Project Report No. 387

February 2006

Price: £4.00

Monitoring the quality and safety of grain and grain-derived co-products destined for animal feed

by

E D Baxter[1] and S Salmon[2]

[1]Brewing Research International, Lyttel Hall, Nutfield, Surrey RH1 4HY
[2]Campden and Chorleywood Research Association, Chipping Campden, Glos., GL55 6LD

This is the final report of a twenty month project that commenced in May 2004. The work was funded by a contract for £109,462 from the Home-Grown Cereals Authority (Project 3033) and a contribution of £5,000 in kind from Agricultural Industries Confederation, making a total of £114,462.

The Home-Grown Cereals Authority (HGCA) has provided funding for this project but has not conducted the research or written this report. While the authors have worked on the best information available to them, neither HGCA nor the authors shall in any event be liable for any loss, damage or injury howsoever suffered directly or indirectly in relation to the report or the research on which it is based.

Reference herein to trade names and proprietary products without stating that they are protected does not imply that they may be regarded as unprotected and thus free for general use. No endorsement of named products is intended nor is it any criticism implied of other alternative, but unnamed, products.

TABLE OF CONTENTS **Page**

LIST OF TABLES **Page**

LIST OF FIGURES

1. ABSTRACT

The overall aim of this project was to monitor the quality and safety of UK animal feed derived from UK-grown cereals. It was carried out jointly by CCFRA, who were responsible for the wheat samples, and BRi (the project co-ordinators), who were responsible for the barley and oat samples. The project ran from April 2004 to December 2005, when it was replaced by new project (HGCA 3100), which also covers malting barley and milling wheat.

The project comprised:

- Collection of representative sample sets of feed cereals, testing them for a range of contaminants, and comparing the results with legal limits or proposed guideline values.
- An ongoing review of proposed and incoming legislation and opinions from food/feed standards bodies (such as the UK Food Standards Agency and European Food Safety Authority) in order to identify any emerging issues which could impact on the safety or quality of feed cereals.

The results indicated that, for the overwhelming majority of animal feed materials derived from UK-grown cereals, levels of contaminants were well below legal limits in the EU.

Levels of **heavy metals** and **arsenic** were, with one exception (a wheatfeed), very low. Cereal products are unlikely to be a significant source of metal contaminants in animal feed.

Aflatoxins were only detected in two samples (both feed barleys), and then only at very low levels compared with the legal limit.

Ochratoxin A was widespread in oatfeed and wheatfeed, but was detected in less than half of grain samples. Maximum levels were well below the proposed Guideline Value for feed materials, but some samples approached the Guideline Values for complete or complementary feedingstuffs in sensitive species such as pigs.

Trichothecene mycotoxins from *Fusarium* were generally widespread in all samples except feed barley. Deoxynivalenol was detected most frequently, except in oats where T-2 and HT-2 were more common. There was also evidence of an increase in the incidence of T-2 and HT-2 in other cereals from 2004 to 2005. Currently there are no legal limits for trichothecenes in animal feed, but all the samples tested were well below the proposed guideline limits for feed materials. However, some of the oats and wheat samples exceeded the proposed guidelines for complete feedingstuffs for sensitive species such as pigs.

Zearalenone was rare in barley and oats but widespread in wheat and wheat feed.

Residues of **Pesticides** were uniformly low. Organochloride insecticides were not detected in any of the samples. Of the insecticides currently used in grain storage, pirimiphos-methyl was detected most frequently, but concentrations were always well below legal MRLs. Cereal products are unlikely to be a significant source of pesticide residues in animal feed.

2. SUMMARY

The overall aim of this project was to monitor the quality and safety of UK animal feed derived from UK-grown cereals. It was carried out jointly by CCFRA, who were responsible for the wheat samples, and BRi (the project co-ordinators), who were responsible for the barley and oat samples. The project ran from April 2004 to December 2005, when it was replaced by new project (HGCA 3100), which also covers malting barley and milling wheat.

The project comprised:

- Collection of representative sample sets of feed cereals, testing them for a range of contaminants, and comparing the results with legal limits or proposed guideline values.
- An ongoing review of proposed and incoming legislation and opinions from food/feed standards bodies (such as the UK Food Standards Agency and European Food Safety Authority) in order to identify any emerging issues which could impact on the acceptability of cereals for use as animal feed.

Four sets of samples were collected and tested for the presence of a range of contaminants. These sets comprised two sets of freshly harvested samples (harvests 2004 and 2005) and two sets of samples from batches which had been stored for approximately 6 months after harvest (harvests 2003 and 2004), plus one set of samples of pelleted malt culms. Each set included feed barley, feed oats, oatfeed, feed wheat and wheatfeed, selected to cover the relevant geographical areas of the UK. Samples were provided by all the major animal feed companies in the UK. In all, a total of 366 samples were collected and analysed.

The stored samples were tested for the presence of:

- pesticide residues, in particular those insecticides used in grain storage, but some samples were also tested for fungicides applied during earring
- aflatoxins and ochratoxin A, which are produced by moulds which can grow in stored grain

The freshly harvested samples were tested for the presence of:

- mycotoxins (zearalenone and trichothecenes such as deoxynivalenol or DON), produced by *Fusarium* fungi which can infect cereals growing in the field
- persistent organochloride insecticides which are no longer used in agriculture but which can still be detected in trace quantities in the environment and can therefore contaminate crops
- heavy metals and arsenic, traces of which can be taken up from the soil by growing plants

The results indicated that the overwhelming majority of animal feed materials derived from UK-grown cereals are legally compliant, safe and of good quality.

In the EU, statutory limits are in place for **pesticide** residues, certain **heavy metals, arsenic** and **aflatoxins** in animal feed materials. Levels of these contaminants were well below the legal limits, with the exception of only one sample (a sample of wheatfeed which exceeded the statutory limit for lead). **Mercury** was rarely detected, but **arsenic, lead and cadmium** were detected in most samples. However, actual concentrations were, with the one exception noted above, very low. Cereal products are unlikely to be a significant source of metal contaminants in animal feed. **Aflatoxins** were only detected in two samples (both feed barleys), and then only at very low levels compared with the legal limit. Residues of **pesticides** were uniformly low. Organochloride insecticides were not detected in any of the samples. Of the insecticides currently used in grain storage, pirimiphos-methyl was detected most frequently, but concentrations were always well below legal MRLs. Cereal products are unlikely to be a significant source of pesticide residues in animal feed.

Currently, there are no statutory limits for ***Fusarium*** **toxins** or for **ochratoxin A** in feed materials, but Guideline Values are under discussion. The current proposals would recommend separate guidelines for feed materials and for complete and/or complementary feedingstuffs, with lower recommendations for particularly sensitive species.

Ochratoxin A was widespread in oatfeed and wheatfeed, but detected in less than half of grain samples. Maximum levels were well below the proposed Guideline Value for feed materials, but some samples approached the Guideline Values for completed feedingstuffs in sensitive species such as pigs.

Trichothecene mycotoxins from *Fusarium* were generally widespread in all samples except feed barley. **Deoxynivalenol** was detected most frequently, except in oats where **T-2** and **HT-2** were more common. There was also evidence of an increase in the incidence of **T-2** and **HT-2** in barley and wheat from 2004 to 2005. **Nivalenol** was also detected frequently in wheat, although mean concentrations were substantially lower than for deoxynivalenol. **Nivalenol** was rare in barley and oats. All the samples tested were well below the proposed guideline limits for feed materials. However, some of the oats and wheat samples exceeded the proposed guidelines for complete feedingstuffs for sensitive species such as pigs.

Zearalenone was rare in barley and oats but widespread in wheat and wheatfeed. Although none of these samples exceeded the proposed Guideline Value for zearalenone in feed materials, some exceeded the lower Guideline Values suggested for complementary and complete feedingstuffs for sensitive species such as young pigs.

Overall, the results from the project suggested that UK-grown cereals are safe and for use in animal feed and do not contain hazardous levels of contaminants. None of the contaminants tested exceeded statutory limits except for one sample out of the 366 tested. UK cereals and cereal products are unlikely to be significant sources of heavy metals, aflatoxins or pesticide residues in animal diets.

Mycotoxins produced by *Fusarium* moulds are common in cereals, particularly in wheat. However, concentrations appear to be well within proposed Guideline Values for feed materials in general, although

care may be needed when formulating complete or complementary feedingstuffs for particularly sensitive animal species. The increase in incidence of T-2 and HT-2 toxins observed in cereals from the 2005 harvest is worrying, since these compounds are significantly more toxic than deoxynivalenol, and they should therefore continue to be monitored.

It is also noteworthy that over the lifetime of this project, the highest levels of *Fusarium* toxins did not coincide with particularly wet harvests. Specifically, maximum levels for trichothecenes were higher in 2005 (a dry year) than in 2004, when there was a particularly wet harvest. These observations support the generally held belief that levels of mycotoxins in cereal grain and cereal by-products are affected by a number of factors other than harvesting conditions.

SECTION 3. TECHNICAL DETAIL

1. BACKGROUND AND SCOPE OF PROJECT

This project was based on two existing HGCA projects developed for assuring the food safety of malting barley and milling wheat (projects 2804 and 2819 respectively) and complements those projects by addressing the requirements of animal feed, which is another major market for UK-grown cereals.

The main part of the project involved surveying the incidence of key contaminants in UK-produced cereals destined for animal feed in order to demonstrate the safety and legal compliance of UK-produced feedstuffs and to highlight any problem areas.

A parallel activity was to identify any emerging issues or legislation which could impact on feed safety by regularly monitoring scientific and legislative publications. The samples collected for this surveillance are being stored in order to provide an archive of appropriate samples for use in reacting to emerging cereal contaminant issues**.**

The scope of the current project includes:

- to provide ongoing access to information on food safety and legislative issues which might impinge on the market acceptability of UK-grown cereals for animal feed
- to draw the attention of the appropriate personnel in the AIC to any particularly urgent issues
- to carry out regular surveillance for potential contaminants of barley, wheat and oats destined for animal feed on two sets of samples per year.
    - One set of freshly harvested cereals, collected in September-October
    - A second set of stored cereals, collected in April-May

The number and source of samples, together with the sampling regimes and the analytes to be tested on each sample set, were decided through discussion and consultation with appropriate technical committees of the AIC. It was decided that each set would comprise approximately 50 samples of wheat and wheatfeed, 30 samples of barley and 20 samples of oats and oatfeed. Analytes to be tested for were as follows:

Freshly harvested samples

- Mycotoxins (trichothecenes and zearalenone)
- Heavy metals (mercury, lead and cadmium, plus arsenic)
- Organochlorine insecticides (a representative selection of samples)

Stored samples

- Mycotoxins (ochratoxin A and aflatoxins)
- Post-harvest insecticides

- Dioxins (if a suitable method could be identified)

The project commenced in May 2004 and finished at the end of December 2005. It is replaced by a combined project which covers malting barley, milling wheat and animal feed.

