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Monitoring contaminants in wheat grain

by

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ABSTRACT

The overall aim of this project was to monitor the safety and quality of wheat entering UK flour mills. This project covered three calendar years i.e. from January 2003 to December 2005. However, due to the importance of climate conditions during crop growth, results are expressed in terms of harvest years. For simplicity, August intakes are considered to be new crop wheat and as a result, this report includes data for the following periods: 2002 crop (January-July 2003); 2003 crop (August 2003-July 2004); 2004 crop (August 2004-July 2005); and 2005 crop (August-December 2005).

The project involved collection of representative sample sets of wheat entering UK flour mills. Most wheat samples were UK-grown (93%), some were imported wheat and included samples of Canadian, French, German, American and Spanish origin.

Samples were analysed routinely for the mycotoxin ochratoxin A (OTA) and organophosphorus storage pesticides. Specific analyses were carried out to investigate the levels of the following contaminants:

- *Fusarium* mycotoxin analyses, for tricothecenes plus zearalenone, were performed on freshly harvested wheat taken from the first 4-5 months of the new crop year (August – December) for the 2003, 2004 and 2005 harvest material.
- Analysis, after harvest, of UK-grown wheat only for the growth regulator chlormequat was carried out on samples from the 2004 and 2005 harvests.
- Analysis, after harvest, of UK-grown wheat only for the broad leaf weedkiller glyphosate was carried out on samples from the 2004 harvest.
- Samples of UK-grown wheat from the 2003 harvest were subjected to analysis for the heavy metals arsenic, cadmium, lead and mercury.

In summary, the results indicated that the overwhelming majority of wheat accepted by UK flour millers was legally compliant and safe for use.

Over the period January 2003-December 2005, only 18.9% of samples contained detectable levels of OTA, i.e. at or above the Limit of Quantification or LOQ of 0.1ppb. This means that more than 80% of samples surveyed contained no detectable OTA. In harvest years 2002 and 2004, where higher percentages of positive OTA were recorded, 25-50% of positive samples were reported as being on the LOQ. Low mean OTA values of between 0.27 and 0.44ppb were recorded across four very different harvest years. A small percentage of wheat samples (less than 2%) were found to have OTA levels above the EU Action Limit of 5ppb. Regional biases in OTA level tend to be related to growing and harvesting conditions in specific crop years and the data suggested that millers tended to adjust their buying decisions to take account of known

regional issues. Length of storage only appeared to have an impact on the occurrence and concentration of OTA in the 2002 and 2004 harvest material.

Surveillance data indicated that nearly 90% of samples surveyed contained no detectable pesticide residues and that no sample contained residues of any OP pesticide above the Maximum Residue Level (MRL) Pirimiphos-methyl was consistently identified as the main OP pesticide detected. Chlorpyrifos-methyl occurred infrequently. Malathion was detected in two samples from the 2002 and 2003 harvests only and in both cases, the samples originated from France. The survey data shows that approximately one third of the samples that were declared as treated with post-harvest pesticides contained detectable pesticide residues.

In terms of the trichothecene mycotoxins, deoxynivalenol (DON) was fairly ubiquitous in UK wheat being detected at levels above the LOQ in 88-91% of samples from all three harvest years (2003 - 2005). However, in only one sample out of the 155 tested was DON detected at a level that would exceed the forthcoming legislative limit for wheat grain of 1250ppb. Interestingly, DON levels did not increase in samples from the difficult 2004 harvest suggesting that, even under these difficult conditions, UK millers were able to source wheat of suitable quality for the production of bread, biscuit and household flour.

The second most frequently detected *Fusarium* mycotoxin was nivalenol (NIV), but the data suggest that the occurrence of this trichothecene is declining in UK wheat destined for the flour milling market. The incidence pattern for T-2 and HT-2 toxins differed between the three crop years. T-2 toxin was most prevalent in 2004 when it was detected in 70% of samples whilst the highest incidence of the HT-2 toxin occurred in 2003 where this toxin was detected in 75% of the samples tested.

Zearalenone was not detected in the majority of wheat destined for use by the UK milling industry. Incidence levels varied between 26.7% and 34% over the three very different crop years (2003-2005).

Chlormequat residues were detected widely in UK-grown wheat samples: it was found in between 80 and 95.6% of samples from the 2004 and 2005 crops respectively. However, the levels detected were very low and significantly below the MRL for wheat for this product.

Only 32% of the samples under test contained glyphosate residues and all samples contained significantly less than the MRL.

Arsenic and heavy metal levels were found to be low in all samples of UK wheat destined for the human food chain from the 2003 harvest.

This surveillance work has been replaced by a new project, (HGCA 3100), which covers wheat and barley entering both the human food chain and the animal feed sector. This has the advantage of facilitating cross sector comparison and discussion in relation to common contaminant issues.

1. SUMMARY

The overall aim of the project was to provide representative survey data on pesticide and mycotoxin levels in wheat used for flour production. In addition, sample retention for the lifetime of the project provided the opportunity to react quickly to any emerging contaminant issue.

The project represents intake wheat from January 2003 to December 2005. However, samples were analysed with respect to harvest years in order to assess the impact of climatic conditions during growth on contaminant levels. Samples were provided on a monthly basis by UK milling companies, according to a pre-arranged schedule. Approximately 12 samples per month were received and were selected to represent the typical intake of each particular mill. This approach ensured that the bulk of analyses were carried out on UK-grown wheat but also guaranteed that imported wheat was properly represented within the survey. Where available, a copy of the grain passport and Grain Storage Audit Form were supplied with the sample. These permitted data relating to mill and location, supplier, variety, location of supply, previous loads carried by the haulier and declaration of pesticide treatment to be logged into the database and facilitate data analysis.

Routine sample analyses involved:

- pesticide residues, in particular those insecticides used in grain storage
- ochratoxin A, a mycotoxin that is produced by moulds which can grow in stored grain.

Additional analyses, were selected by the members of the Technical & Regulatory Affairs Committee (TRAC) of the National Association of British & Irish Millers (**nabim**), and included:

- trichothecene mycotoxins, particularly deoxynivalenol (DON), produced by *Fusarium* fungi which can infect cereals growing in the field
- zearalenone
- the growth regulator chlormequat
- the weedkiller glyphosate
- 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 wheat destined for use by UK flour millers over the period January 2003 to December 2005 was legally compliant and safe. Results for the specific contaminants included in this project follow.

- **Ochratoxin A (OTA)**

The occurrence of OTA was relatively low in wheat used by the milling industry over the period in question with only 18.9% of samples containing OTA levels at or above the limit of quantification (LOQ). Thus, in more than 80% of samples in the survey, the mycotoxin OTA was not detected. Low mean OTA values of between 0.27 and 0.44ppb were also recorded across four very different harvest years.

The data shows that more than 98% of the wheat used by UK millers in the period January 2003-December 2005 was safe with respect to the storage mycotoxin OTA. A small percentage of wheat samples were found to have OTA levels above the EU Statutory Limit of 5ppb. Isolated samples with high OTA levels from the 2003 and 2004 harvest were of UK origin, whereas in the 2005 material the high OTA sample was imported from Canada.

