfully kept in check by storage conditions. Occasional samples exceeded legal limits but the levels were not always replicated upon re-sampling: the well-recognised difficulties with obtaining representative samples remain a problem. Incidence in compounded samples (wheatfeed, oatfeed) was significantly higher but levels were invariably well below guideline levels.

The situation with trichothecenes was very different from that of Ochratoxin A. Concentrations of these toxins varied from year to year. Over the short term, concentrations followed changes in climatic conditions. DON was the commonest trichothecenes in barley and wheat, whilst T-2 and HT-2 toxins predominated in oats.

**Pesticides:** although many samples tested contained detectable residues of agrochemicals, concentrations were very low, and were invariably well below legal MRLs for all chemicals sought. The residue detected most frequently was the growth regulator chlormequat, which was found in the majority of samples tested, though residues were well below the MRL. The only other pesticide detected with any frequency was the storage insecticide pirimiphos-methyl, which was detected in upwards of 10% of food grain samples and over 20% of feed samples. Levels, however, were in the vast majority of cases only marginally above the detection limit. Overall, the generally low concentrations detected for all pesticides relative to legal limits suggested that pesticides in UK-grown cereals present no health hazard.

**Heavy metals:** Concentrations of metals were generally low in the samples tested and well below current legal limits. Proposing new lower limits for cadmium, however, may pose a problem with some samples of wheat approaching the limit.

**Emerging issues:** Ergot alkaloids have arisen as an issue in the last couple of years following calls for data from both the FSA and EFSA.

The establishment by EFSA of a tolerable daily intake (TDI) for T-2 + HT-2 has focused attention on setting legal limits. Proposed “discussion levels” for food and feed were put forward in early 2012: some of the UK grain harvest and processed cereal products produced from it might have been close to these, however they have subsequently been withdrawn.

The recent EFSA Opinion on alternaria toxins has highlighted the lack of data on these toxins in cereals. Grains and grain-based products are identified as one of the primary sources of these toxins but none of the available data derive from the UK.

Historically, the vast majority of samples have fallen below legal limits for cadmium. However, the potential lowering of the limits for cereals and in particular wheat may cause problems, suggesting that more extensive monitoring might be required.

Incidences of dioxin contamination of animal feed have been reported in Ireland in 2008 and Germany in 2011. Neither was ascribed to cereal sources but there is a lack of data on incidence in cereals, particularly in the feed sector. No analysis has been carried out in the monitoring programme since 2003 hence there is a need to incorporate into future monitoring programmes.

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