2. METHODS

2.1 Information gathering

2.1.1.BRi

BRi has an established system for scanning and collecting scientific and technical literature published world-wide relating to malting and brewing, including barley-related information. This information is stored on fully searchable electronic databases for ready access. For the purposes of this project, this system was supplemented by searching a number of additional information sources for references relating to animal feed, which included (but were not limited to):-

- UK's Food Standards Agency newsletters, web site and publications
- UK's Pesticides Safety Directorate circulars, web site and publications
- HMSO web site
- The Official Journal of the European Commission (L and C series)
- The European Food Safety Authority (EFSA) web site
- Codex web site and reports
- The FAO/WHO Joint Expert Committee on Food Additives and Contaminants (JECFA) web site and reports

2.1.2.CCFRA

The following publications support CCFRA's ability to scrutinise the scientific, technical and legislative matters relating to wheat-based products.

These are:

- CCFRA MRL Alert which provides details of changes to legal or recommended MRLs (UK, EU and Codex) and relevant news relating to pesticide MRLs, including proposed changes to legislation.
- CCFRA Flour Milling & Baking Abstracts which are prepared by information scientists who regularly scan over 200 journals, patents other sources for articles which are of interest to the milling and baking industries worldwide. Specific sections exist covering "Cereal growing & Agriculture" and "Toxicology & Contaminants"
- CCFRA Food Law Alert which provides prompt and succinct updates on legislative developments with links to relevant websites.

2.2 Surveillance

2.2.1 Sample sets collected

Because of the timing of the project, only four sample sets could be collected:

- Stored samples from the 2003 harvest, collected in May-June 2004
- Freshly harvested samples from the 2004 harvest, collected September-December 2004
- Stored samples from the 2004 harvest, collected May – June 2005

- Freshly harvested samples from the 2005 harvest, collected September-November 2005.

Details of the sample sets are given in Appendix 1.
In addition, one set of processing co-products (pelleted malt culms) were collected, in September/October 2004. Only one sample was provided by the animal feed companies, so additional samples had to be obtained directly from the malting companies. These were analysed for *Fusarium* toxins.

2.2.2 Mycotoxin analysis

- **Trichothecenes.**

Barley and oat samples were analysed at BRi for DON, 3-acetyl-DON, 15-acetyl-DON, NIV, HT-2 toxin and T-2 toxin using an in-house procedure based on a published method *(Patel et al, 1996)*. From the 2004 harvest onwards, this was extended to also include fusarenone-X, neosolaniol and diacetoxyscirpenol. These mycotoxins have been identified by the European Commission as potentially serious contaminants of cereals and cereal products *(Verstraede, 2003)* and BRi is currently investigating their occurrence in UK cereals and behaviour in processing under a separate HGCA project. The mycotoxins were extracted using acetonitrile/water, partially purified using trichothecene clean-up columns, then derivatised and analysed by GC-mass spectrometry. The limit of quantification (LOQ) was 5 μg/kg for each toxin. BRi participates in FAPAS proficiency tests. Z scores are available on request.

Wheat samples were sub-contracted to RHM Technology, High Wycombe for analysis of the full range of trichothecenes: DON, diacetoxyscirpenol (DAS), 3-acetyl DON, 15-acetyl DON, fusarenone X (FUS X), NIV, neosolaniol (NEO), T-2 triol (T-23), T-2 and HT-2 toxin (HT2) in both years. This laboratory is UKAS accredited for trichothecene analysis. The method used was developed by Patel *et al*., 1996 and hence is a GC-mass spectrometry method that is equivalent to that used at BRi. The limit of quantification (LOQ) was 10 μg/kg for each toxin.

- **Zearalenone.**

Barley and oat samples were analysed at BRi by an in-house procedure based on a published method as before *(Patel et al, 1996)*. After extraction with acetonitrile/water, specific immuno-affinity columns were used for the clean-up stage. Detection and quantification was by HPLC. The limit of quantification was 2 μg/kg. BRi participates in FAPAS proficiency tests for zearalenone and Z scores are available on request.

Wheat samples were sub-contracted to RHM Technology, High Wycombe which is UKAS accredited for this analysis. The method used was developed by Patel *et al*., 1996 and is equivalent to that used at BRi. The LOQ for zearalenone was 3 μg/kg.

- **Fumonisins**

FB1 and FB2 in **oats** were analysed by a similar method to that used for zearalenone, except that fumonisin-specific immuno-affinity columns were used. As with the other mycotoxins, BRi participates in FAPAS proficiency tests for fumonisins and Z scores are available on request.

- **Ochratoxin A.**

Barley and oat samples were analysed at BRi by HPLC with fluorescence detection, following extraction and clean-up with immuno-affinity columns *(Baxter, Slaiding and Kelly, 2001)*. The limit of quantification was 0.1µg/kg. BRI has UKAS accreditation for this analysis and also participates in FAPAS proficiency tests. Z scores are available on request.

Wheat samples were analysed at CCFRA for OTA using an in house HPLC method with fluorescence detection after solvent extraction and sample clean-up using immuno-affinity columns. The LOQ for OTA was 0.1µg/kg. CCFRA has UKAS accreditation for this analysis and participates in FAPAS proficiency schemes and Z scores are available on request.

- **Aflatoxins.**

Barley and oats samples were analysed at BRi for aflatoxins B_1, B_2, G_1 and G_2 by an in-house procedure based on a published method *(Patel et al, 1996)*. After extraction with acetonitrile/water, specific immuno-affinity columns were used for the clean-up stage. Detection and quantification was by HPLC with post-column derivatisation. The limit of quantification was 0.1µg/kg for each toxin. BRi participates in FAPAS proficiency tests for aflatoxins and Z scores are available on request.

Wheat samples were analysed at CCFRA for aflatoxins B_1, B_2, G_1, G_2 by an in-house procedure. After blending with methanol/water, sample clean–up was carried out using an immuno-affinity column. Aflatoxin measurement was by HPLC with fluorescence detection and post-column derivatisation. The LOQ for all four aflatoxins is 0.1µg/kg. CCFRA has UKAS accreditation for this analysis and participates in FAPAS proficiency schemes and Z scores are available on request.

2.2.3 Pesticides

- **Barley and oat samples**

Barley and oat-based samples were analysed at BRi for storage insecticides (mainly organophosphorus compounds but also including the organochlorine insecticide benzene hexachloride or Lindane) and late-acting fungicides, using a multi-residue pesticide analysis developed for cereals and malted cereals. Currently this includes all insecticides which are currently, or have until recently, been used on cereals post-harvest or on empty stores, as well as several late acting fungicides, including cyprodinil and some of the new strobilurin fungicides. Samples were milled and extracted with acetone/methanol. The extract was

concentrated and fractionated by gel permeation chromatography. The fraction containing pesticide residues was concentrated and analysed by GC-MS. Results are reported uncorrected for recovery.
Barley and oat samples were subcontracted to Mountainheath Services Ltd, Letchworth, for analysis of organochlorine insecticides. The method involved hexane extraction, followed by evaporative concentration, and finally detection and quantification by GC-MS.

- **Wheat samples**

Wheat samples were analysed at CCFRA for organophosphorus and organochlorine pesticides by gas chromatography - mass spectrometry (GC-MS) with mass selective detection by an in house method. The method used was based on the following published methods: Unilever plc., Undated; Food & Drug Administration (U.S.A) Pesticide Analytical Manual, 1982; Luke *et al*., 1981;Baker & Bottomley, 1984; Chamberlain, 1990; Anon., 1979. Following solvent extraction sample and solvent partitioning, clean-up involved gel-permeation chromatography or solid phase extraction columns.

Details of individual pesticides included in each screen, together with their Limits of Quantification and legal MRLs are given in Tables 4 and 5.

2.2.4 Heavy metals and arsenic

Wheat, barley and oat samples were analysed by atomic absorption spectroscopy at CCFRA using an in house method based on the following published methods: Rees, 1978; Dixon & Hill, 1976; and Louie, 1983.

Methods vary according to the heavy metal under consideration. For lead, cadmium and arsenic, the first step was to incinerate the sample to destroy the organic material. The residue was dissolved in hydrochloric acid the metal was measured by atomic absorption spectroscopy (AAS). For lead and cadmium, the metals were extracted from the acid solution as their iodide complexes using an ion exchange resin before AAS. For arsenic, the hydride generation technique was used where As^{v} was reduced to As^{III} by the addition of potassium iodide which was converted to the hydride using sodium borohydride. For mercury, the sample was digested using a mixture of nitric, sulphuric and hydrochloric acids and an aliquot of the digest reduced with sodium borohydride. Mercury was measured by cold vapour AAS.

CCFRA is UKAS accredited for heavy metal analysis and participates in FAPAS proficiency tests for lead, cadmium, arsenic and mercury. Z scores are available on request.

The Limit of Quantification (LOQ) for each metal is as follows:
Lead – 0.02mg/kg
Cadmium, arsenic, mercury – 0.01mg/kg

2.2.5 Dioxins

An ah-immunoassay dioxin kit obtained from Biosense Laboratories AS, Thormøhlensgt. 55, Bergen, N-5008, Norway (http://www.biosense.com/comweb.asp?articleno=26&segment=3) was investigated as a potential alternative to traditional analysis as part of a parallel HGCA-funded project (2804). Essentially this system mimics an enzyme immunoassay although it is not a typical ELISA. The dioxin responsive elements from human cells (which are DNA) are bound to a microtitre plate. The reaction mixture includes the ah-receptor which binds dioxins as well as other components of the cellular reactive complex. In the presence of dioxin the complex is formed, binds to the DNA and remains in the plate. An antibody linked to an enzyme recognises and binds to the complex in the plate. The presence or absence of this enzyme can then be readily measured colorimetrically. Since the screening kit is essentially a bioassay (it depends upon the binding of dioxins to specific genes called Dioxin Responsive Elements (DRES) via an intracellular receptor) and expresses results on a weight basis for total dioxins rather than in terms of WHO Toxin Equivalents for individual dioxin species, which are used in legislation and by most analytical laboratories offering dioxin analysis, interpretation of the results is not straightforward. In this case, we compared the results obtained by this kit for brewery spent grains with those obtained for the same sample by traditional analysis (high resolution GC.MS) by TNO laboratories in the Netherlands. This laboratory quoted values below the limit of quantification for each of 17 dioxin congeners, giving an upper bound concentration of 22 pg/g, compared with the 42 pg/g given by the Biosense kit. We therefore assumed that the dioxins detected by the kit in the other samples tested would also be below or close to the limits of quantification by traditional analysis.

The experience suggests that the kit could potentially be used for semi-quantitative screening of at-risk cereal samples for dioxins, however, it would be of limited value for assessing legal compliance unless significant further work was carried out in order to calibrate the values obtained in terms of WHO-TEQ values and to identify a pass/fail threshold. We did not therefore continue with testing any of the samples from the current project for dioxins using this kit.