Some regional trends in OTA levels were observed in some harvest years. In the 2002 harvest, the highest incidence of positive OTA samples and the highest levels were observed in the Northern region. In the 2003 harvest, the highest incidence of positive OTA samples and the highest levels were observed in the Midlands region (including one sample above the statutory limit with an OTA level of 8.3ppb). In the difficult harvesting year of 2004, the highest incidence of positive OTA samples and the highest levels were observed in the Midlands region (including 2 samples above the statutory limit at 9.1 and 16.3ppb. The regional split of samples within the survey differed in 2004 with over 10% of samples being supplied from Scotland and 25% from the Eastern region. The incidence of positive OTA was found to be 25% in Scottish samples and 19.4% in samples from the Eastern region, but all samples exhibited levels below 1ppb. This suggested that limited availability of suitable grain or an adjustment in purchasing decisions by millers had occurred in order to avoid potential mycotoxin hotspots.

The length of the storage period is known to have an impact on OTA levels in certain years. Analysis of the data indicated that only in the 2002 and 2004 harvest material did the intake date to have any impact on the occurrence of OTA.

- **Storage pesticides**

Nearly 90% of wheat samples in the survey, used by the milling industry between January 2003 and December 2005, contained no detectable pesticide residues (only 10.5% contained detectable residues). In addition, no sample contained residues of any OP pesticide that was above the maximum residue level or MRL. As in previous surveys of UK wheat, pirimiphos-methyl was the main OP pesticide detected and chlorpyrifos-methyl occurred infrequently. Residues of malathion occurred in samples of imported French wheat from the 2002 and 2003 harvests only.

- **Trichothecenes**

Only four of the 10 trichothecenes included in the analysis were detected at levels above the limit of quantification in each year. These were deoxynivalenol (DON), nivalenol (NIV), T-2 and HT-2 toxins. Based on the limited number of samples surveyed in this project, the results suggested that DON was fairly ubiquitous in UK wheat being detected in more than 88% of samples. However, in only one sample in this survey (from a total of 155 samples collected from the 2003, 2004 and 2005 harvests) was DON detected at a level which would exceed the forthcoming legislative limit for wheat grain of 1250ppb. Mean DON levels in UK-grown wheat were relatively constant at 113-123ppb over the three harvest years tested. The survey data suggests that, even under the difficult harvesting conditions that occurred in 2004, UK millers were able to source wheat of suitable quality with respect to DON for the production of bread, biscuits, breakfast cereals and household flours.

The survey data suggested that the occurrence of NIV was declining in UK wheat destined for the flour milling market. Mean NIV levels were 29.6 in 2003 and 9.0ppb in 2005.

T-2 toxin was most prevalent in 2004 when it was detected in 70% of samples, but mean values were low at 7.1- 9.1ppb over the three harvest years.

For HT-2, the highest incidence occurred in 2003 where this toxin was detected in 75% of the samples tested. Again, mean values were low at 9.6-13.5ppb.

- **Zearalenone**

Zearalenone was not detected in the majority of wheat used by the UK milling industry between January 2003 and December 2005 (the incidence of zearalenone was found to be 26.7- 34%). In addition, mean values at 3.48-5.26ppb were consistently low in mill intake samples across the three crop years. However, the maximum level for each harvest year showed a peak at 86.1ppb for samples from the 2004 harvest suggesting that the wet harvesting conditions in 2004 had an effect on zearalenone levels.

➢ **Chlormequat**

Chlormequat residues were detected in 80-95.6%of UK-grown wheat samples selected from this survey for testing, but the levels detected were very low and significantly below the MRL for wheat for this product.

➢ **Glyphosate**

The widely-used agrochemical, glyphosate, was the subject of a small survey of samples from the 2004 crop only. Residues were detected in only 32% of samples and the mean level was also low at less than 0.1ppm.

➢ **Heavy metals**

Arsenic, cadmium, lead and mercury were found at very low levels in wheat samples selected from the 2003 harvest indicating that UK wheat was safe for use with respect to these potential metal contaminants.

2. INTRODUCTION

Pesticide residue analysis of wheat has been carried out on behalf of the UK milling industry since 1970. Organophosphorus (OP) storage pesticides have traditionally accounted for the majority of pesticide residues detected in this surveillance work and, therefore, effort has been concentrated in this area. The data generated provided a comprehensive picture of milling wheat in the UK over this period and an extremely valuable database that has been used by UK government in support of negotiations within the EU. On a routine basis, data has been used by UK millers to demonstrate quality assurance in raw material entering UK mills and due diligence in complying with Maximum Residue Levels (MRL's). Changes in EU regulations have resulted in the withdrawal of EU approval for a number of storage pesticides. European Directive 91/414/EEC required that all new active ingredients, as well as existing pesticides, were authorised by the same procedures (Stanley, 2002).

Mycotoxins are typically produced as a result of secondary metabolism e.g., when the plant is invaded by specific fungi in the field, it produces chemical compounds to defend itself from attack. The *Fusarium* mycotoxins, trichothecenes and zearalenone, are produced by various *Fusarium* moulds. These moulds cause infection within wheat crops grown in temperate climates. Essentially, *Fusarium* mycotoxins are considered to be field contaminants and there is little evidence of significant effects in grain during storage. Disease created by *Fusarium* species can affect one or more parts of the growing plant. *Fusarium* ear blight (FEB) is frequently found in UK and leads to the production of pink and shrivelled or deformed grains with consequent effects on crop yield and grain quality and safety. Interest in trichothecene levels in cereals has also been generated by a number of surveys of retail cereal products commissioned by the Food Standards Agency (FSA). Such food surveys are carried out to monitor consumer dietary exposure to potential contaminants or to inform discussion and negotiations in Europe.

A typical analytical screen for trichothecenes detects deoxynivalenol (DON), diacetoxyscirpenol (DAS), 3-acetyl DON (3-AcDON), 15-acetyl DON (15-AcDON), fusarenone X (FUS X), nivalenol (NIV), neosolaniol (NEO), T2 triol (T_23), T2 and HT-2 toxin (HT2). All 10 compounds have been detected in UK wheat. However, typically DON predominates followed by NIV with T2 and HT-2 as minor components of the total mycotoxin content.

A wide range of *Fusarium* species are known to be associated with wheat grain and are capable of producing several important toxins. In relation to the above trichothecenes, the following species have been identified: DON producers - *F.culmorum* and *F.graminearum*; DAS producers – *F.equisetti, F.poae and F.sambucinu* FUS X producers – *F.cerealis (crookwellense)* and *F.poae;* NIV producers - *F.cerealis (crookwellense)* and

F.poae; T-2 and HT-2 toxin producers – *F.acuminatum*, *F.sporotrichioides* and *F.langsethiae* (Alldrick, 2004).

