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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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# CONTENTS

<b>1.</b>	<b>ABSTRACT .....</b>	<b>4</b>
<b>2.</b>	<b>SUMMARY.....</b>	<b>5</b>
<b>2.1.</b>	<b>Introduction/Background and aims.....</b>	<b>5</b>
<b>2.2.</b>	<b>Materials and methods.....</b>	<b>5</b>
<b>2.3.</b>	<b>Results .....</b>	<b>6</b>
<b>2.4.</b>	<b>Discussion/Conclusions and implications .....</b>	<b>12</b>
<b>3.</b>	<b>TECHNICAL DETAIL.....</b>	<b>14</b>
<b>3.1.</b>	<b>Introduction .....</b>	<b>14</b>
3.1.1.	Storage mycotoxins .....	14
3.1.2.	Field mycotoxins.....	15
3.1.3.	Masked mycotoxins .....	16
3.1.4.	Pesticide residues .....	17
3.1.5.	Heavy metals.....	18
3.1.6.	Ergot alkaloids.....	18
<b>3.2.</b>	<b>Materials and methods.....</b>	<b>19</b>
3.2.1.	Samples .....	19
3.2.2.	“Horizon scanning” for emerging issues.....	22
3.2.3.	Analysis of mycotoxins .....	22
3.2.4.	Analysis of pesticide residues.....	23
3.2.5.	Analysis of heavy metals .....	24
3.2.6.	Analysis of ergot alkaloids .....	25
3.2.7.	Analysis of “masked” mycotoxins.....	25
<b>3.3.</b>	<b>Results .....</b>	<b>25</b>
3.3.1.	Storage mycotoxins .....	25
3.3.2.	Field mycotoxins.....	29
3.3.3.	Masked mycotoxins .....	42
3.3.4.	Pesticide residues .....	47
3.3.5.	Heavy metals.....	50
3.3.6.	Ergot alkaloids.....	52

<b>3.4.</b>	<b>Discussion .....</b>	<b>55</b>
<b>3.5.</b>	<b>References.....</b>	<b>56</b>

## 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<sup>th</sup> 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.