3. RESULTS

3.1 Mycotoxins

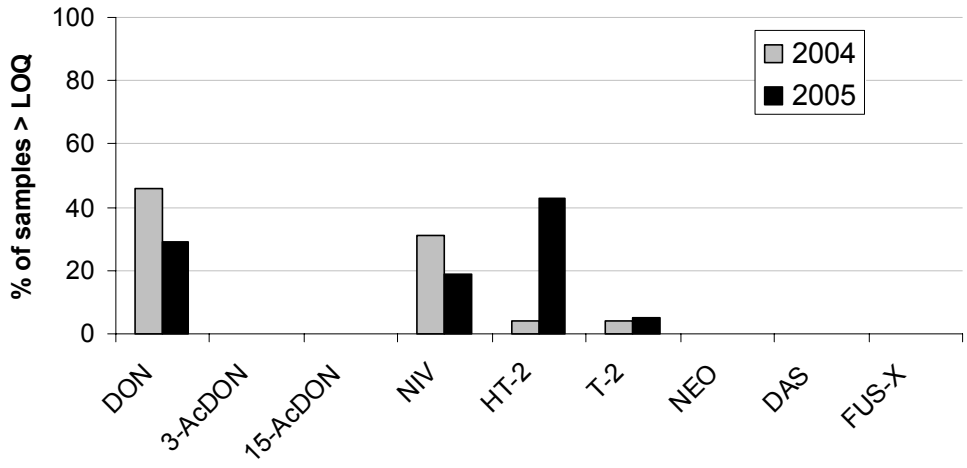
3.1.1 Trichothecenes in freshly harvested samples

Samples sets collected immediately after harvest were tested for a range of trichothecene mycotoxins. All samples were tested for deoxynivalenol (DON), Nivalenol (NIV), T-2 toxin and HT-2 toxin. Some samples were tested for additional trichothecenes, including the acetylated DONs, neosolaniol, fusarenone-X, diacetoxyscirpenol or T-2 triol.

BARLEY

Data for trichothecenes in feed barleys from the 2004 and 2005 feed barleys immediately after harvest are shown in **Figures 1-3**. The limit of quantification was 5µg/kg. In 2004 deoxynivalenol (DON) and Nivalenol (NIV) were the commonest species, being detected in almost 50% and around 30% of samples respectively. However, in 2005 HT-2 replaced DON as the commonest species, being found in over 40% of samples. The number of samples is too low to tell whether this is a significant change; however, similar increases were also observed for feed wheat and wheatfeed, and for malting barley in a separate project.

Figure 1. Incidence of trichothecenes in feed barley



Concentrations were relatively low for all trichothecenes, with mean concentrations being less than 20 µg/kg for each toxin. Maximum levels being well below proposed guidelines limits for feed materials (**Figures 2 and 3**).

Figure 2. Mean concentrations of trichothecenes in feed barley

(means are calculated by assuming that samples below the limit of quantification contain half that limit)

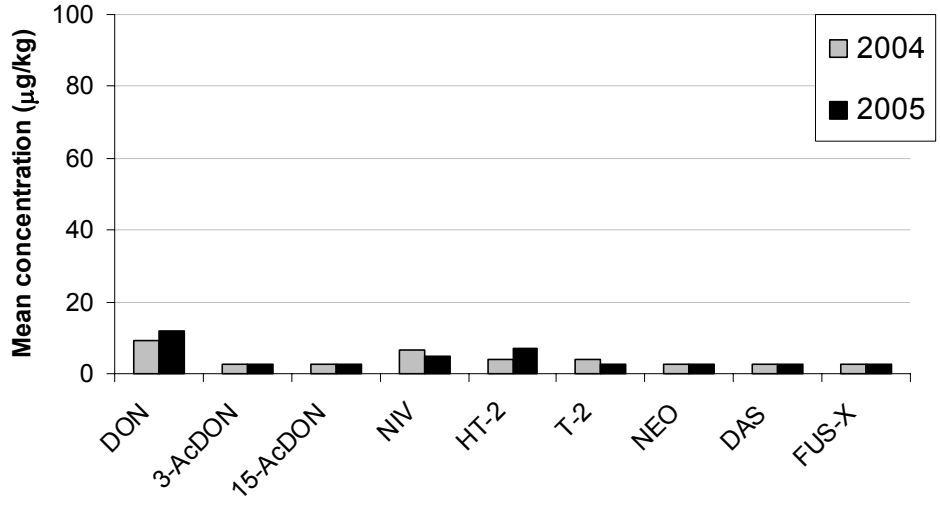
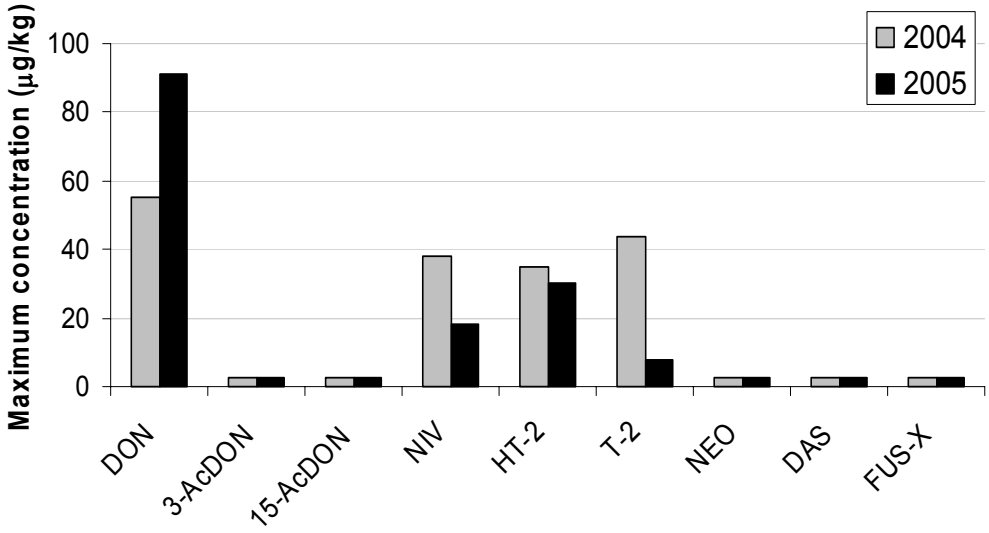


Figure 3. Maximum concentrations of trichothecenes in feed barleys

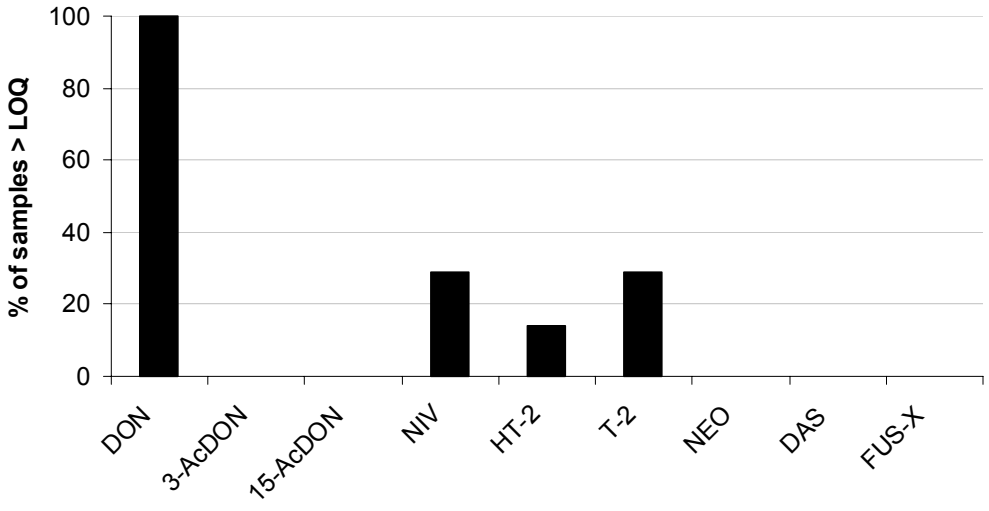


The acetylated DON species, and the other trichothecenes sought (neosolaniol, fusarenone-X and diacetoxyscirpenol (DAS) which are emerging issues in the EU) were not detected in any samples, either in 2004 or 2005.

PELLETED MALT CULMS

Results for the analysis of trichothecenes in pelleted malt culms are shown in **Figures 4-6.** The incidence of trichothecene mycotoxins was higher in pelleted malt culms that in barley grains (either raw or malted), and concentrations were also higher, although still below guideline limits (see also section on zearalenone).

Figure 4. Incidence of trichothecenes in pelleted malt culms



As with the barley samples, the acetylated DON species, and the other trichothecenes sought (neosolaniol, fusarenone-X and diacetoxyscirpenol (DAS)) were not detected in any samples.

Figure 5. Mean concentrations of trichothecenes in pelleted malt culms

(means are calculated by assuming that samples below the limit of quantification contain half that limit)