ADAS carry out an annual survey of *Fusarium* species infecting the UK cereal crop pre-harvest, details of which are accessible via their website (www.adas.co.uk). This provides industry with scientific data to support mycotoxin analysis. This survey has identified a number of *Fusarium* species responsible for FEB on wheat in the UK: *Fusarium avenaceum* (*Gibberella avenacea*); *Fusarium culmorum*; *Fusarium graminearum* (*Gibberella zeae*); *Fusarium poae*; *Microdochium nivale* (*Monographella nivalis* formerly *Fusarium nivale*); and the recently described *Fusarium langsethiae*. In addition, Crop Monitor from CSL which is accessible via their website (www.csl.gov.uk) provides information on the incidence of *Fusarium* species in UK crops. Incidence data for *Fusarium* species in UK winter wheat over the period 2002 to 2005 shows that the dominant *Fusarium* species changed from year to year as follows: *Microdochium nivale* in 2002; *Fusarium poae* in 2003; *Fusarium langsethiae* in 2004; and *Fusarium graminearum* in 2005. In addition, the CSL survey indicates that, only in the 2004 harvest did a single species occur in more that 50% of the samples tested. As a result of differences in the pattern and levels of infection, it is difficult to accurately predict DON levels in harvested grain. Work carried out at Harper Adams between 2001 and 2005 (Edwards, 2005) has examined the effects of agronomic practice on mycotoxin levels in winter wheat. In addition to assessing the impact of region, previous crop, cultivation and varietal resistance to FEB on *Fusarium* mycotoxin levels, this work has provided practical advice to farmers and useful data on mycotoxin levels in wheat over the relevant period of this work.

Another mycotoxin which became of increasing interest during the lifetime of this project was zearalenone. In addition to receiving considerable attention within the European community zearalenone has also been the subject of FSA commissioned surveys of retail cereal products. Zearalenone is a non-steroidal oestrogenic compound that can cause fertility problems in a number of animals. The following *Fusarium* species are known zearalenone producers: *F. cerealis* (*crookwellense*), *F.culmorum, F.equisetti*, *F.graminearum* and *F.semitectum* (*incarnatum*). EU limits were set for unprocessed wheat in July 2006 at 100ppb.

The mycotoxin, ochratoxin A (OTA), is produced by the fungus *Penicillium verrucosum* as a result of poor drying and storage conditions. OTA is a potential genotoxin and hence low limits have been set for its presence in raw cereal grains of 5 micrograms per kilogram (µg/kg) or parts per billion (ppb). Following concerns relating to the poor wheat harvest in 2004 and in order to ensure that cereals harvested and stored in 2004 did not contain unacceptable OTA levels, the Food Standards Agency undertook a survey of wheat entering the flour milling industry at various locations across England (FSA, 2005). The survey showed that OTA was detected in 30% of samples, but in all cases was below the regulatory limit of 5ppb.

Chlormequat or chlorocholine, the active ingredient in products such as 5C Cycocel, is commonly used in the cultivation of wheat in the UK as a growth regulator. This growth regulator acts by reducing the lower internode length hence reducing plant height, the possibility of crop lodging and optimising yield and quality potential. The chemical acts systemically and thus active ingredient is likely to persist in plant material.

Changes in crop cultivation could possibly result in an accumulation of heavy metals such as arsenic, cadmium, lead and mercury. Previous surveys commissioned by **nabim** provided a snapshot of metal levels in UK-grown wheat and indicated that elevated metal contamination did not appear to be an issue for wheat entering the human food chain in the UK. This project provided an opportunity to update this dataset and examine the current position with respect to metal contamination.

The following sources were used to search the scientific and technical literature and to keep abreast of changes in legislation relating to contaminants in cereals, particularly wheat.

- CCFRA participation in the **nabim** Technical & Regulatory Affairs Committee (TRAC). Contaminant issues feature highly in the work of this committee and decisions on the use of contingency funds to screen samples for potential contaminant threats were taken by TRAC
- 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 that provides prompt and succinct updates on legislative developments across the food industry with links to relevant websites.

3. PROJECT OBJECTIVES

The overall aim of the project was to provide representative survey data on pesticide and mycotoxin levels in wheat used for flour production. Sample retention for the 3-year period of the project was built-in to provide the opportunity to react quickly to any emerging contaminant issue that surfaced during the project lifetime. This archive was intended, if required, to permit analysts to work retrospectively to collect representative data over current and past wheat crops and, thus, respond quickly at the National or European level.

Specific objectives within the project included:

- co-ordination of sample selection to ensure that the survey represented UK and imported wheat intake used for flour production in the UK
- creation of a sample database to permit retrospective data analysis
- collation and interpretation of analytical data for specific contaminants for **nabim** members.

4. MATERIALS & METHODS

4.1 Sample collection and supporting information

Samples were provided, on a monthly basis by UK milling companies, according to a pre-arranged schedule. Approximately 12 samples per month were received which were intended to represent the typical intake of each particular mill. Due to the regional distribution of UK mills and differences in their intake pattern due to product requirements, this approach ensured that the bulk of analyses were carried out on UK-grown wheat but also guaranteed that imported wheat was properly represented within the survey. Mills were requested to ensure that their sampling methods complied with BS ISO 13690:1999 "Cereals, pulses and milled products- Sampling of static batches" and were required to provide a representative sample (~1.5kg) taken from the bulk supply to CCFRA for testing. Where possible, a copy of the grain passport and Grain Storage Audit Form were supplied with the sample. This permitted data relating to mill and location, supplier, variety, location of supply, previous loads carried by the haulier and declaration of pesticide treatment to be logged into the database and facilitate data analysis.

The project represents intake wheat from January 2003 to December 2005. However, it has been analysed with respect to harvest years in order to assess the impact of climatic conditions during growth on contaminant levels. For simplicity, August intakes are considered to be new crop wheat and as a result, this report includes data for the following periods: 2002 crop (January-July 2003); 2003 crop (August 2003-July 2004); 2004 crop (August 2004-July 2005); and 2005 crop (August-December 2005).

On arrival at CCFRA, the sample was divided into two representative sub-samples of approximately 700g using a riffle divider. The first sub-sample was used for testing and the second whole grain sub-sample was placed in controlled storage conditions (~10^{o}C) to provide an archive of samples that would enable the milling industry to respond quickly to any emerging contaminant issue. Samples for testing were ground to produce a fine wholemeal using a KT-3100 mill prior to analysis. Sub-samples of this material were used for all analyses shown in 4.2.

4.2 Analytical methods

The project contained a routine screening element where all samples were monitored for storage pesticides and ochratoxin A (OTA).

4.2.1 Routine screening methods

Ochratoxin A (OTA)

Samples were analysed for OTA using an in house high-performance liquid chromatography (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.

Pesticides

Wheat samples were analysed at CCFRA for organophosphorus storage 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. The organophosphorus pesticides screened for routinely are shown in Table 1.

