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REVIEW OF FOOD SAFETY ISSUES RELATING TO THE SUPPLY AND MARKET ACCEPTABILITY OF UK MALTING BARLEY AND UK MALT

by

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This is the final report of a twenty-four month project that commenced in October 2002. The work was funded by a contract for £98,188 from the Home-Grown Cereals Authority (Project 2804).

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1. ABSTRACT

The aims of this project were:

(1) to provide reassurance that UK grown malting barleys, and the malts prepared from them, complied with EU and UK law relating to safety and wholesomeness.

(2) to establish a robust data set which could be used on behalf of growers and processors in any negotiations with official organisations such as the UK's Food Standards Agency or the European Food Safety Authority.

(3) to identify any emerging issues which might have an effect either on the wholesomeness of UK cereals and cereal products, or on the perception of wholesomeness by customers and consumers, and to initiate responses as appropriate.

The project included barleys from the 2002 harvest up to and inclusive of the 2005 harvest. One set of barley samples was collected soon after harvest. This set was representative of (a) commercial malting companies in the UK, (b) malting barley varieties on the market, and (c) the main growing regions for malting barley in the UK. These samples were mainly tested for the presence of mycotoxins produced by *Fusarium* moulds (for example, deoxynivalenol (DON)). Another set of samples was collected around March-April, and included both barleys (which had been stored for approximately 6-8 months after harvest) and the commercial malt made from each batch of barley. These sets were sampled according to the rigorous protocol set by the European Commission for Official Control of non-homogenous contaminants such as ochratoxin A in cereals. These sample sets were tested for mycotoxins produced by moulds which can grow on stored grain (for example, ochratoxin A), as well as for pesticides, growth regulators and other potential contaminants of raw and processed cereals.

Over the duration of the project, no samples exceeded legal limits for *Fusarium* mycotoxins, ochratoxin A, pesticides or growth regulators.

The majority of samples, either barley or malt, did not contain detectable *Fusarium* mycotoxins. When they were found, DON was detected most frequently, but at levels well below the legal limit introduced in the EU in 2005. The related toxin nivalenol was detected infrequently. T-2 and HT-2 toxins were not detected at all during the first 2 years of the project, but were found at low levels in several samples of barley from the 2005 harvest.

Similarly, the majority of samples did not contain detectable ochratoxin A. Stored samples were more likely to test positive than freshly harvested ones, and malts tended to contain slightly higher levels than raw barleys.

Most samples tested contained growth regulators (chlormequat and/or mepiquat) but at low levels. The post-harvest insecticide pirimiphos-methyl was detected at trace levels in some samples. The fungicide cyprodinil was also detected occasionally at trace levels.

The data accumulated by this project supports the image of UK-grown malting barley as a safe and wholesome cereal, which complies with existing limits for contaminants. The data will also be used to inform discussions relating to the setting of new limits by the Commission.

2. SUMMARY

This project is a continuation of HGCA project 2279, which established a system by which:
(1) published information in the medical, scientific and agricultural press, as well as official information circulated by governmental bodies in the UK, the EU and elsewhere, was routinely scanned for emerging issues which might impact upon the wholesomeness of UK barley and malt,
(2) relevant information was communicated to the industry,
(3) relevant information was also used to guide a detailed surveillance programme for UK-grown malting barley and malt.

In the current project, the original surveillance programme has been amended slightly from that used for Project 2279. Two sets of samples are now collected routinely - the first is of barleys and is collected soon after harvest, whilst the second is collected around April and represents malts made from barleys which will have been in storage for at least 6 months. For the samples from the 2003 and 2004 harvests, barley/malt pairs were collected, that is samples of stored barleys plus the malts made from them. Analytes tested have mainly been mycotoxins and pesticides, reflecting the current priorities of the European Commission and the UK's Food Standards Agency. However, the range of species sought has been extended in each case.

The range of mycotoxins sought has been extended from the 2004 harvest to include certain other mycotoxins. This has been in response to specific concerns expressed either by the EU commissioner responsible for contaminants, or in some cases by customers for cereal products.

The range of pesticide residues sought has also been extended in order to cover growth regulators, the pre-harvest desiccant glyphosate and certain late fungicides. These have been targeted as a result of a risk assessment exercise, also conducted as part of this current project. This exercise, which aimed to identify which legally used cereal pesticides were most likely to leave significant residues on barley, and thus should be included in surveillance exercises, took into account agronomic, chemical and physiological parameters.

The overwhelming majority of the tests carried out support the view that UK malting barley and UK malts are wholesome foodstuffs, and generally contain only very low concentrations of contaminants. The majority of samples contained no detectable residues of the mycotoxins deoxynivalenol and no samples exceeded the legal limit in the EU (Figure 1). Similarly, three quarters of samples contained no detectable ochratoxin A and no samples exceeded the then current legal limits (Figure 2). Two samples from 2000 and 2001 exceeded the 3 µg/kg limit which was subsequently introduced in 2002. Pesticide residues, if present, were also only at low levels. Only a minority of samples contained detectable residues of insecticides or fungicides, and, where

present, these were well below MRLs (Figure 3). Several samples contained detectable residues of growth regulators, but always at levels well below legal limits.