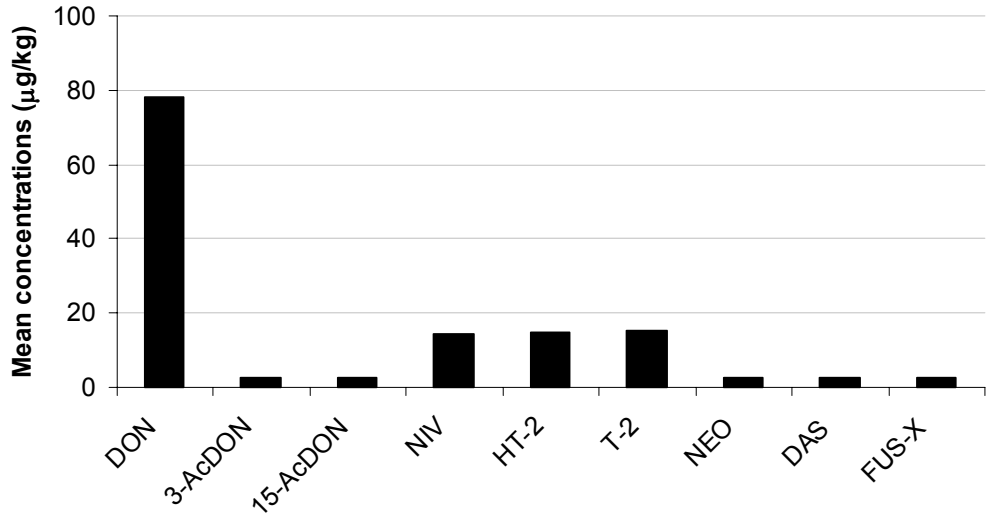
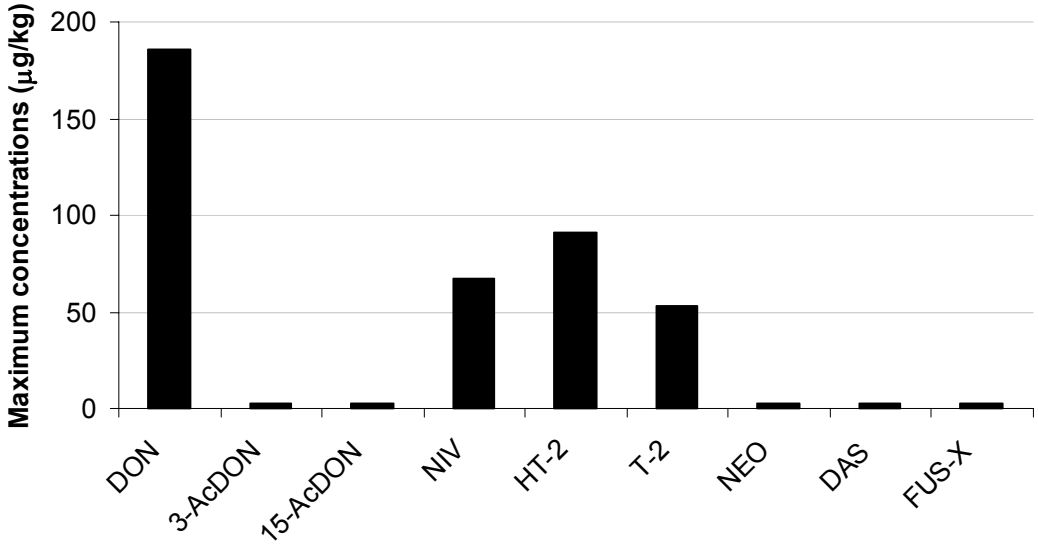


Figure 6. Maximum concentrations of trichothecenes in pelleted malt culms



OATS

Data for oats are shown in **Figures 7-10**. Because of the small number of samples, data for oats and oatfeed have been combined. Although there was a trend for both the incidence and the concentrations of trichothecenes to be higher in oatfeed than in oat grain, the differences were not substantial and there was considerable overlap. Analysis of samples from the first set collected in September 2004 indicated that the incidence and the concentrations of T-2 and HT-2 toxins in oats could be quite high, substantially higher than for DON. Because the sample set was small, the set of stored samples collected in May 2005 were tested for trichothecenes as well as for storage mycotoxins. These results, together with those from the 2005 harvest, confirmed the initial observations. No guideline limits have yet been suggested for T-2 and HT-2 toxins in animal feed.

Figure 7. Incidence of trichothecenes in oats

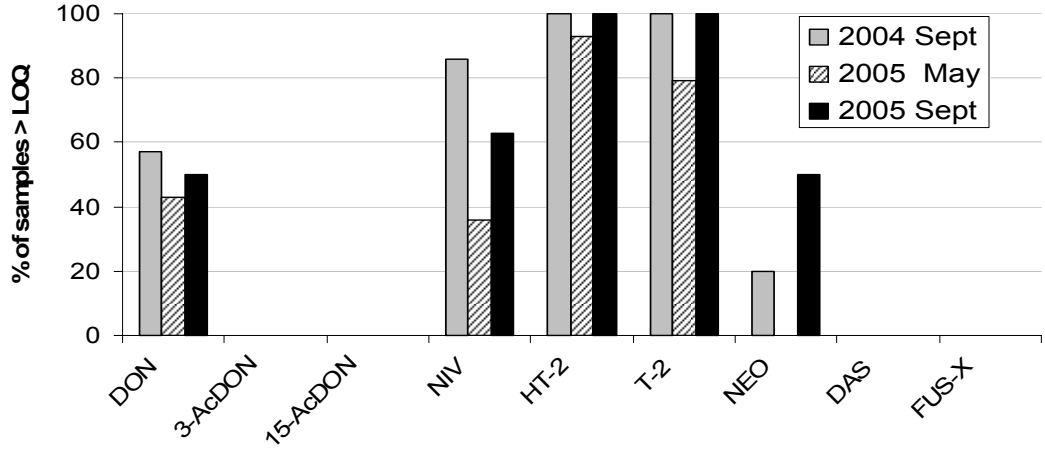


Figure 8. Mean concentration of trichothecenes in oats

(means are calculated by assuming that samples below the limit of quantification contain half that limit)

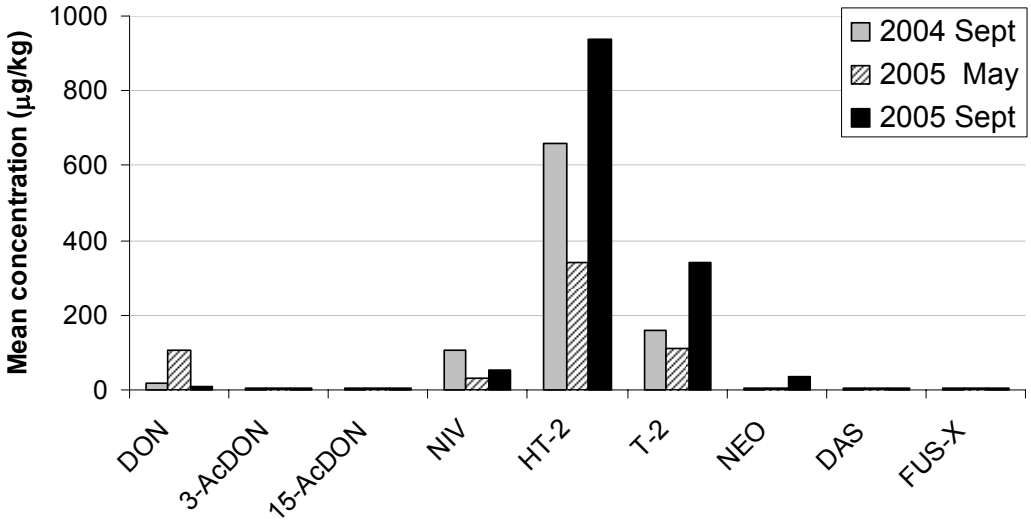
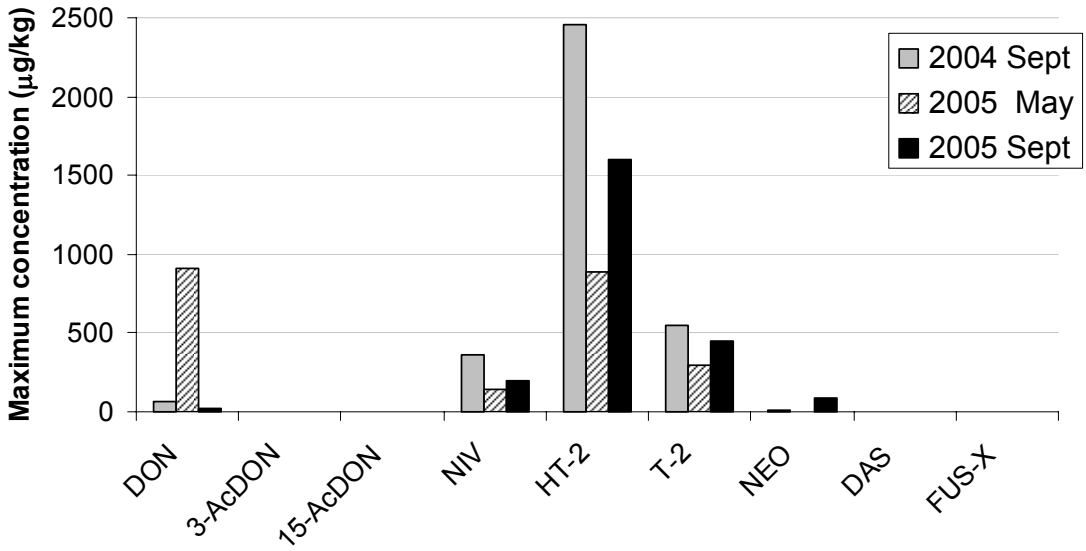


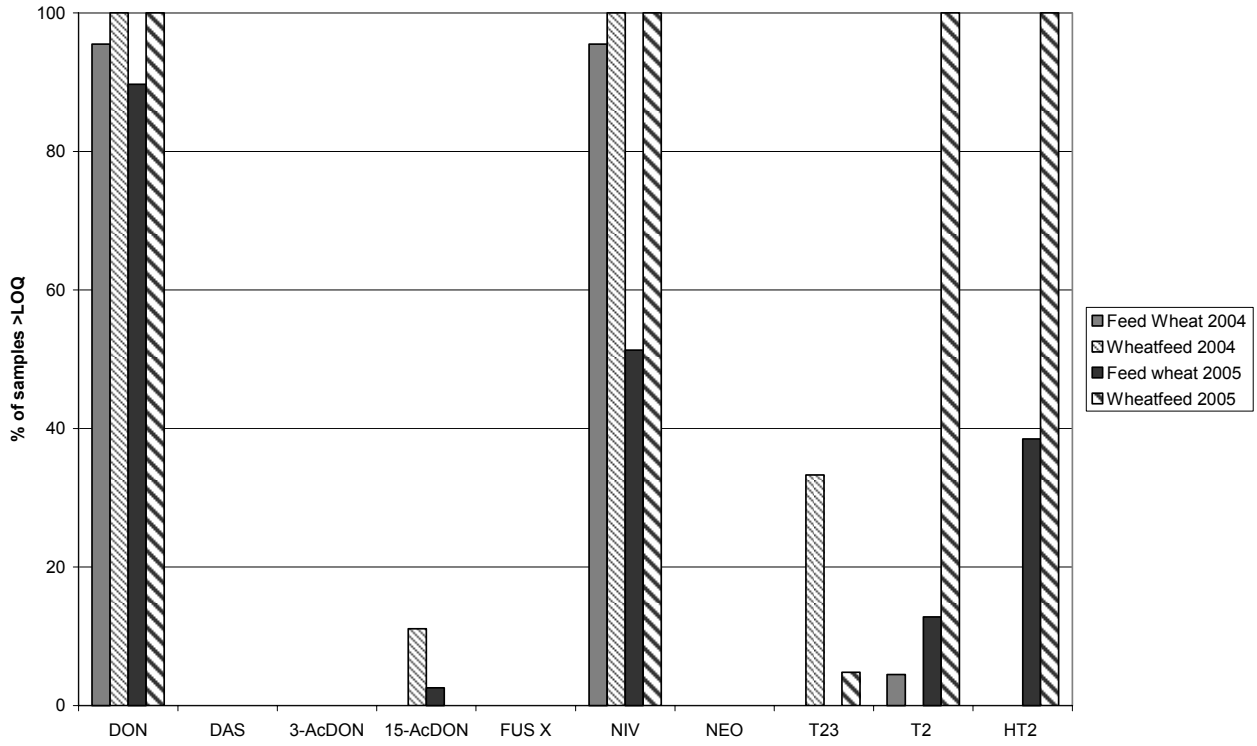
Figure 9. Maximum concentration of trichothecenes in oats.