Table 1 Organophosphorus insecticides included in routine pesticide screen of wheat samples

Active ingredient	Limit of Detection (mg/kg; ppm)	EU MRL (mg/kg; ppm)
Chlorpyrifos	0.02	0.05
Chlorpyrifos-methyl	0.02	3
Diazinon	0.01	0.02
Dichlorvos	0.05	2
Etrimfos	0.05	No MRL
Fenitrothion	0.05	No MRL
Malathion	0.05	8 (Malathion + malaoxan)
Malaoxan	0.05	See Malathion above
Methacrifos	0.02	0.05
Phosphamidon	0.02	0.05
Pirimiphos-methyl	0.02	5
Trichlorfon	0.05	0.1

4.2.2 Additional contaminant studies

The project contained a contingency fund that was established to enable the milling industry to respond to emerging issues. The decision, regarding which potential contaminants should be investigated within this project in a particular crop year, was taken at the October meeting of the Technical & Regulatory Affairs Committee (TRAC) of **nabim**. The following topics were investigated in one or more years during the lifetime of this project.

***Fusarium* mycotoxin screen**

The project permitted the inclusion of a limited survey of *Fusarium* mycotoxins to be performed on freshly harvested wheat taken from the first 4-5 months of the new crop year (August – December). Due to the start date of the project this measurement was made for 2003 to 2005 harvest material only. The method used for this is provided below.

Trichothecenes

Ground 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), T2 triol (T_23), T2 and HT-2 toxin (HT2) in both years. This laboratory is UKAS accredited for trichothecene analysis. The method is based on the use of GC-MS and was developed by Patel *et al*., 1996. The limit of quantification (LOQ) for all 10 trichothecenes is 10µg/kg i.e., 10ppb.

Zearalenone

In all three crop years, wheat samples were sub-contracted to RHM Technology, High Wycombe who are UKAS accredited for this analysis. The method used was developed by Patel *et al*., 1996. The LOQ for zearalenone is 3.0µg/kg i.e., 3ppb.

Chlormequat

Samples of ground wheat were supplied to CSL, York for chlormequat analysis. The sample was extracted with methanol, filtered and analysed by HPLC-MS/MS with electrospray ionsiation. Collisionally induced dissociation product ions at *m/z* 122>58 and 124>58 were monitored. The LOQ for chlormequat is 0.01mg/kg i.e., 0.01ppm.

Glyphosate

Samples of ground wheat were supplied to CSL, York for glyphosate analysis. Samples were extracted with an acidified aqueous solution. An aliquot of the extract was derivatised prior to analysis using a gas chromatograph connected to a mass selective detector (GC-MSD). This analysis is not a UKAS accredited method at CSL. The LOQ for glyphosate is 0.05mg/kg i.e., 0.05ppm.

Heavy metals

Metal analyses involved atomic absorption spectroscopy using an in house procedure based on a published method (Rees and Hilton, 1978).

Methods vary according to the 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 that 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 the metal analyses conducted and participates in FAPAS proficiency tests for lead, cadmium, arsenic and mercury and Z-scores are available on request. The LOQ for each metal tested is shown in Table 2.

Table 2 Limits of quantification for metal analysis

Metal	LOQ (mg/kg; ppm)
Arsenic	0.01
Cadmium	0.01
Lead	0.02
Mercury	0.01

5. RESULTS & DISCUSSION

The data has been presented to be consistent with HGCA Project Report No 387 " Monitoring the quality and safety of grain and grain derived co-products destined for animal feed" (Baxter & Salmon, 2006) that describes similar surveillance studies for representative samples entering the animal feed chain. This differs from the way in which the data was reported to the milling industry. In particular, for all contaminants other than pesticide residues the mean value is calculated by assigning a value to all samples where no contaminant was detected as follows: any sample recorded as "not detected" or below the "LOQ" was given a value of half the LOQ.

The data has been scrutinised for potential effects of intake date, region and variety on OTA and pesticide levels. For the majority of UK-grown samples the growing location was identified by the supplier which enabled the data to be analysed on a regional basis. However, the data indicated, that in most years, the majority of samples originated in the Midlands and Northern region of the UK. This finding was slightly unexpected, as the main wheat growing regions are located in the East and South of the country. However, a number of the largest UK flour mills are located in the Midlands region and therefore the survey also reflects the mill buying patterns and possibly the current pressures to reduce road miles. Due to the limited number of samples in some regions, it is not possible to provide a full statistical analysis of the data and therefore only trends are provided within this report.

Ochratoxin A (OTA)

Table 3 presents a summary of OTA results for wheat samples representing UK mill intake over the period January 2003 to December 2005. Where the OTA level was reported as not detected i.e. below the LOQ of 0.1ppb, half this value or 0.05ppb was used to calculate the mean value.

Table 3 Ochratoxin levels in wheat used by UK millers between January 2003 and December 2005

Harvest year	% of samples > LOQ	Mean (ppb)	Maximum (ppb)	% >Action Limit
2002	27.1	0.27	4.5	0
2003	14.0	0.38	16.0	1.8
2004	23.3	0.33	16.3	1.2
2005	4.6	0.44	24.3	1.5

Results show that the occurrence of OTA was relatively low in wheat used by the milling industry over this period. Over the entire period, 18.9% of samples contained OTA levels at or above the LOQ i.e., more than 80% of samples surveyed contained no detectable OTA. In harvest years 2002 and 2004, where higher percentages of positive OTA were recorded, between 25 and 50% of these samples were 0.1ppb i.e., on the limit of detection. Low mean OTA values of between 0.27 and 0.44ppb across four very different harvest years provided further confirmation of the generally low levels detected.

The data shows that more than 98% of the wheat used by UK millers in the period January 2003-December 2005 was safe with respect to the storage mycotoxin OTA. Only a small percentage of wheat samples were found to have OTA levels above the EU statutory limit of 5ppb. In the 2003 and 2004 harvest material, samples with high OTA levels were of UK origin whereas in the 2005 material the high OTA sample was imported from Canada. These data do not support the view that variety plays a role in OTA levels.

For UK regions where sufficient data existed, the following trends were observed:

- 2002 harvest - the highest percentage of positive OTA samples (20.0%) and the highest levels were observed in the Northern region. The second highest incidence of OTA was observed in the Eastern region, but levels were very low and none exceeded the statutory limit.
- 2003 harvest - the highest percentage of positive OTA samples (12.9%) and the highest levels were observed in the Midlands region. This included one sample with an OTA level of 8.3ppb that was above the statutory limit of 5ppb. No specific issues were observed in any other region in this year.
- 2004 harvest – in this difficult harvesting year, the highest percentage of positive OTA samples (25.6%) and the highest levels were observed in the Midlands region. This included two samples above the action limit of 5ppb. The regional split suggested that millers had purchased wheat from different areas of the country as over 10% of the survey samples were grown in Scotland. The second highest incidence of OTA was observed in wheat from this region at 25%, but the levels were all below 1ppb. The occurrence of OTA was also significant in the Eastern region at 19.4%. This region constituted over 25% of the survey samples for this harvest, but as for Scotland all samples exhibited levels below 1ppb.
- 2005 harvest – no issues were observed for any region.