Figure 1. Occurrence of deoxynivalenol in malting barleys and malts

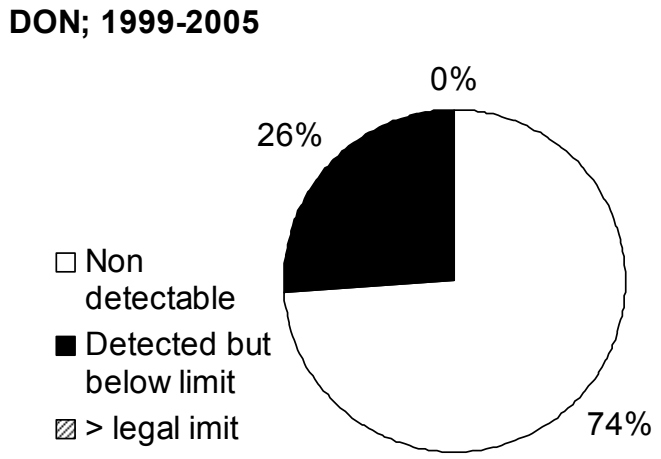


Figure 2. Occurrence of ochratoxin A in malting barleys and malts

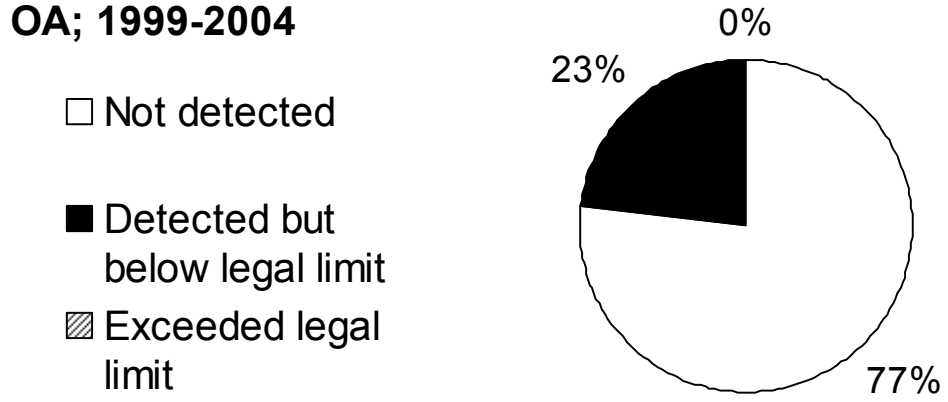
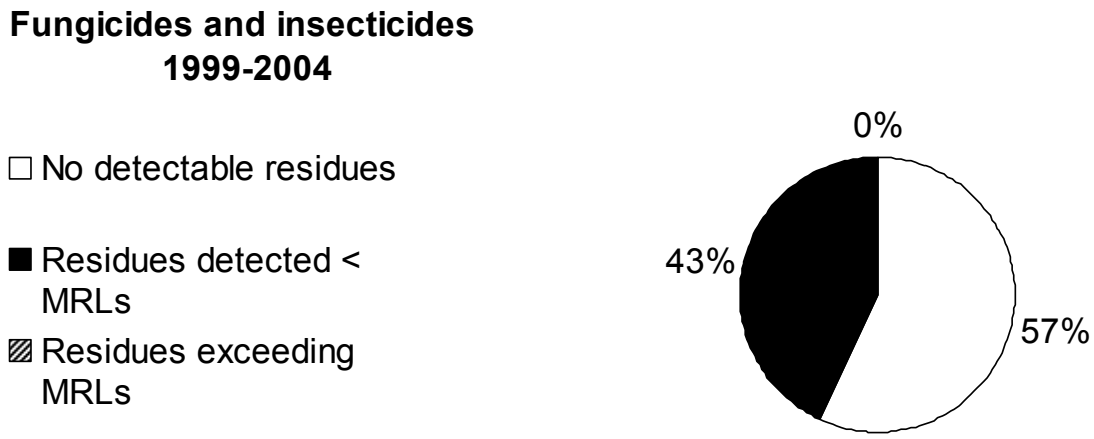


Figure 3. Residues of insecticides and fungicides in malts



However, some potential trends have been observed. The gradual increase in the incidence of deoxynivalenol which was observed during the initial project (which covered the harvest years 1999, 2000 and 2001) now appears to have stabilised but from the 2004 and 2005 harvest, there has been an apparent increase in the incidence of the more toxic trichothecenes, T2 and HT-2. Since these toxins have previously been rare in UK barleys, this may indicate a shift in mould species. Further surveillance will be necessary to confirm if this is real, and whether it is sustained.

The data obtained has been communicated back to the malting industry and other levy payers via the MAGB's web site, but has, with the permission of the HGCA, also been provided to governmental bodies such as the UK's Food Standards Agency, the Pesticides Safety Directorate, and the European Commission, as appropriate.

SECTION 3. TECHNICAL DETAIL

1. BACKGROUND AND SCOPE OF PROJECT

Project 2279 was set up as a response to the increasing importance of issues relating to food safety in the marketplace for cereals, both in order to comply with an increasing burden of legislation, and to satisfy customer demands, both for entry into new markets, and in existing markets, for maintaining a competitive advantage over similar products from different sources. It took account of the continuing refinement of analytical methodology and instrumentation, using mass spectrometry together with new methods based on immunology (ELISA, immuno-affinity) and molecular biology (PCR), which together mean that traces of unwanted materials can be detected in foods at ever lower concentrations. In many cases these materials are naturally occurring or widespread in the environment, so that elimination is difficult if not impossible.

The formula established by Project 2279, involving "horizon scanning" for emerging issues, supported by regular surveillance of representative sample sets for established contaminants together with the flexibility to expand the analytical scope to encompass newly identified issues, emphasised the value of such surveillance data to the cereals industry. It has since been adopted by the milling industry and the animal feed industry. The current project has built on this established pattern and developed it further.

Scope of this project.