WHEAT and WHEATFEED

Results for trichothecenes in feed wheat and wheatfeed from the newly harvested 2004 and 2005 crop are shown in **Figures 10-12.** The limit of quantification was 10µg/kg in each case and where a value of <10µg/kg was recorded, half this value i.e. 5µg/kg was used to calculate the mean value Within the 10 trichothecenes measured, only four consistently produced levels above the limit of quantification in either year. These were deoxynivalenol (DON), nivalenol (NIV), T-2 and HT-2 toxins.

Figure 10. Incidence of trichothecenes in wheat and wheatfeed



The mycotoxin DON was detected at levels above the LOQ in over 90% of feed wheat samples and 100% of wheatfeed samples (**Figure 10**). The majority of any mycotoxin contaminant is concentrated in the outer layers of the wheat grain and therefore it is to be expected that trichothecene levels would be higher in wheatfeed than in whole grain entering the animal feed chain. Mean DON levels confirm this difference between the two raw materials (**Figure 11**). For feed wheat, the mean DON levels were 96 µg/kg and 591 µg/kg in 2004 and 2005 respectively and the maximum levels observed were 597 µg/kg and 1940 µg/kg, respectively. As expected, in general, higher DON levels were observed in wheatfeed: mean values were 419 and 472 µg/kg in 2004 and 2005 samples respectively; and maximum values were 2420 and 1745 µg/kg, respectively. The exception to this is that the maximum DON value observed in 2005 feed wheat was higher that that for wheatfeed. The sample at 1940 µg/kg was quite extreme within this population with the next highest value standing at nearly 900 µg/kg lower.

Figure 11. Mean concentration of trichothecenes in wheat and wheatfeed.

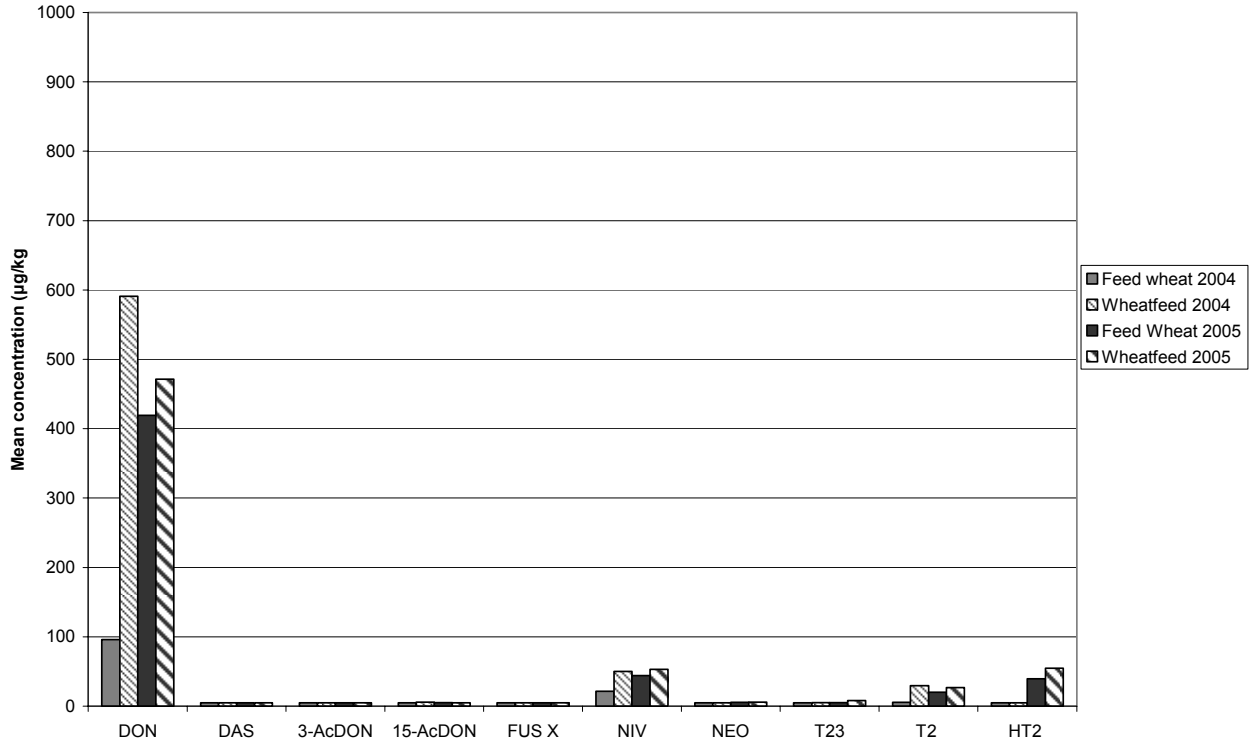
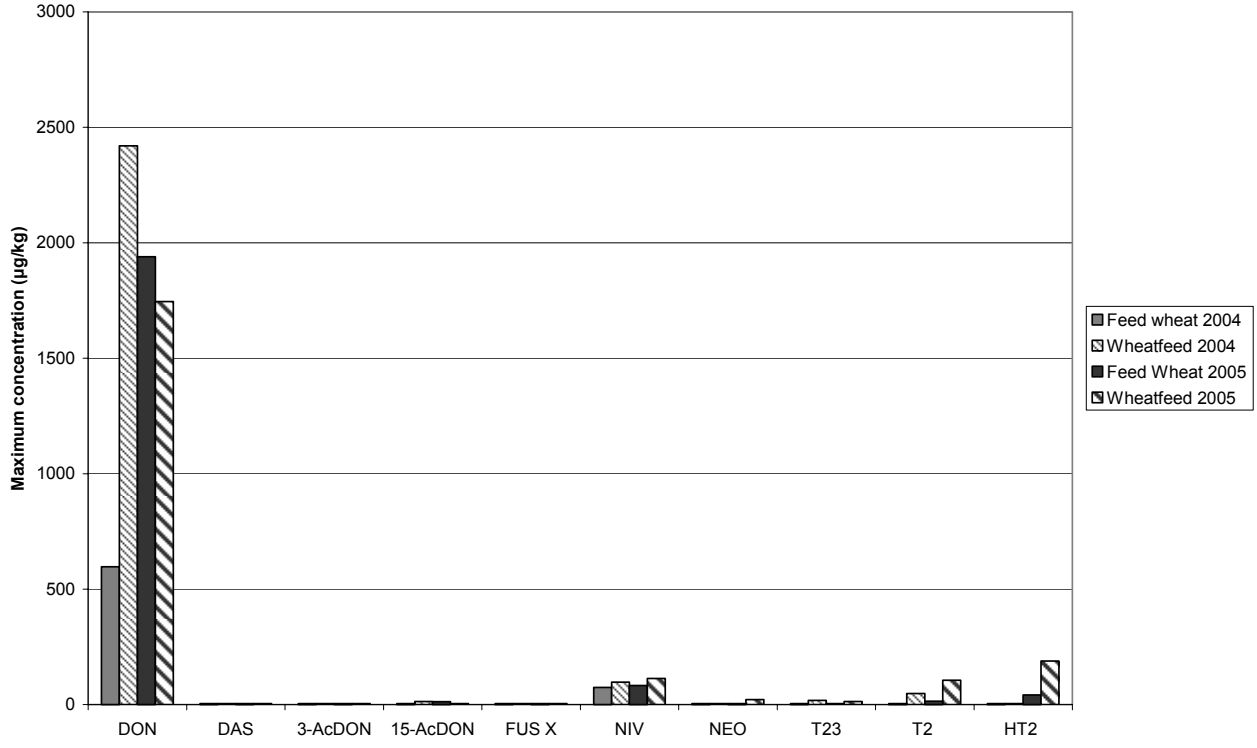


Figure 12. Maximum concentration of trichothecenes in wheat and wheatfeed



It is interesting to note that values for feed wheat in 2005 exceed those for 2004. 2004 was considered to be a problem year where the crop contained high levels of pink grains (*Hook and Williams, 2004*) and the potential to contain high trichothecene levels (particularly DON). This survey suggests that even under these difficult conditions, animal feed manufacturers have been able to source wheat of suitable quality for the production of animal feed.

The second most prevalent trichothecene nivalenol (NIV) was detected in all wheatfeed samples in both years and observed incidence was higher in feed wheat in 2004 than 2005. For NIV the mean levels, were 22 and 44 μg/kg in feed wheat in 2004 and 2005 respectively while the respective maximum values were 75 and 83 μg/kg. As for DON, NIV values tend to be higher in wheatfeed than feed wheat. The equivalent values for wheatfeed were: means 50 and 53 μg/kg and maximum values 98 and 113 μg/kg in 2004 and 2005 respectively.

The incidence pattern for T-2 and HT-2 toxins also differed between the 2004 and 2005 crops. These toxins were detected in 100% of wheatfeed samples in 2005, their incidence was also increased in feed wheat within this crop. For T-2 the mean values were 20 and 27 μg/kg for feed wheat and wheatfeed in 2005 and for HT-2 the respective values were 40 and 55 μg/kg.

Of the other trichothecenes only 15Ac-DON and T2-triol were detected in any sample of feed wheat or wheatfeed. As with other Fusarium mycotoxins, the incidence of 15Ac-DON and T2-triol tended to be concentrated in the wheatfeed samples

3.1.2 Zearalenone in freshly harvested samples

Data for zearalenone in all samples tested is shown in **Table 1**.

Table 1. Zearalenone in feed cereals and cereal by-products

(means are calculated by assuming that samples below the limit of quantification contain half that limit)

Sample	% of samples > LOQ		Mean µg/kg		Maximum µg/kg	
Harvest year	**2004**	**2005**	**2004**	**2005**	**2004**	**2005**
Barley	19	0	3.3	1.0	41	< 2.0
Pelleted malt culms	71	not done	26	not done	128	not done
Oats and oatfeed	33	13	6.0	1.1	23	< 2.0
Feed wheat	77	64	41.0	11.8	444	136
Wheatfeed	100	95	50.5	19.0	147	42.3

BARLEY

Zearalenone was relatively rare in barleys, occurring in only 19% of samples in 2004 (which was a particularly wet harvest) and not at all in 2005 (**Table 1**). Concentrations were also low, with the highest concentration detected being 41µg/kg.