As OTA is generated during grain storage, the length of the storage period should have an impact on OTA mycotoxin levels in certain years and, therefore, the data was examined with respect to intake date. Only in the 2002 and 2004 harvest material, did intake date appear to have any impact on the occurrence of OTA. In the 2002 harvest material, over 80% of the positive OTA samples were received by mills between April and July (i.e., during the latter part of the crop year). Similarly, in the 2004 material there was a bias towards lower OTA occurrence in the first 4 months, as shown by 18.4% of positive OTA samples detected in August to November intakes.

Pesticides

Table 4 presents a summary of OP pesticide residue results for wheat samples, representing UK mill intake over the period January 2003 to December 2005. Results are reported as mg/kg or ppm.

Results indicate the low occurrence of OP pesticide residues in wheat used by the milling industry over this period. Over the entire period, only 10.5% of samples contained measurable OP pesticide residues i.e., nearly 90% of samples surveyed contained no detectable pesticide residues. The highest incidence occurred in 2002 harvest samples and the data suggests that the number of samples with detectable OP pesticide residues was reduced in all subsequent harvests. Pirimiphos-methyl was consistently identified as the main OP pesticide detected. Chlorpyrifos-methyl occurred infrequently. Malathion was detected in samples from the 2002 and 2003 harvests only and in both cases the samples originated from France.

Comparing the recorded mean and maximum pesticide values with the MRL, it is clear that the levels were low and no sample contained residues of any OP pesticide that was above the MRL. The maximum level of pirimiphos-methyl detected was less than half the MRL and, in fact, this was not associated with UK-grown wheat and was detected in a cargo of imported German wheat. The picture was similar for malathion, where the maximum level was less than one sixth of the MRL and this pesticide was associated with imported French wheat. In all cases, only very low levels of chlorpyrifos-methyl were detected: the maximum was less was than one fiftieth of the MRL.

Table 4 Storage pesticide residues detected in wheat used by UK millers during the period January 2003 and December 2005

Sample/analyte	EU MRL (ppm)	No of samples > LOQ	Mean (ppm)*	Range (ppm)
2002 harvest				
Any pesticide		18		
Pirimiphos-methyl	5	18	0.38	0.09-1.71
Chlorpyrifos-methyl	5	1	0.06	
Malathion	8	1	0.21	
2003 harvest				
Any pesticide		11		
Pirimiphos-methyl	5	9	0.26	0.06-0.53
Chlorpyrifos-methyl	5	1	0.09	
Malathion	8	2	0.88	0.56-1.2
2004 harvest				
Any pesticide		11		
Pirimiphos-methyl	5	10	0.64	0.06-2.3
Chlorpyrifos-methyl	5	1	0.05	
Malathion	8	0		
2005 harvest				
Any pesticide		7		
Pirimiphos-methyl	5	6	0.20	0.07-0.43
Chlorpyrifos-methyl	5	1	0.06	
Malathion	8	0		

*In contrast with other contaminant data reported in this survey, where no OP pesticide was detected a value of zero was used to calculate the mean value.

Where provided, post-harvest pesticide usage was recorded from the pesticide passport. Samples were recorded in three categories as “treated”, “untreated” or “unknown”. Analysis of the data indicated that between 12.3 and 21.9% of the survey samples had received post-harvest pesticide treatment. Treatment with some form of Actellic was the most prevalent store treatment and applications were primarily reported to have taken place prior to the introduction of new crop into store. Across all four harvest years included in this survey, approximately one third of the samples that were declared as treated with post-harvest pesticides contained detectable pesticide residues. The highest reported post-harvest pesticide treatment occurred in samples from the 2003 harvest. The data suggested a decline in store treatment in subsequent years, but the percentage of samples which were recorded as unknown with respect to post-harvest pesticide treatment also increased from 10.5% in 2003 to 26.4% in 2004 and therefore this observation cannot be taken as conclusive.

As for OTA, the length of the storage period appeared to have an impact on the number of samples with detectable OP pesticide residues. This was particularly true for 2002 and 2003 crops where 60 and 54% of positive samples occurred in intake samples received in the last 4 months i.e., April-July. This observation slightly contradicts the records of treatment prior to placing new crop in storage.

Due to the limited number of positive pesticide samples detected within the survey, analysis of the regional and varietal data does not produce any useful information.

Trichothecenes

Only UK-grown wheat was selected from intake samples from the first 5 months after each harvest for *Fusarium* mycotoxin analysis. This amounted to 60 samples from the 2003 harvest, 50 samples from the 2004 harvest and 45 samples from the 2005 harvest. For each trichothecene included in the analysis, the limit of quantification (LOQ) was 10ppb. Where a value of below the LOQ was recorded i.e. <10ppb, half this value or 5ppb was used to calculate the mean value.

Results for the three harvest years are presented as the percentage above the limit of quantification (Figure 1), mean values (Figure 2) and maximum level detected (Figure 3). Within the 10 trichothecenes measured, only four consistently produced levels above the limit of quantification in each year. These were deoxynivalenol (DON), nivalenol (NIV), T-2 and HT-2 toxins. This picture is similar to that observed for UK feed wheat (Baxter & Salmon, 2006).

Figure 1. Percentage of UK-grown wheat with trichothecene levels above the LOQ

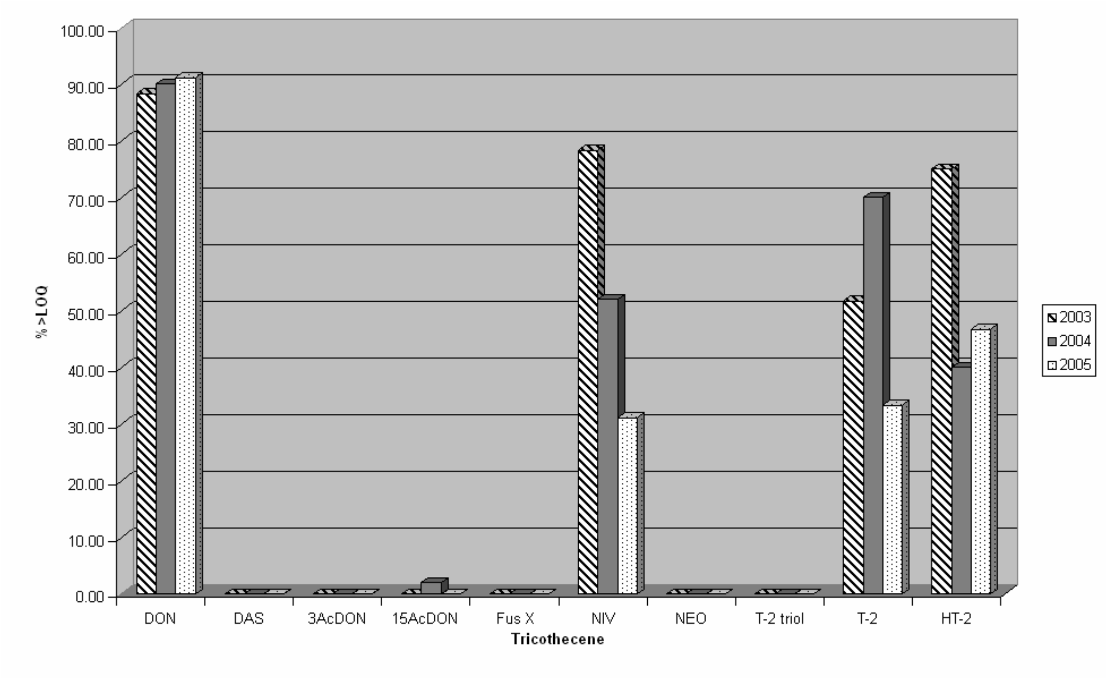


Figure 2. Mean trichothecene levels in UK-grown wheat, 2003-2005.