The scope of the current project was;-

- to continue to provide ongoing access to information on food safety and legislative issues which might impinge on the market acceptability of malting barley and malt in general and of UK-sourced malt in particular
- to draw the attention of the appropriate personnel in the malting industry or HGCA to any particularly urgent issues
- to carry out a risk assessment in order to identify those plant protection products most likely to leave detectable residues in UK barley and , where possible, to develop analytical methods for those chemicals
- to carry out regular surveillance for potential contaminants of barley and malt on two sets of barley and/or malt samples per year.
    - One set of freshly harvested barleys, collected in September-October
    - A second set collected in April-May, comprising barleys stored for at least 6 months after harvest and malts prepared from them.

- to identify any issues or trends in the incidence or concentration of these contaminants and bring them to the notice of the malting industry
- to carry out initial exploratory work, including method development where necessary, on any emerging issues which could pose a threat to wholesomeness or market acceptability of UK barley or malt

This project commenced on January 1st 2003 and finishes on 31st December 2005. It is envisaged that from January 2006 the surveillance work will be continued as part of a larger project including both milling wheat and animal feed, and carried out jointly by CCFRA and BRi.

2. METHODS

2.1 Information sources

As in Project 2279, BRi utilised its established system for scanning and collecting scientific and technical information published world-wide relating to malting and brewing. 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, 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
- TNO-BIBRA Toxicology and Regulatory News
- Codex web site and reports
- The FAO/WHO Joint Expert Committee on Food Additives and Contaminants (JECFA) web site and reports
- World Food Law Monthly
- US FDA's Center for Food Safety and Applied Nutrition web site
- Australia-New Zealand Food Standards Agency web site

2.2 Surveillance

2.2.1 Sample sets collected

Each year representative sets of samples were collected with the collaboration of the Maltsters Association of Great Britain (MAGB), whose members represent the majority of commercial maltsters in the UK.

- The first sample set was collected in September-October (except in the first year of the project when samples from the 2002 harvest were collected in January 2003) and consisted of about 20 freshly harvested barleys, drawn from all members of the MAGB, with the number of samples per company governed by its production volumes. The companies were instructed to take several sub-samples from the bulk and to mix them to produce the final sample submitted. These samples were analysed principally for *Fusarium* mycotoxins.
- The second sample, also drawn from all members of the MAGB, was collected in April-May and 10kg samples were taken according to the protocol set down in Directive 2002/26 for the official control of ochratoxin A in foods. The set comprised about 20 samples of malts made from barleys from the previous

year's harvest, stored for at least 6 months. From 2003, samples of both the barley and the resultant malt were collected.

Table 1. Summary of sample sets collected

(for further details see Appendix 1)

Harvest year	Date Collected	Barley (No. samples)	Malt (No. samples)
2002	**Jan 03**	**Yes (17)**	**No**
	May 03	**NO**	**Yes (26)**
2003	**Oct 03**	**Yes (17)**	**No**
	April 04	**Yes (19)**	**Yes (19)**
2004	**Oct 04**	**Yes (20)**	**No**
	April 05	**Yes (20)**	**Yes (20)**
2005	**Oct 05**	**Yes (18)**	

- **Speciality malts.**

In 2004, a set of speciality malts (including roast barley) was collected in order to monitor heat generated toxins (chloropropanols, acrylamide and furan)

2.2.2 Mycotoxin analyses.

- **Trichothecenes.**

DON, 3-acetyl-DON, 15-acetyl-DON, NIV, HT-2 toxin and T-2 toxin were analysed by 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 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 for each toxin was 5 µg/kg. BRi participates in FAPAS proficiency tests. Z scores are available on request.

- **Zearalenone.**

This was analysed 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.

- **Ochratoxin A.**

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

- **Aflatoxins.**

Aflatoxins B1, B2, G1 and G2 were analysed 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 0.1 µg/kg. BRi participates in FAPAS proficiency tests for aflatoxins and Z scores are available on request.

- **Citrinin**

Samples were milled and extracted with a dichloromethane/phosphoric acid mixture. The extract was cleaned up on a polyamide SPE column and eluted with methanol/formic acid. The eluate was concentrated and analysed by LC-MS-MS. The limit of quantification was 0.1 µg/kg. Results are reported corrected for recovery.

- **Cytochalasin**

Samples were milled and extracted by homogenisation with methanol. The extract was loaded onto a C18 SPE column and residues eluted with acetonitrile/water. The eluate was concentrated and analysed by LC-MS-MS. The limit of quantification was 0.5 µg/kg for 2003 harvest and 0.1 µg/kg for 2004 and subsequent harvests. Results are reported corrected for recovery.

2.2.3 Pesticides

- **Storage insecticides and late-acting fungicides**

A multi-residue pesticide analysis was developed at BRi 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 cyprodanil and some of the new strobiluron 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. The limit of quantification for each residue was 0.01 mg/kg. Results are reported uncorrected for recovery.

- **Growth regulators**

Samples were milled and extracted by homogenisation with methanol/water. The extract was loaded onto a C18 SPE column and residues eluted with buffer. The eluate was analysed by LC-MS-MS using the deuterated analogues of chlormequat and mepiquat as internal standards. The limit of quantification was 0.01 mg/kg. Results are reported corrected for recovery.

- **Glyphosate**

Analysis of residual glyphosate and its metabolite aminomethyl phosphonic acid in barley was carried out at BRi using an in-house method. The sample was ground and then shaken with ammonium hydroxide solution for 60 minutes. The resulting extract was centrifuged and a portion evaporated to dryness. The components of interest were derivatised with 9-fluorenylmethyl chloroformate and separated and quantified by HPLC using fluorescence detection. The limit of detection was 0.5 mg/kg.

2.2.4 Chloropropanols

3-Mono-chloro-propanediol (3-MCPD) was analysed using a method developed and validated by CSL (*Brereton et al, 2001)*. This method depends upon GC-mass spectrometry, following derivatisation with heptafluorobutyrylimidazole. BRi has UKAS accreditation for this method and participates in FAPAS proficiency tests for 3-MCPD. Z scores are available on request.