PELLETED MALT CULMS

In spite of the low levels of zearalenone in barley, pelleted malt culms contained noticeably higher levels. It was also noted that they contained higher levels of trichothecenes than those generally found in barleys, whether feed or malting grade. This has been further investigated by examining the individual components of pelleted malt culms. Like oatfeed and wheatfeed, pelleted malt culms also contain fines and screenings of grain, in addition to the malt rootlets (culms). The highest levels of mycotoxins were found in the fines and screening of raw grain (particularly where this was a mixture of both wheat and barley). No evidence was found that the mycotoxins were being formed during the malting process, and fines and screenings of malted grain contained only low levels of mycotoxins. The surveillance studies reported in this project suggest that wheat can be more heavily contaminated with zearalenone than barley.

OATS and OATFEED

Oats, like barley, contained relatively little zearalenone. Again, both incidence and concentrations were higher in 2004 than in 2005.

WHEAT and WHEATFEED

Zearalenone was prevalent in both feed wheat and wheatfeed, occurring in almost all wheatfeed samples in both years. The incidence of zearalenone was higher in 2004, where the wet harvesting conditions hit the later maturing wheat crop more heavily than the other cereals (**Table 1**). Mean values for zearalenone confirmed the effect of wet harvesting conditions in 2004 on this mycotoxin and the normal pattern of higher values in wheatfeed. However, the maximum levels observed were actually found in feed wheat in

both 2004 and 2005. These were caused by extreme values within the populations namely 444 µg/kg in 2004 and 136 µg/kg in 2005. These values were over 3 times higher than any other sample within the survey in that year. These samples were, well within the current proposed Guideline Value for zearalenone in cereals and cereal products destined for feed materials (2000 µg/kg) but did exceed the lower Guideline Values proposed for complete and complementary feedingstuffs for sensitive species.

3.1.3 Aflatoxins

Aflatoxins do not occur in growing crops in the UK but can be formed in stored cereals if storage conditions are unsuitable (moisture and temperature levels are too high). The sample sets collected in May 2004 and 2005, which would have incorporated grain from the previous year's harvest, were therefore tested for aflatoxins B_1, B_2, G_1 and G_2 , with the limit of detection being 0.1µg/kg for each. Results are expressed as total aflatoxins. Results are shown in **Table 2.**

Table 2. Aflatoxins in stored cereals and cereal by-products

(means are calculated by assuming that samples below the limit of quantification contain half that limit)

Sample	**% of samples > LOQ**		**Mean µg/kg**		**Maximum µg/kg**	
Harvest year	**2003 (stored)**	**2004 (stored)**	**2004 (stored)**	**2004 (stored)**	**2005 (stored)**	**2004 (stored)**
Barley	7	0	0.12	0.05	1.1 (all B1)	< 0.1
Oats + oatfeed	0	0	0.05	0.05	< 0.1	< 0.1
Wheat	0	0	0.05	0.05	< 0.1	< 0.1
Wheatfeed	0	0	0.05	0.05	< 0.1	< 0.1

BARLEY

Aflatoxins were detected in two barley samples (7% of the sample set) in stored samples from the 2003 harvest and in no samples from the 2004 harvest. Aflatoxin B_1 was the only species detected, at a maximum of 1.1 µg/kg, compared with a legal limit for feed materials of 20 µg/kg.

OATS and OATFEED

None of the samples tested contained aflatoxins above the quantification limit of 0.1 µg/kg.

WHEAT and WHEATFEED

None of the samples tested contained aflatoxins above the quantification limit of 0.1 µg/kg.

3.1.4. Ochratoxin A (OA) in stored samples

Although ochratoxin A can be formed in growing crops in some parts of the world, in Western Europe it is only produced by the mould *Penicillium verrucosum,* which can infect stored cereals at moisture contents in excess of 16-20% (depending upon the temperature). The sample sets collected in May 2004 and 2005, which would have incorporated grain from the previous year's harvest, were therefore tested for ochratoxin A. The limit of detection was 0.1 μg/kg. Results are shown in **Figures 13-16** for all the cereals tested.

Whole grain samples (wheat, barley and oats) were generally less likely to contain detectable OA than was wheatfeed or oatfeed.

Figure 13. Incidence of OA in stored feed cereals and cereal by-products

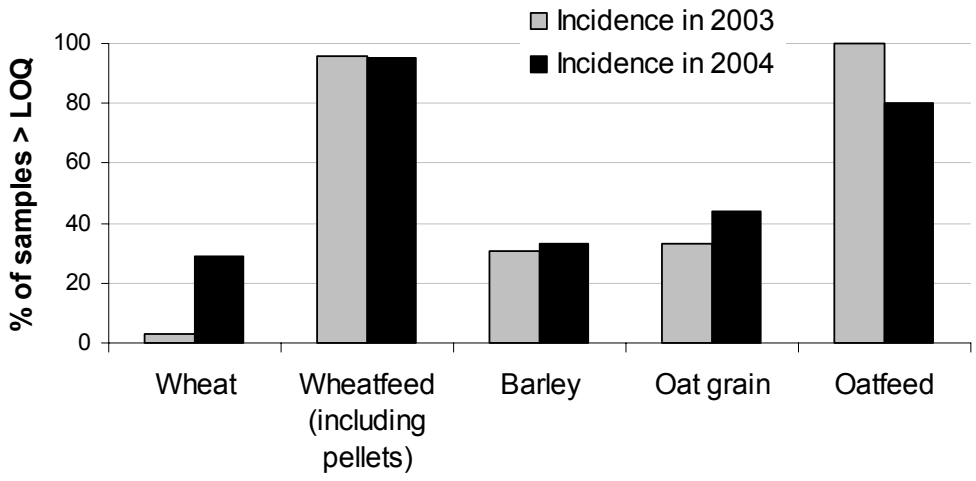
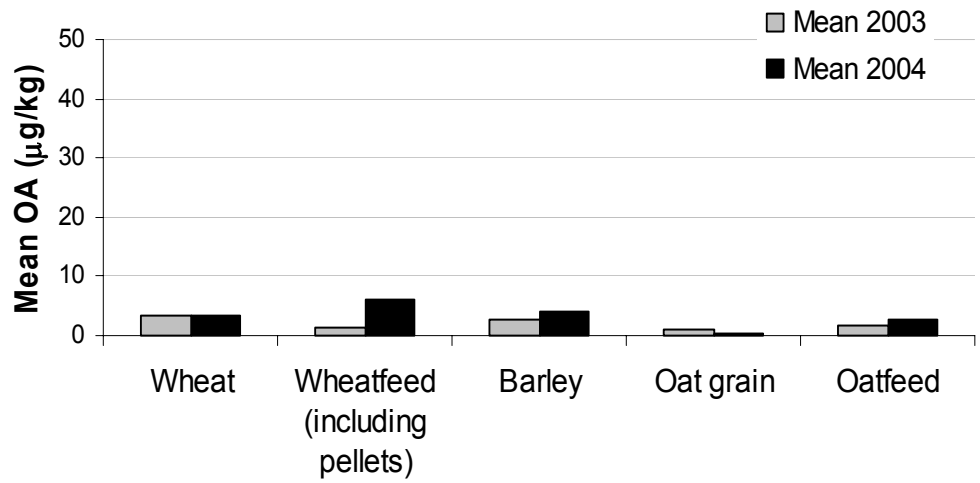


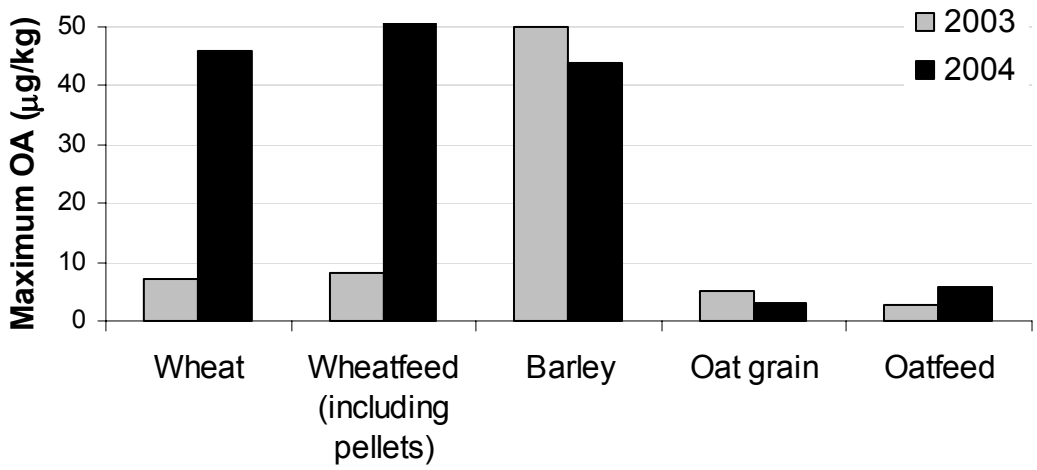
Figure 14. Mean concentrations of OA in stored feed cereals and cereal by-products

(means are calculated by assuming that samples below the limit of quantification contain half that limit)



Mean concentrations were low for all categories, and well below the proposed guidelines for OA in feed materials (250 μg/kg). However, maximum concentrations in some samples approached the guideline of 50 μg/kg proposed for complementary and complete feedingstuffs for the most sensitive animal species (pigs).

Figure 15. Maximum levels of OA in stored feed cereals and cereal by-products



3.1.5 Fumonisins

Fumonisins are mycotoxins which are also produced by *Fusarium* species, but are generally associated with maize. However, they are sometimes found on other cereals, although there is little available data. In view of the proposed guideline limits for fumonisins, not only on maize and maize products for animal feed, but also for substantially lower limits on all complete and complementary feedingstuffs for certain species (including pigs and horses) it was decided to extend the surveillance to include fumonisins, particularly for oats. Four of the oats samples with particularly high levels of other *Fusarium* mycotoxins (DON, T-2 and/or HT-2) were also tested for fumonisins B1 and B2. None of the samples contained either B1 or B2 above the quantification limit of 5 μg/kg.

3.2 Pesticides

The samples sets collected were tested for a number of pesticides, which were grouped according to their chemical structure, agronomic use, and/or current approval status in the EU.

FRESHLY HARVESTED SAMPLE SETS

The freshly harvested set was tested for a range of organochloride pesticides, mainly insecticides (see **Table 3**). Most of these chemicals are no longer approved for use in the UK and the EU, but traces can still be found occasionally in crops because of their persistence in the environment. Legal limits for many of these organochloride pesticides in animal feedstuffs are set specifically by Directive 2002/32/EC and its amendments.