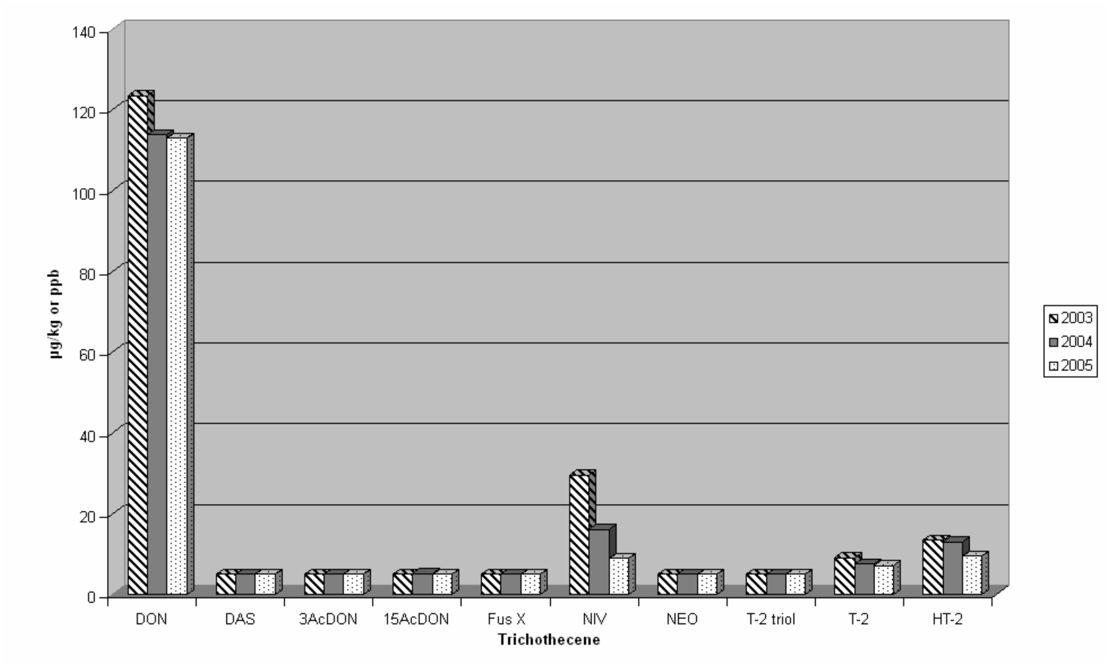
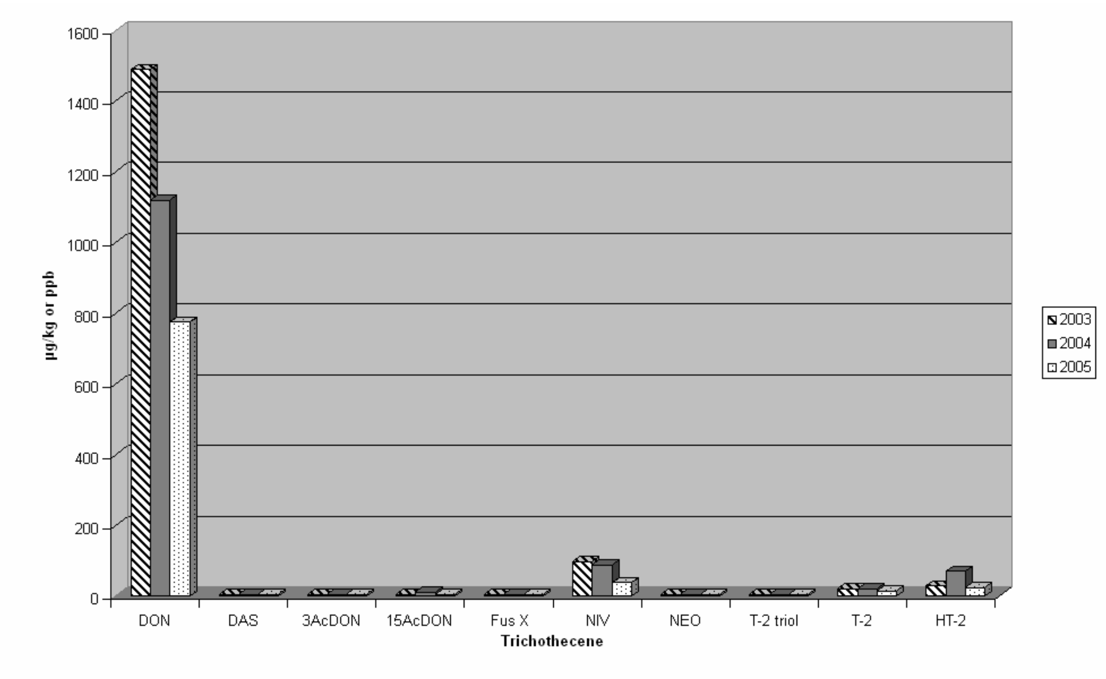


Figure 3: Maximum trichothecene levels in UK-grown wheat, 2003-2005.



The results show that deoxynivalenol (DON) was fairly ubiquitous in UK wheat being detected at levels above the LOQ in between 88 and 91% of samples from all three harvest years (Figure 1). However, in only one sample out of the 155 tested (included in the harvest 2003 sample set) was DON detected at a level which would exceed the forthcoming legislative limit for whole wheat grain of 1250ppb.

Maximum DON levels detected in 2004 and 2005 were 1119 and 775ppb respectively. Mean DON levels in UK-grown wheat were relatively constant at 123, 114 and 113ppb for 2003, 2004 and 2005 respectively. It is interesting to note that DON levels did not increase in samples from the 2004 harvest. 2004 was considered to be a difficult year where it was feared that significant pockets of the wheat crop would contain high levels of pink grains and hence *Fusarium* mycotoxins (Hook and Williams, 2004). The perceived risk within the human and animal feed chains was that wheat processors would have difficulty obtaining safe raw material with acceptable trichothecene levels (particularly DON). This survey suggests that, even under these difficult conditions, UK millers were able to source wheat of suitable quality for the production of bread, biscuits, breakfast cereals and household flours. Again, this finding supports the conclusion of contaminant monitoring work carried out on behalf of the animal feed sector (Baxter & Salmon, 2006).