2.2.5 Acrylamide

This was analysed using a GC-MS method based on that developed by Castle and others at CSL, York *(Castle, 1993).* BRi participates in FAPAS proficiency tests for acrylamide and Z scores are available on request.

Highly processed samples (roast barley and malts with a colour greater than about 100°EBC) are widely recognised as presenting analytical difficulties and it was necessary to modify the Castle method to some extent for these samples.

2.2.6 Dioxins

Dioxins were extracted by Soxhlet extraction for 16 hours, using 20% acetone in hexane. The solvent was removed using a rotary evaporator and the residue dissolved in DMSO.

The isolated dioxins were then measured using ah-immunoassy purchased from Biosense Laboratories AS, Thormøhlensgt. 55, Bergen, N-5008, Norway

(http://www.biosense.com/comweb.asp?articleno=26&segment=3).

Mode of action.

In the body, dioxins react with a particular intracellular receptor (ah-receptor) which then binds to specific genes called the dioxin responsive elements (DREs). This binding causes specific changes in gene expression leading to changes in metabolism including altered growth patterns, oncogensis and other deleterious effects. These biological components have been isolated and used as a basis for the measurement of dioxins. Essentially the system mimics an enzyme immunoassay although it is not a typical ELISA. The dioxin responsive elements (which are DNA) are bound to a microtitre plate. The reaction mixture includes the ah-receptor as well as other components of the 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. The reaction must be calibrated using α-naphthalene and a number of positive and negative controls must also be included. **Results are reported on a weight basis, for total dioxins**. This differs from most instrumental methods, in which each dioxin species is measured separately, and then the weight of each species transformed to a WHO TEQ value, using a factor based on the toxicity of that species. The total dioxin content in terms of TEQ numbers appears to be a significantly lower value than the total dioxin weight as estimated by this kit.

Each kit costs approximately £1000. Although the microtitre plate contains 48 wells, many of these are taken up by controls and calibration standards. In addition, serial dilutions of each sample must be run. We were therefore only able to test 4 unknown samples. If the kit was being used routinely for a limited number of matrices, thus reducing the number of controls needed, and a pass/fail threshold had been identified, a maximum of 10 unknown samples could be analysed with each kit, giving a cost/assay of £100 compared with between £800 and £1000 per sample for traditional analysis.

3. RESULTS AND DISCUSSION

3.1. Surveillance

3.1.1. Mycotoxins

This project has covered surveillance for toxins from both field fungi (mainly *Fusarium spp.)* and storage fungi *(Penicillium verrucosum* and *Aspergillus flavus). Fusarium* species can give rise to a range of structurally related toxins known as trichothecenes, as well as the structurally unrelated oestrogenic toxin zearalenone and the fumonisin group of toxins. The profile of toxins formed depends both upon the mould species and the environmental conditions. Fumonisins mainly occur in maize and have not therefore been included in this project. Legal limits for deoxynivalenol (DON), the commonest of the trichothecenes, have just been introduced in the EU, and limits for other *Fusarium* toxins are being considered.
Penicillium verrucosum infections can give rise to ochratoxin A and also to citrinin, while *Aspergillus flavus* can give rise to aflatoxins. There are legal limits for both ochratoxin A and aflatoxins on cereals in the EU.
Analyses have been carried out on freshly harvested barleys and on stored barleys plus the malts made from them.

3.1.1.1. *Fusarium* toxins: trichothecenes

A. Freshly harvested barleys

Since this project commenced in January 2003, barleys harvested in 2002 were not sampled until January 2003. In subsequent years, freshly harvested barleys were collected in October.

Results are shown in Table 2 and Figure 1. The commonest trichothecene detected was deoxynivalenol (DON), which was detected in approximately one third of samples, although this varied from year to year, ranging from 6% in 2005 to 40% in 2004 (which was a wet year). Concentrations were always low, with the highest level detected being 71 µg/kg, compared with the EU legal limit for raw barley of 1250 µg/kg *(Regulation 856/2005).* The acetylated species, 3-acetyl-DON and 15-acetyl-DON, which can be formed from DON in living tissues under some circumstances, were not detected in these raw barleys.

Nivalenol (NIV), which was detected in approximately 10% of samples, was generally only found in samples which were also positive for DON, at concentrations significantly lower than for DON.
T-2 and HT-2 toxins are type A trichothecenes, which are significantly more toxic than DON, but generally rarer in the UK. Since HT-2 is a metabolite of T-2 and since a legal limit is expected to be set in the EU for the sum of

T-2 + HT-2 *(Reg 856/2005)* values here are also quoted as the sum of these two toxins. Neither toxin was detected in barleys from the 2002 and 2003 harvests, continuing the pattern found in malted barleys from 1999 to 2001 *(HGCA project 2279, final report)*. However, traces of HT-2 were detected in barleys from 2004 harvest, and in 2005 around one third of samples contained detectable T-2 or HT-2, at concentrations similar to those found for DON in previous years. This shift in incidence is displayed graphically in Figures 4A and 4B.