Table 3. Organochloride insecticides sought

Pesticide active ingredient	**Limit of detection (mg/kg)**		**Limit in feed materials (mg/kg)**
	Barley/Oats	Wheat	
Aldrin and dieldrin (singly or combined)	0.01	0.005	0.01
Chlordane	0.01	Not tested	0.02
Camphechlor	Not tested	Not tested	0.1
DDT	Barley/oats 0.02 (sum of isomers) Wheat – separate limits for each isomer		0.05
Endosulfan	0.02	Not tested	0.1
Endrin	0.01	Not tested	0.01
Heptachlor	0.01	0.005	0.01
Hexachlorobenzene (HCB, lindane)	0.01	0.005	0.01
Hexachlorocyclohexane (HCH)			
α isomers	0.02	0.005	0.02
β isomers	0.01	0.005	0.01
γ isomers	0.01	0.005	0.2

Barley, Oats and Malt culms

Six samples of barleys, 1 sample of oats and 2 samples of oatfeed from the 2004 harvest were analysed for the organochloride pesticides as listed in **Table 3.** A similar set from the 2005 harvest were also tested. In addition, two samples of pelleted malt from the 2004 harvest were tested. No residues were detected in any sample from either harvest.

Wheat

Samples were selected for measurement of organochloride pesticides. 20 samples were chosen from both the 2004 and 2005 crop to be representative of raw material entering the animal feed chain. No organochloride pesticides were detected in either sample set.

STORED SAMPLE SETS

Table 4. Insecticides and fungicides sought on stored sample sets

Active ingredient	Limit of Detection mg/kg Barley/oats	Wheat	EU Maximum Residue Level (MRL) (mg/kg)	Type
Azoxystrobin	0.01	Not tested	0.3 (barley, oats, wheat)	Late strobilurin fungicide
Bifenthrin	0.01	Not tested	0.5 (barley, oats, wheat)	Post harvest and field insecticide
Chlorpyrifos	0.01	0.02	0.2 (barley) 0.05 (oats, wheat)	Late insecticide
Chlorpyrifos-methyl	0.01	0.02	3 (barley, oats, wheat)	Post-harvest insecticide
Cypermethrin	0.01	Not tested	0.2 (barley, oats) 0.05 (wheat)	Late field insecticide
Cyprodinil	0.01	Not tested	Not yet set	Late fungicide
Deltamethrin	0.01	Not tested	1 (barley, oats, wheat)	Field insecticide
Diazinon	0.01	0.01	0.02 (barley, oats, wheat)	Field insecticide
Dichlorvos	0.01	0.05	2 (barley, oats, wheat)	Post harvest insecticide
Etrimfos	0.01	0.05	No MRL	Post harvest insecticide; now withdrawn
Fenitrothion	0.01	0.05	No MRL	Post harvest insecticide ; now withdrawn
Fenvalerate	0.01	Not tested	0.2 (barley, oats) 0.05 (wheat)	Late field insecticide;
Kresoxim-methyl	0.01	Not tested	0.05 (barley, oats, wheat)	Strobilurin fungicide
Lindane	0.01	Not tested	0.01 (barley, oats, wheat)	Post harvest and field insecticide; withdrawn in UK
Malathion	0.01	0.05	8 (Malathion + malaoxan (barley, oats, wheat)	Post harvest insecticide
Malaoxan	Not tested	0.05	Included in malathion MRL	Metabolite of malathion in animals
Methacrifos	0.01	0.02	0.05 (barley, oats, wheat)	Post harvest insecticide ; now withdrawn
Permethrin	0.01	Not tested	0.05 (barley, oats, wheat)	Insecticide was used in cereal stores
Phosphamidon	Not tested	0.02	0.05 (barley, oats, wheat)	Field insecticide Withdrawn in UK
Pirimiphos-methyl	0.01	0.02	5 (barley, oats, wheat)	Post harvest insecticide; widely used
Trichlorfon	Not tested	0.05	0.1 (barley, oats, wheat)	Insecticide Withdrawn in UK
Trifloxystrobin	0.01	Not tested	Not yet set	Late applied strobilurin fungicide

Barley and Oats

The samples sets of stored barleys and oats were tested for insecticides and fungicides as listed in **Table 4**. Results are shown in **Table 5.** Residue levels were generally very low, and no residues greater than existing EU Maximum Residue Levels (MRLs) were detected. In both years the post harvest insecticide pirimiphos-methyl (Trade name Actellic) was detected most frequently (detected in 50-60% of barley samples and 70-90% of oat/oatfeed samples). The fungicide cyprodinil (trade name Unix) was detected in around 10% of barleys, at levels of up to 0.14 mg/kg. No EU MRL has yet been set for this compound.

Table 5. Pesticide residues found in barley and oat samples

(Means are calculated as the mean of samples containing residues at or above the limit of quantification)

Sample/analyte	EU MRL mg/kg	% of samples > LOQ	Mean mg/kg	Maximum mg/kg
BARLEY				
2003 harvest (26 samples)				
Any pesticide		65	-	0.68
Chlorpyrifos-methyl	3	15	0.03	0.32
Pirimiphos-methyl	5	58	0.10	0.68
Dichlorvos	2	0	-	< 0.01
Cyprodinil	not set	8	0.035	0.06
2004 harvest (23 samples)				
Any pesticide		78	-	1.49
Bifenthrin	0.5	4	0.09	0.09
Chlorpyrifos-methyl	3	9	0.015	0.02
Pirimiphos-methyl	5	52	0.41	1.49
Dichlorvos	2	0	-	<0.01
Cyprodinil	not set	13	0.08	0.14
OATS and OATFEED				
2003 harvest (10 samples)				
Any pesticide		90	-	0.38
Chlorpyrifos-methyl	3	20	0.01	0.05
Pirimiphos-methyl	5	90	0.08	0.38
Dichlorvos	2	10	0.01	0.01
Cyprodinil	not set	0	-	<0.01
2004 harvest (8 samples)				
Any pesticide		75	-	1.86
Chlorpyrifos-methyl	3	0	-	< 0.01
Pirimiphos-methyl	5	75	0.63	1.86
Dichlorvos	2	0	-	< 0.01
Cyprodinil	not set	0	-	< 0.01

WHEAT and WHEATFEED

Table 6. Pesticide residues sought in wheat and wheatfeed samples
(Means are calculated as the mean of samples containing residues at or above the limit of quantification)

Sample/analyte	EU MRL mg/kg	% of samples > LOQ	Mean mg/kg	Maximum mg/kg
WHEAT				
2003 harvest (31 samples)				
Any pesticide	-	19	-	-
Chlorpyrifos-methyl	3	3	0.07	0.07
Pirimiphos-methyl	5	16	0.12	0.27
Malathion	8	3	0.05	0.05
2004 harvest (31 samples)				
Any pesticide	-	6	-	-
Pirimiphos-methyl	5	6	0.18	0.28
WHEATFEED (including wheatfeed pellets)				
2003 harvest (23 samples)				
Any pesticide	-	17	-	-
Pirimiphos-methyl	5	17	0.36	0.99
2004 harvest (19 samples)				
Any pesticide	-	63	-	-
Pirimiphos-methyl	5	63	0.27	0.77

Details of the pesticide residues found are given in **Table 6**. All residues found in whole wheat and in wheatfeed were well below the EU MRLs for wheat grain.

Of the wheat grain samples tested, 19% from the 2003 harvest and 6% from the 2004 harvest contained at least one detectable pesticide.

No residues were detected in the organic wheat samples, but the number of samples (3) was too small for the results to be statistically significant. The organic samples have not therefore been separated out in Table 6.

The proportion of wheatfeed samples containing detectable residues was similar in 2003 but significantly higher in 2004 (63%). It is not surprising that more wheatfeed should contain detectable residues, since any residues are likely to be concentrated in the bran layers and hence find their way into wheatfeed. There is no obvious reason for the difference between the 2003 and 2004 findings.

The most commonly detected pesticide was pirimiphos-methyl in both years. In samples from 2003, two other pesticide residues were detected in wheat grain samples: chlorpyrifos-methyl on its own in one sample and malathion detected in combination with pirimiphos-methyl in a single wheat sample. Pirimiphos-methyl was the only residue detected in 2004.

3.3 Heavy metals and arsenic

Table 7. Residues of heavy metals and arsenic in feed barley and pelleted malt culms

A. BARLEY

Metal	Limit of quantification (mg/kg)	% of samples > LOQ	Mean (mg/kg)	Maximum (mg/kg)	Legal limit for feed materials (mg/kg)
2004 harvest					
Arsenic	0.01	73	0.014	0.05	2
Cadmium	0.01	95	0.016	0.04	1
Lead	0.02	96	0.056	0.11	10
Mercury	0.01	0	0.005	<0.01	0.1
2005 harvest					
Arsenic	0.01	85	0.032	0.20	2
Cadmium	0.01	85	0.015	0.05	1
Lead	0.02	100	0.053	0.09	10
Mercury	0.01	0	0.005	<0.01	0.1

B. PELLETED MALT CULMS

Metal	Limit of quantification (mg/kg)	% of samples > LOQ	Mean (mg/kg)	Maximum (mg/kg)	Legal limit for feed materials (mg/kg)
2004 harvest					
Arsenic	0.01	100	0.042	0.09	2
Cadmium	0.01	100	0.026	0.04	1
Lead	0.02	100	0.25	0.21	10
Mercury	0.01	0	0.005	< 0.01	0.1

Although residues of arsenic, cadmium and lead were detected in a high proportion of samples, mean concentrations were very low, and the maximum levels detected were well below legal limits in all cases. Mercury was not detected in any of the samples.

Table 8. Residues of heavy metals and arsenic in oats and oatfeed

OATS and OATFEED

Metal	Limit of quantification (mg/kg)	% of samples > LOQ	Mean (mg/kg)	Maximum (mg/kg)	Legal limit for feed materials (mg/kg)
2004 harvest					
Arsenic	0.01	86	0.029	0.06	2
Cadmium	0.01	43	0.01	0.02	1
Lead	0.02	86	0.07	0.16	10
Mercury	0.01	0	0.005	< 0.01	0.1
2005 harvest					
Arsenic	0.01	86	0.016	0.04	2
Cadmium	0.01	86	0.011	0.02	1
Lead	0.02	100	0.034	0.06	10
Mercury	0.01	0	0.005	<0.01	0.1

Results for oat feed were not significantly different from those for oat grain, and therefore the two categories have been combined in **Table 8.** Results were very similar to those for feed barley and malt culm pellets, with the incidence of arsenic, lead and cadmium residues being relatively high, but actual concentrations being very low, well below legal limits. Mercury was not detected in any of the samples.