As expected, the second most frequently detected *Fusarium* mycotoxin was nivalenol (NIV). The data suggested that the occurrence of this trichothecene was declining in UK wheat destined for the flour milling market: it was detected in 78% of samples in 2003, in 52% of the sample set in 2004 and only in 31% of samples from the 2005 harvest. This was borne out by reduced mean and maximum NIV levels over the three harvest years. Mean NIV values were 29.6, 16.1 and 9.0ppb in 2003, 2004 and 2005 respectively and the maximum NIV levels detected were 97, 88 and 38 in the same three harvest years. This finding contrasts with results for raw material entering the animal feed chain (Baxter & Salmon, 2006). No reason for this discrepancy can be identified. It is unlikely to be varietal as the sample set in this project included a significant proportion of Group 3 wheat and a small proportion of Group 4 wheat, both of which could equally have found a market in the animal feed sector.

The incidence pattern for T-2 and HT-2 toxins differed between the three crop years. T-2 toxin was most prevalent in 2004 when it was detected in 70% of samples. The incidence of this toxin was 53% in 2003 and 33% in 2005. However, the data was very consistent with respect to levels of T-2 detected: mean values were 9.1ppb in 2003, 7.5ppb in 2004 and 7.1ppb in 2005. The maximum T-2 levels were also low for each crop year at 20ppb in 2003, 21ppb in 2004 and 14ppb in 2005. For HT-2, the highest incidence of this toxin was in 2003 where this toxin was detected in 75% of the samples tested. The figure was reduced to 40% in 2004 and 47% in 2005. Mean values were relatively consistent at 13.5ppb in 2003, 13ppb in 2004 and 9.6ppb in 2005, but there was more variation in the maximum levels detected with 29pbb in 2003, 70ppb in 2004 and 24ppb in 2005. Previously suggested limits for HT-2 and T-2 were set at 100ppb. No sample within the set of 155 contained combined levels of HT-2 + T-2 above this suggested limit.

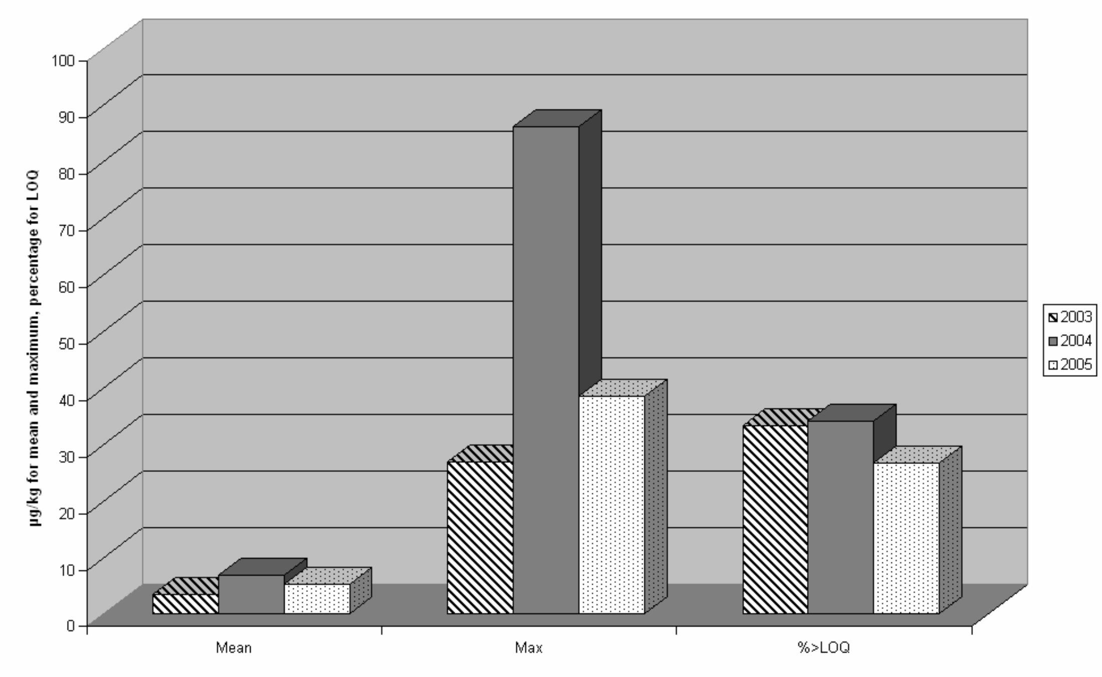
Of the other trichothecenes, only 15Ac-DON was detected in any sample of mill intake wheat and this was found in a single sample at a level of 11ppb i.e. only just above the level of quantification.

Zearalenone

The level of quantification for zearalenone is 3.0ppb. Where a value of below the LOQ was recorded i.e., <3ppb, half this value or 1.5ppb was used to calculate the mean value.

The incidence, mean and maximum levels of zearalenone in UK-grown wheat from the 2003-2005 harveste are presented in Figure 4. Zearalenone was not detected in the majority of wheat destined for use by the UK milling industry (incidence levels varied between 26.7% and 34% over the three crop years). This contrasts with the survey carried out of wheat entering animal feed chain where in 2004 some 77% and in 2005 just over 64% of the samples had zearalenone levels above the LOQ (Baxter & Salmon, 2006). Mean values were consistently low in mill intake samples across the three crop years: 3.48ppb in 2003, 6.82ppb in 2004 and 5.26ppb in 2005. However, the maximum level observed in each year showed more variation with 28.6ppb in 2003, 86.1ppb in 2004 and 38.5ppb in 2005. The wet harvesting conditions, which hit the later maturing wheat crops in the 2004 harvest, appeared to have affected zearalenone levels in intake wheat to flour mills. A more significant effect was observed in feed wheat entering the animal feed chain in 2004 (Baxter & Salmon, 2006). Forthcoming legislation due to come into force on July 1st 2006 will set an EU limit of 100ppb for unprocessed wheat.

Figure 4: Zearalenone levels in UK-grown wheat from the 2003-2005 harvests



Chlormequat

Chlormequat, the active ingredient in commonly used growth regulators acts sytemically and thus is likely to persist in plant material. Therefore, it is not surprising that residues of chlormequat were detected widely in UK-grown wheat samples: it was found in between 80 and 95.6% of samples from the 2004 and 2005 crops respectively. However, the levels detected were very low and significantly below the MRL for wheat for this product of 2.0 mg/kg i.e., 2ppm Where a value of below the LOQ was recorded for chlormequat i.e. <0.01ppm, half this value or 0.005ppm was used to calculate the mean value.

Results of chlormequat analyses carried out on samples of UK-grown wheat from the 2004 and 2005 harvests. Mean chlormequat values were 0.08ppm in samples from the 2004 crop and 0.13ppm in samples from the 2005 samples, while the maximum chlormequat level detected in 2004 harvest samples was 0.16 ppm and in 2005 harvest samples was 0.51ppm.