Table 2. Trichothecenes in freshly harvested barleys
(samples below the limit of detection are regarded as containing half that limit)

Mycotoxin *(Limit of quantification is 5 µg/kg for all toxins)*	**Harvest year**			
	2002 *N = 17*	**2003** *N = 17*	**2004** *N = 20*	**2005** *N = 18*
DON				
Mean (µg/kg)	5.3	7	10.8	3.3
Maximum (µg/kg)	14	26	71	16
% of samples > LOQ	35	35	40	6
NIV				
Mean (µg/kg)	3.1	2.8	3.5	2.8
Maximum (µg/kg)	9	7	16	8
% of samples > LOQ	12	6	15	6
HT-2 + T2				
Mean (µg/kg)	2.5	2.5	2.6	8.1
Maximum (µg/kg)	<5	<5	5	56
% of samples > LOQ	0	0	5	33
Others*				
Mean (µg/kg)	2.5	2.5	2.5	2.5
Maximum (µg/kg)	<5	<5	<5	<5
% of samples > LOQ	0	0	0	0

** In 2002, 2003 and 2004, this included 3-actyl-DON and 15-acetyl-DON*

In 2005, neosolaniol, diacetoxyscirpenol and fusarenon-X were included in addition to the acetylated DONs

Freshly harvested barleys from the 2005 harvest were also tested for the three additional *Fusarium* toxins, neosolaniol, fusarenon-X and diacetoxyscirpenol, since it has been suggested by EU Commissioners that these toxins could also occur in cereals in Western Europe and could survive processing. None of these toxins was detected in any of the samples.

Figure 4A. Concentrations and incidence of deoxynivalenol in barleys

(samples below the limit of detection are regarded as containing half that limit)

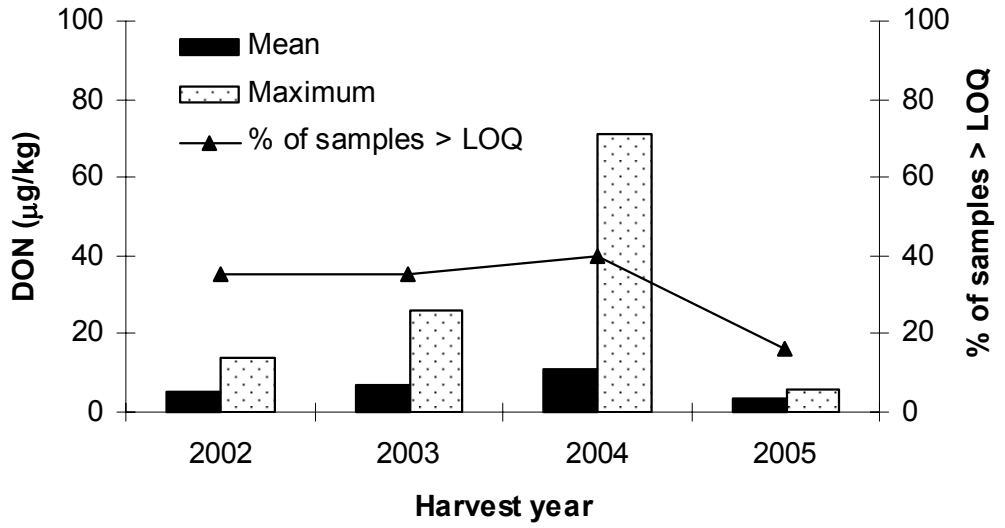
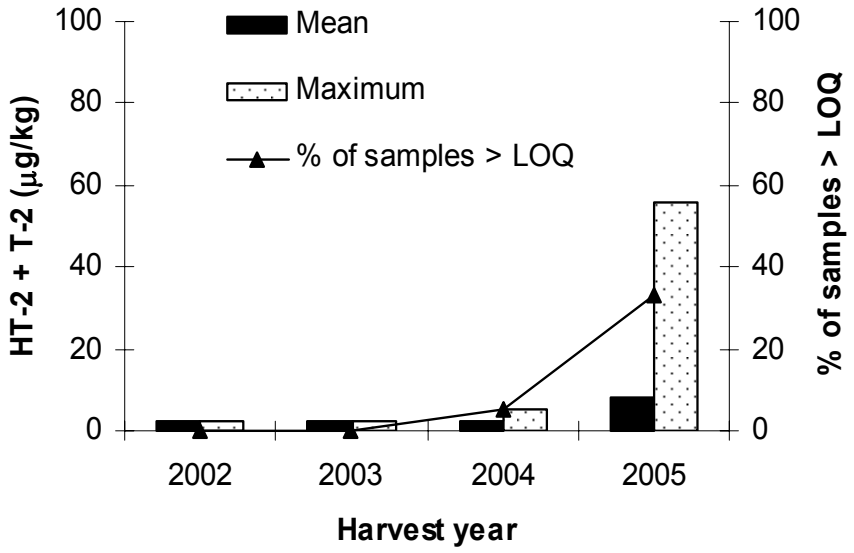


Figure 4B. Concentrations and incidence of T-2 and HT-2 in barleys



B. Stored barleys and malts prepared from them

The main reason for testing stored barleys was to monitor the occurrence of toxins such as ochratoxin A, which is produced by moulds such as *Penicillium verrucosum*. Such moulds can grow under relatively dry conditions and can therefore sometimes be found in stored cereals. However, the stored samples collected were also tested for *Fusarium* toxins. In 2002, samples of malts prepared from barleys from the 2002 harvest stored for approximately 6 months were collected according to the recommended protocol for official control in the EU *(Directive 2002/26/EC)*. (This requires defined number of sub-samples to be taken (depending upon the weight of the bulk being sampled) and combined to give an aggregate sample of at least 10kg. The entire 10kg is then milled finely and mixed before being sub-sampled for analysis). In subsequent years, both the barleys and the malts prepared from them were analysed in order to give some indication of the persistence of these toxins under commercial malting conditions.