Table 9. Residues of heavy metals and arsenic in feed wheat and wheatfeed

A. WHEAT

Metal	Limit of quantification (mg/kg)	% of samples > LOQ	Mean (mg/kg)	Maximum (mg/kg)	Legal limit for feed materials (mg/kg)
2004 harvest					
Arsenic	0.01	50	0.009	0.03	2
Cadmium	0.01	100	0.035	0.06	1
Lead	0.02	86	0.036	0.11	10
Mercury	0.01	5	0.005	<0.01	0.1
2005 harvest					
Arsenic	0.01	20	0.011	0.14	2
Cadmium	0.01	100	0.049	0.10	1
Lead	0.02	93	0.041	0.14	10
Mercury	0.01	0	0.005	<0.01	0.1

B. WHEATFEED

Metal	Limit of quantification (mg/kg)	% of samples > LOQ	Mean (mg/kg)	Maximum (mg/kg)	Legal limit for feed materials (mg/kg)
2004 harvest					
Arsenic	0.01	100	0.028	0.06	2
Cadmium	0.01	100	0.074	0.12	1
Lead	0.02	100	1.29	12.3	10
Mercury	0.01	0	0.005	<0.01	0.1
2005 harvest					
Arsenic	0.01	100	0.021	0.04	2
Cadmium	0.01	100	0.099	0.15	1
Lead	0.02	100	0.066	0.18	10
Mercury	0.01	0	0.005	<0.01	0.1

Results of heavy metal analysis for wheat and wheatfeed are shown in **Table 9, A and B**. Although arsenic, cadmium and lead were detected widely in wheatfeed and feed wheat samples, the levels recorded were generally very low (mean values were <0.1mg/kg for all heavy metals over both years and products). Only one sample presented any cause for concern. This involved a sample of wheatfeed in 2004 where the lead content was measured at 12.3mg/kg. The dataset did not provide sufficient information to identify a reason for this elevated lead result and the only conclusion can be that it is an outlier within this population. As with the barley and oat samples, mercury was rarely detected.

4. INFORMATION UTILISATION

4.1 Communications to levy payers

- Companies who provided samples for the project have been provided with details of the results for their own samples for each sample set collected.
- In addition, a summary report for the AIC has been compiled for each sample set.
- Technical committee meetings of the AIC have also been attended (May 2004 and March 2005) in order to agree sampling protocols and to present summary data.
- Summary data has been presented to levy payers at the HGCA monitoring meeting in April 2005.
- A poster explaining the project was presented at Cereals 2005
- A talk outlining the aims of the project and summarising the findings and implications was presented to a conference on Pig Performance, organised by the HGCA and the Society of Feed Technologists in November 2005.

In addition, the project has featured at the following events at CCFRA:

- Lecture to the Cereal Variety Working Party (December 2004) where a summary of data generated on all cereals was provided "Contaminant monitoring in cereals"
- Lecture to the Cereal Variety Working Party (11th January 2006) "Monitoring contaminants in wheat" covering HGCA projects 2819,3033 and 3150
- Powerpoint presentation at Campden Day, June 2005
- A CCFRA Research Summary Sheet will be produced summarising HGCA funded project work on contaminant issues within the animal and human food chain plus rapid assessment of DON at grain intake.

4.2 Dissemination of information to other organisations

Comments on the potential implications of proposed mycotoxin guidelines (taking into account results obtained from this project) were provided to the AIC for use in discussions with the Food Standards Agency.

4.3 Issues which arose during this project

4.3.1 Trichothecene levels in oats

Monitoring of the first set of freshly harvested samples (2004 harvest) for mycotoxins indicated that some samples of oats and oatfeed could contain relatively high levels of trichothecenes, particularly of T-2 and HT-2. EFSA is currently collecting data on the occurrence of these two mycotoxins, with a view to setting legal limits for cereals/cereal products used for human food, and there is a similar interest in setting limits or guidelines for animal feed (see section 4.3.2). Since the actual number of oats/oatfeed samples tested from

the 2004 harvest was small, it was decided to also test oats from the next sample set collected (stored samples from the 2004 harvest) for trichothecenes in order to obtain more data. (See also Section 4.2).

4.3.2 Draft guideline limits for mycotoxins in animal feed

In August 2005 a Draft Commission Recommendation (on the presence of deoxynivalenol, zearalenone, ochratoxin A and fumonisins in products intended for animal feeding) was circulated. This document:

- Proposed guidelines for deoxynivalenol, zearalenone and ochratoxin A in cereals and cereal products used as feed materials
- Suggested lower limit for complementary and/or complete feedingstuffs for certain more sensitive species (especially pigs)
- Noted an urgent need for more data on T-2 and HT-2 in animal feedingstuffs and feed materials
- Proposed guidelines limits for fumonisins in maize and lower limits for all complementary and complete feedingstuffs for certain specified species
- Noted a need to generate reliable data across the EU on the presence of mycotoxins in cereals and cereal products intended for use in animal feed

The data generated from this project could help to fulfil this need, at least for the UK.

4.3.3 Ergot

Reports in the farming press and personal communications from end-user companies suggest that there is at least a subjective impression amongst both cereal growers and processors that ergot infections in cereals are increasing in Western Europe. The European Commission's Scientific Committee on Animal health has concluded that existing EU legislation on ergot is insufficient to protect animal health due to the difficulty of accurately separating contaminated grain on the basis of size, and the importance of the alkaloid content of individual ergot sclerotia on actual toxicity, The Contaminants Panel of the European Food Safety Authority (EFSA) has issued an Opinion on the occurrence of Ergot in animal feed *(EFSA, 2005)*. Ergot sclerotia contain a number of structurally related alkaloids (ergolines), of which 6 (ergocristine, ergotamine, ergocryptine, ergometrine, ergosine and ergocornine), together with their –inine- metabolites (ergocristinine, ergotaminine etc) are the most common. However, the Panel noted that there were big differences, not only between the total alkaloid content of individual sclerotia, but also between the relative proportions of specific alkaloids. Since there are significant differences between the toxicity and bioavailability of individual ergolines, the Panel felt unable to identify marker alkaloids which would provide a reliable marker of ergot contamination. Subsequently, the Scientific Committee on Animal health, following the lead announced by Germany, has agreed that the total concentration of ergometrine, ergotamine and ergocryptamine would as suitable as a marker of ergot contamination. Ergocrystine should ideally be included in this monitoring, but some analytical problems might need to be overcome *(SCFCAH, animal nutrition section, 2005)*.
It is therefore recommended that a selection of samples of cereals should be tested for these ergot alkaloids.

5. CONCLUSIONS AND RECOMMENDATIONS

Although the period covered by this project is limited to 3 harvests, the results to date indicate that UK-grown cereals are safe for use as feed materials for animals. Levels of contaminants such as heavy metals, pesticide residues and aflatoxins, were low and, apart from one exception, well within statutory limits. Only one sample out of 336 contained a contaminant (lead) at a level which exceeded the legal limit. In general, cereals are unlikely to be significant sources of these contaminants in animal diets. The procedures put in place for the replacement project will allow exceptional samples such as the one noted above to be followed up so that the cause can, if possible, be identified.

Statutory limits have not been set for ochratoxin A and *Fusarium* mycotoxins in animal feeds, and the current indications are that Guideline Values rather than legal limits will be recommended. None of the samples tested for this project contained mycotoxins at levels which would exceed the current proposals for these Guideline Values for general feed materials. However, some samples did exceed the proposed lower Guideline Values for complete and/or complementary feedingstuffs for particularly sensitive species. The results also suggest that the relative incidence of the different Fusarium mycotoxins might be changing, with an increase in the incidence of the more toxic T-2 and HT-2 toxins. It is recommended that continued monitoring of *Fusarium* toxins is particularly important in order to determine whether this is a real trend.

An investigation of a cheaper alternative for screening of dioxins identified a kit which could theoretically be used for this purpose. However, the very different basis of quantification means that the kit is unsuitable for routine use for assuring legal compliance without substantial further work to develop and validate correlations.

Scanning of information sources for emerging issues has revealed an increasing concern in relation to potential contamination with ergot and ergot alkaloids, and some sources suggest that ergot infection is increasing in cereals in Western Europe. Further investigation into the incidence of ergot and the levels of ergot alkaloids in cereal and their by-products is recommended.

6. REFERENCES

Baxter, E.D., Slaiding, I.R. and Kelly, B., *J. Am. Soc. Brew. Chem*., 2001, **59 (3),** 98-100.

Bottomley, P and Baker P.G., *Analyst,* 1984, **109(1),** 85-90.

Chamberlain, S.J., *Analyst,* 1990, 115(9), 1161-1165.

European Commission; The Standing Committee on the Food Chain and animal health; animal nutrition section; Minutes of the meeting of 30th November 2005.

European Food Safety Authority; Opinion of the CONTAM Panel related to ergot as undesirable substance in animal feed. Adopted 19th April, 2005.

Hook, S. and Williams, R. (2004) Investigating the possible relationship between pink grains and Fusarium mycotoxins in 2004 harvest feed wheat. HGCA Project Report No. 354, pp16.

Luke, M.A., Froberg, J.E., Doose, G.M., Masumoto, H.T., *J.Assoc. Off. Anal. Chem,* 1981, **64(5):**1187-95.

Patel, S., Hazel, C.M., Winterton, A.G.M., Mortby, E., *Food Adit. Contam*., 1996, **13(7),** 833-841.

Rees, T D; Hilton, J. *Laboratory Practice*. 1978, **27 (4),** pp. 291.

Verstraede, EBC Symposium on Mycotoxins and other contaminants in the malting and brewing industries. January 2003, Brussels

Appendix 1. Details of sample sets collected

Harvest year	Date collected	Cereal type	Number of samples	No of companies	Growing areas covered
2003 (Stored)	June 2004	Wheat Wheatfeed	31 (inc 3 organic) 23	5	Scotland (5), Wales (4), NE (3), NW (7), Midlands (10), East (16), SW (9)
		Barley	29	5	Scotland (3), Wales (1), NE (4), NW (4), East (9), SW (8)
		Oats Oatfeed	6 6	4	Wales (1), NW (2), Midlands (2), East (2), SW (5)
2004 (freshly harvested)	Sept 2004	Wheat Wheatfeed	41 (inc 2 organic) 21	5	Scotland (7), NE (11), NW (5), Midlands (9), East (12), SE (2), SW (12)
		Barley	26	4	Scotland (5), NE (4), Midlands (5), East (4), SW (5)
		Malt culms	7	7	NE (1), Midlands (1), East (5)
		Oats Oatfeed	6 6	1	Midlands (4), East (1), SW (1)
2004 (stored)	July 2005	Wheat Wheatfeed	37 (inc 3 organic) 17	5	Scotland(5), Wales(2), NE (7), NW (3), East (10), Midlands(9), SW(9)
		Barley	24	3	Scotland(3), Wales (2), NE (2), NW (5), Midlands (1), SE(4), SW (7)
		Oats Oatfeed	8 (inc 1 organic) 5	2	Wales(1), East (3), NE (6), NW (2), SW (1)
2005 (Freshly harvested)	Sept 2005	Wheat Wheatfeed	30 14		Scotland (2), NW (1), NE (10), Midlands (4), East (5), SE (1), SW (7). NW (3), NE (4), East (2), Wales (1), SE (1), SW (3)
		Barley	21	3	Wales(1), East (3), NE(2), NW(3), Midlands(3), SW(3)
		Oats Oatfeed	3 5	3	Scotland(2), East (1), NW92), Midlands(1), SW(1)