Table 5 Chlormequat levels (ppm or mg/kg) in UK-grown wheat from harvest years 2004 & 2005

	Chlormequat		
	Mean	**Max**	**%>LOQ**
2004	0.08	0.16	80
2005	0.13	0.51	95.65

Glyphosate

Glyphosate, the active ingredient in herbicides designed to control annual and perennial weeds, was the subject of a small survey in samples from the 2004 crop. Given the weather conditions during harvest in 2004, members of TRAC considered that glyphosate use was more widespread in order to desiccate the crop and facilitate harvest. A sub-set of 25 freshly harvested samples from the 2004 crop were selected for glyphosate analysis. Where a value of below the LOQ was recorded for glyphosate i.e. <0.05mg/kg or 0.05ppm, half this value or 0.025ppm was used to calculate the mean value.

In fact, glyphosate residues were not detected in the majority of samples. Only 32% of the samples under test contained residues of this widely used agrochemical. Mean and maximum glyphosate levels were also correspondingly low: mean value was 0.099ppm and the maximum level observed in any of the 25 samples tested was 0.63ppm. As all samples contained significantly less than the MRL for wheat of 5.0ppm for glyphosate, this survey was not repeated in 2005 harvest samples.

Heavy metals

60 samples of freshly harvested wheat for the 2003 crop were analysed for arsenic, cadmium, lead and mercury. For each metal where the value quoted was below the LOQ half the LOQ was used for the purposes of calculating a mean value.

Table 6 Heavy metal and arsenic levels in wheat ex 2003 harvest

Metal	% of samples > LOQ	Mean (mg/kg)	Maximum (mg/kg)	Legal limit (mg/kg)
Arsenic	10	0.006	0.01	2
Cadmium	100	0.041	0.22	1
Lead	53.3	0.02	0.06	10
Mercury	0	0.005	<0.01	0.1

The data in Table 6 shows that, in samples from the 2003 harvest, heavy metal contamination was not an issue for UK-grown wheat. For all four metals the mean level detected was very low and significantly below any legal limit. No mercury was detected in any of the 60 samples tested. Arsenic was detected in only 10% of samples. Cadmium and lead were the most frequently occurring metals, but even for these the mean and maximum levels detected were very low. The data is consistent with that generated within an HGCA project covering raw materials entering the animal feed chain.

6. CONCLUSIONS

- Results of routine ochratoxin A (OTA) and organophosphorus (OP) pesticide screening, obtained within this 3 year survey, are consistent with previous **nabim** generated surveillance data and have enabled **nabim** members to show due diligence in monitoring for potential contaminants of wheat entering the human food chain.

- On average, OTA was detected in 18.9% of the survey samples representing UK milling industry usage between January 2003 and December 2005. Over this period, which included four very different harvest years, the mean OTA level was less than 0.5ppb suggesting significant success with respect to storage and control of this mycotoxin in wheat being accepted by the UK milling industry for human consumption. A few isolated samples were found to have OTA levels above the legal limit. It is normal practice for millers to conduct an audit of the grain store/ farm records to investigate the cause of any high OTA levels.
- As sampling has a significant effect on the measured levels of this non-homogenously distributed mycotoxin, it is also common practice to take additional samples to verify the OTA level measured on a sample above any legal limit. **nabim** have produced guidelines for their members to ensure a consistent approach to any samples containing a contaminant level above statutory limits.

- Over the period covered by this project, OP storage pesticides were detected in 10.5% of the survey samples, but the levels found never exceeded legal limits. Data has been used by UK millers to demonstrate quality assurance in raw material entering UK mills and due diligence in complying with pesticide MRL's.

- As in previous surveillance work, pirimiphos-methyl was the most commonly detected pesticide in UK wheat. Only one other OP pesticide, chlorpyrifos-methyl was detected in any UK wheat sample. The list of pesticides detected has clearly reduced over the last three years. European Directive 91/414/EEC required that all existing approved pesticides in use in member states before the Directive came into force on 25th July 1993 were reviewed in order to ensure that all new active ingredients, as well as existing pesticides, were authorised by the same procedures. As a result, a number of storage pesticides were withdrawn from use following the restrictions on the use of certain active ingredients. An example of the impact of this EU regulation on UK usage of storage pesticides was shown in the absence of malathion residues in UK-grown wheat. In fact, malathion was only detected in samples from the 2002 and 2003 harvests and only in imported samples from France.

- This project provided the opportunity to investigate emerging contaminant issues and hence generate representative data which could be used to assist in discussions at the National or European level. In order of priority, the key contaminants addressed under this category were the *Fusarium* mycotoxins (trichothecenes and zearalenone), the growth regulator chlormequat, the weedkiller glyphosate and heavy metals.

- Deoxynivalenol (DON) was detected in the majority of wheat samples tested, but the level found exceeded the forthcoming EU legislative limit of 1250ppb in only 1 of the 155 samples tested over three harvest years.
- Nivalenol (NIV) was the second most frequently detected *Fusarium* mycotoxin. Over the three-year survey period, the occurrence of this trichothecene was in decline in UK- grown wheat. Levels found were consistently low and the maximum never exceeded 100ppb.
- The T-2 toxin was found to be most prevalent in samples from the 2004 crop when it was detected in 70% of samples, but in all three years the average T-2 toxin level was below 10ppb.
- The highest incidence of the HT-2 toxin occurred in 2003 where it was detected in 75% of the samples tested. The average HT-2 level found was below 15ppb across all three crops tested, but the maximum levels varied between 24 and 70ppb.
- Zearalenone was detected in less than 35% of UK-grown wheat destined for use by the UK milling industry and mean values were less than 7ppb in mill intake samples across all three harvest years.
- The highest level of zearalenone observed in the 155 samples tested was 86.1ppb in a sample from the 2004 harvest. Thus, no UK-grown sample was found to contain zearalenone at levels that would exceed forthcoming legislative limit of 100ppb for unprocessed wheat.
- Residues of the persistent and widely-used growth regulator, chlormequat, were detected in the majority of UK-grown wheat samples, but the maximum level found was approximately 25% of the MRL demonstrating compliance with respect to this agrochemical.
- In a limited survey of samples from the 2004 crop, the herbicide glyphosate was detected in only 32% of samples and the levels found were consistently low. In fact the maximum level observed was only 12.6% of the MRL, thus demonstrating compliance with respect to this agrochemical. The results of this survey provided the milling industry with some reassurance with respect to levels of glyphosate and this survey was not repeated in 2005 harvest samples.
- Results of heavy metal analysis indicated that contamination with arsenic, cadmium, lead or mercury was not an issue in UK-grown wheat from the 2003 harvest and as a result the survey was not repeated in future harvest material.

➢ The project generated a 3-year archive of samples in order to permit analysts to produce data over current and past wheat crops and, thus, to respond to any emerging contaminant issue that surfaced during the project lifetime. This facility was not utilised within the project lifetime.

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