Table 3. Trichothecene mycotoxins in stored barleys and malts prepared from them
(samples below the limit of detection are regarded as containing half that limit)

Toxin	Harvest year	Number of samples	Barley			Malt		
			Mean µg/kg	Max µg/kg	%>LOQ %	Mean µg/kg	Max µg/kg	%>LOQ %
DON	**2002**	26	Not done			8.1	56	42
	2003	19	5.8	13	10	6.1	27	5
	2004	20	6.9	28	40	4.0	17	20
3 + 15 acetyl DON	**2002**	26	Not done			4.9	31	15
	2003	19	2.5	<5	0	2.5	<5	0
	2004	20	2.5	<5	0	2.5	<5	0
NIV	**2002**	26	Not done			2.5	<5	0
	2003	19	3.4	11	3	2.5	<5	0
	2004	20	5.6	8	11	2.5	<5	0
T-2 + HT-2	**2002**	26	Not done			2.5	<5	0
	2003	19	2.5	<5	0	2.5	<5	0
	2004	20	5.7	64	5	3.3	14	6

Results are shown in Table 3. Values for stored barleys were in line with those obtained for freshly harvested samples from the same year, with DON, while being the commonest of the Fusarium mycotoxins detected, being present only at low levels. Acetylated DON species were detected in one year only (2002 harvest). T-2 and HT-2 were only detected in one barley and its corresponding malt from the 2004 harvest.

Both stored barleys and malts from the 2004 harvest were also analysed for other *Fusarium* mycotoxins (neosolaniol, fusarenon-X and diacetoxyscirpenol). None of these toxins was detected in any of the samples.

Both concentrations and incidence of mycotoxins were generally lower in malts than in the corresponding barleys. Although the number of samples involved is too small to be statistically significant, the results would support the widely held supposition that *Fusarium* moulds tend to die off in storage. Thus, although pre-formed toxins would still be present in the starting barleys, some of these would be washed out during steeping and the amount of inoculum present would generally be too low for substantial re-growth during malting.

3.1.1.2 Zearalenone

Although zearalenone (ZEA) is a mycotoxin also produced by similar *Fusarium* species to the trichothecenes, it is structurally unrelated to them. Its toxicity profile is also very different, in that while general toxicity is low, ZEA is potent oestrogen and can affect reproduction, especially for domesticated animals. Pigs are the most sensitive species, and guideline limits being suggested for zearalenone for animal diets for pigs are likely to be in within the range which could be found in cereals.

Results are shown in Table 4. In these samples, ZEA occurred only infrequently, and only at trace levels, well below those being suggested as guideline limits for animal feed materials. There were too few positive samples to give any reliable indication of whether the ZEA was derived from field infection or infection during storage, or whether it is likely to increase during the malting process.

Table 4. Zearalenone in barleys and malts
(Limit of quantification was 2 µg/kg for all samples)
(samples below the limit of detection are regarded as containing half that limit)

Toxin	Harvest year	Number of samples	Barley			Malt		
			Mean µg/kg	Max µg/kg	%>LOQ %	Mean µg/kg	Max µg/kg	% >LOQ %
Freshly harvested samples								
ZEA	**2002**	26	Not done					
	2003	19	Not done					
	2004	20	1.2	4	5			
	2005	18	1.0	<2	0			
Stored samples								
ZEA	**2002**	26	Not done			1.0	<2	0
	2003	19	1.9	12	2	1.4	4	3
	2004	20	1.4	8	5	1.0	<2	0

3.1.1.3 Aflatoxins

In the previous project (HGCA No 2279) samples were tested every year for aflatoxins. No positive samples were found (limit of quantification 0.1 µg/kg). In the first year of the current project, selected malts made from stored barleys harvested in 2002 were also tested for aflatoxins. No positive samples were detected.
In view of this continued record of negative results, regular monitoring for aflatoxins was discontinued.

3.1.1.4 Ochratoxin A

In the UK and Western Europe, Ochratoxin A (OA) is produced by a mould *Penicillium verrucosum* which can grow on stored cereals at moistures in excess of around 18-20 % (although the ambient temperature and incubation time also have an effect). In the EU there are legal limits of 5 µg/kg for OA on raw cereals and 3 µg/kg for processed cereals such as malt. This mould can also potentially grow and produce toxin during the

malting process. There are also proposals to introduce limits for OA in cereals for animal feed. Quality assurance both of raw materials and products for OA is thus a high priority for both maltsters and their customers.

All samples of stored barleys and the malts made from them were tested for OA. In some cases freshly harvested samples were also tested. Results are shown in Table 5.

The results suggest that, although OA is relatively common, being detected in some 15-20% of stored barleys, the actual levels remain well below the legal limits. As expected, incidence is frequently greater in stored barleys compared with freshly harvested ones. Incidence also tends to be higher in malts than in raw barleys, but actual concentrations remain comfortably below legal limits.

Table 5. Ochratoxin A in barleys and malts
(Limit of quantification was 0.1 µg/kg for all samples)
(samples below the limit of detection are regarded as containing half that limit)

Toxin	Harvest year	Number of samples	Barley			Malt		
			Mean µg/kg	Max µg/kg	%>LOQ %	Mean µg/kg	Max µg/kg	% >LOQ %
Freshly harvested samples								
OA	**2002**	26	Not done					
	2003	19	0.08	0.5	6			
	2004	20	0.17	2.3	15			
	2005	18	Not done					
Stored samples								
OA	**2002**	26	Not done			0.3	1.8	38
	2003	19	0.18	1.0	20	0.23	1.4	30
	2004	20	0.10	0.6	15	0.28	1.1	50

Effects of sample size

The results quoted above are all obtained from 10kg samples collected according to the official EU method. This is a very onerous regime, and in particular the requirement to mill the complete 10kg samples attracts significant extra costs. In 2002 an experiment was conducted to compare results from 10kg sample and from a smaller

(500g) sample. In each case, the number of sub-samples collected followed the EU official method, based on the weight of the bulk, and a total aggregate sample of 10.5 kg was collected. This was split into two, a 10kg portion and a 500g portion, and each portion milled separately before being sub-sampled for analysis. Results (Table 6)

Table 6. A comparison of OA analyses on 500g and 10kg samples

Sample size	Mean OA µg/kg	Maximum OA µg/kg	Incidence % samples > LOQ
500 g	0.26	2.38	35
10 kg	0.30	1.76	38

indicate that milling the entire 10kg sample did not significantly increase the detection of OA contamination, since the proportion of positive samples was only slightly higher than for the 500g sample. Likewise, mean concentrations were similar in each case. The biggest effect of milling the whole of the 10kg aggregate sample was to reduce maximum values for individual samples, due to the dilution effect. This experiment suggests that, provided the aggregate sample is collected properly, milling the entire 10kg sample does not improve detection rates. The most important factor in detecting heterogeneous contamination is the number of separate sub-samples collected.

3.1.1.5 Citrinin

Citrinin is produced by the same moulds as is OA, therefore could be expected to occur relatively frequently in stored cereals. However, it is rarely tested for. Thirteen samples of stored barleys from the 2004 harvest, together with 14 samples of malts were tested for citrinin. Detectable citrinin was found in only one sample of barley and in its accompanying malt, at concentrations of 3.0 µg/kg in the barley and 2.9 µg/kg. It is noteworthy that OA was not detected in this barley, although small amounts (0.5 µg/kg) were detected in the malt.

3.1.1.6 Cytochalasin

Cytochalasin is a mycotoxin produced by the mould *Aspergillus clavatus*., which has been isolated from several cereals including wheat, rice and barley *(Tanabe, 2003; Demain 1976)* . There are some reports of mould growth during the malting process *(Lopez-Diaz and Flannigan, 1997*) but in general the temperatures required for toxin formation are higher than would be normal in commercial production. There are some reports in the literature of livestock in the UK *(Gilmour, 1989)* and more recently in Brazil *(Loretti, 200)* suffering from mycotoxicosis

which were attributed to consumption of brewery, malting or distillery byproducts which had become visibly infected with *Aspergillus clavatus.* The nature of the actual material consumed is poorly defined in these papers, and no mycotoxin analyses were carried out. As a part of the current project, the stored barley and malt samples have been tested for cytochalasin.

Table 7. Occurrence of cytochalasin by harvest year

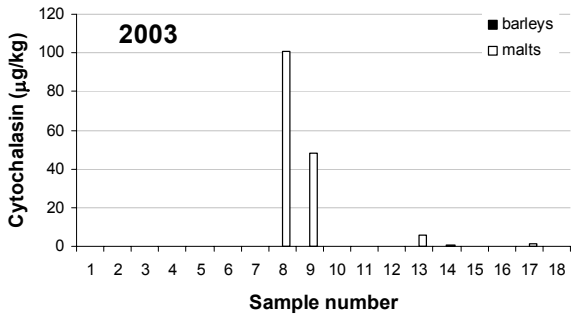
Toxin	LOD µg/kg	% of samples > LOD	Mean* µg/kg	Max µg/kg	Median µg/kg
Barley					
2002 harvest	ND	ND	ND	ND	ND
2003 harvest	0.5	0	0.25	<0.5	0.25
2004 harvest	0.1	10	0.09	0.4	0.05
Malt					
2002 harvest	0.5	16	35	750	0.25
2003 harvest	0.5	25	9	101	0.25
2004 harvest	0.1	15	5	89	0.05

*(ND = not done) * samples below the limit of detection are regarded as containing half that limit*

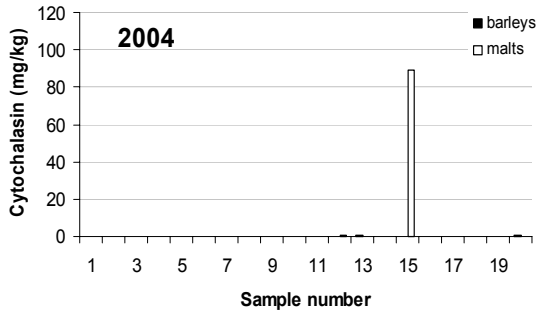
The results, shown in Table 7, indicate that traces of cytochalasin can occur in UK barleys but is rare in the raw grain. In some cases, however, the mould can grow during malting and in these cases detectable amounts of the toxin can be formed (see Figure 5, A and B). In general the levels formed are low. Mean values shown in Table 7 are skewed by higher concentrations in a few samples; consequently median values have also been calculated. These were below the limit of quantification in all sample sets. There is also a trend to reduced maximum concentrations over the three years of the study as companies have become aware of the problem and have taken steps to reduce infection.

Figure 5. Cytochalasin in stored barleys and malts made from them

A. 2003 harvest



B. 2004 harvest



3.1.2. Pesticides

In recent years there has been more concern amongst consumers over the presence of pesticide residues in foodstuffs, as evidence by the FSA's Pesticide Minimisation initiative. Although a large number of chemicals could legally be used on cereals, relatively few residues are routinely detected. In spite of this customer expectations often result in companies having to test for a large number of chemicals, only a few of which are actually used or likely to leave detectable residues in the grain. It was therefore decided to carry out a risk assessment in order to identify those plant protection products which were likely to leave residues in barley grain and which should therefore be included in due diligence monitoring.

The BBPA (British Beer and Pubs Association) / BRi list of Agrochemicals Accepted for use on brewing raw materials was used as a starting point. Each chemical was scored (where data was available) or the following factors:-

- Extent of use on barley in the UK (using the Pesticide Usage Survey Report 187 produced by CSL).
- Timing of use (taken from approval documents on PSD database)
- Solubility characteristics (from the British Crop Protection Council's Pesticide Manual)
- Partition coefficients (from the British Crop Protection Council's Pesticide Manual)
- Residues in supervised trials (BRi confidential data)
- Residues found in surveys (BRi confidential data; FSA surveillance)
- Malting trial data (Miyake 2002 and BRi confidential data)
- Brewing data (Miyake 1999 and BRi confidential data)

Scores were used to estimate the likelihood of residues occurring in barley, malt and beer, and thus to rank each pesticide as high, low or medium priority for residue monitoring in malt. A simplified version of the spreadsheet is given in Appendix 2 to this report.

Residues identified as high priority for including in surveillance exercises are shown in Table 8. In some cases, chemicals have been included because they are known to leave residues and are frequently detected in surveillance exercises. In other cases, particularly with some of the newer fungicides, there is little monitoring data available. Once more data has been accumulated it may be possible to eliminate these residues or reduce the priority.

The samples collected within this project have been tested using BRi's multi-residue pesticide residue method, which has been extended to include most of the insecticides and strobiluron fungicides identified. The growth regulators chlormequat and mepiquat, and the desiccant glyphosate are analysed by separate methods. A new multi-residue method to include the triazole fungicides is being developed. A summary of the residues sought is given in Table 9. Results are given in Table 10 (A and B).

Table 8. Pesticide residues identified as a top priority for surveillance.

Active ingredient	Type	Reason for listing
Azoxystrobin	Late fungicide	Applied post ear emergence ; potential for residues but no data available
Bifenthrin	Insecticide	Applied post-harvest
Chlormequat	Growth Regulator	Widely used ; very soluble can persist into beer
Chlorpyrifos-methyl	Insecticide	Post harvest ; widely used
Cyhalothrin	Insecticide	Widely used ; applied late
Cypermethrin	Insecticide	Frequently used ; applied late
Cyprodanil	Fungicide	Widely used ; applied post ear emergence
Deltamethrin	Insecticide	Applied late post ear emergence
Dichlorvos	Insecticide	Post harvest ; found on some imports
Diquat	Growth Regulator	Applied late ; not allowed by BBPA
Epoxiconazole	Fungicide	Late applications can affect fermentability
Ethephon (cerone)	Growth Regulator	Widely used ; very soluble
Famoxadone	Fungicide	Late application ; little survey data
Glyphosate	Dessicant	Used immediately before harvest. Residues can be relatively high
Lindane	Insecticide	No longer allowed but still detected
Malathion	Insecticide	Used post harvest
Mepiquat	Growth Regulator	Widely used ; very soluble
Picoxystrobin	Fungicide	Widely used, applied late ; little survey data
Trifloxystrobin	Fungicide	Widely used, applied late ; little survey data

Table 9. Pesticide residues sought in current project

Active ingredient	Limit Of Detection mg/kg	Legal limit (mg/kg)	Type	Method
Azoxystrobin	0.01	0.3	Late strobiluron fungicide	GC-MS multiresidue
Chlorpyrifos	0.01		Late insecticide	GC-MS multiresidue
Bifenthrin	0.01		Post harvest and field insecticide	GC-MS multiresidue
Chlorpyrifos-methyl	0.01		Post-harvest insecticide	GC-MS multiresidue
Chlormequat	0.01		Growth regulator	LC-MS method
Cypermethrin	0.01		Late field insecticide	GC-MS multiresidue
Cyprodanil	0.01		Late fungicide	GC-MS multiresidue
Deltamethrin	0.01		Field insecticide	GC-MS multiresidue
Diazinon	0.01			
Dichlorvos	0.01		Post harvest insecticide	GC-MS multiresidue
Etrimfos	0.01		Post harvest insecticide; now withdrawn	GC-MS multiresidue
Fenitrothion	0.01		Post harvest insecticide ; now withdrawn	GC-MS multiresidue
Fenvalerate	0.01		Late field insecticide;	GC-MS multiresidue
Glyphosate + AMPA	0.5		Pre-harvest dessicant + Major metabolit	In house method
Kresoxim-methyl	0.01		Strobiluron fungicide	GC-MS multiresidue
Lindane	0.01		Post harvest and field insecticide; withdrawn in UK	GC-MS multiresidue
Malathion	0.01		Post harvest insecticide	GC-MS multiresidue
Mepiquat	0.01		Growth regulator	LC-MS method
Methacrifos	0.01		Post harvest insecticide ; now withdrawn	GC-MS multiresidue
Pirimiphos-methyl	0.01		Post harvest insecticide; widely used	GC-MS multiresidue
Permethrin	0.01		Insecticide was ; used in cereal stores	GC-MS multiresidue
Trifloxystrobin	0.01		Late applied strobiluron fungicide	GC-MS multiresidue

The pesticides in Table 9, together with chlormequat, mepiquat and glyphosate, cover all the residues most frequently detected in cereals in the UK and France, and 10 of the 14 residues most frequently reported in cereals throughout the EU. All samples from 2002 and 2003 harvests were analysed by the multi-residue method. The only residues which were detected were pirimiphos-methyl and the fungicide cyprodanil, both at very low levels.

Surveillance was reduced in 2004, with only 8 samples being tested. Again, only pirimiphos-methyl and cyprodanil were detected, at low levels.

Table 10. Pesticide residues detected in malts from stored barleys

A. Multiresidue method

Harvest year	Number of samples	Residues detected	MRL mg/kg	Incidence (%>LOQ)	Mean mg/kg	Max mg/kg
2002	26	Pirimiphos-methyl	5	42	0.07	0.76
2003	18	Pirimiphos-methyl	5	39	0.04	0.22
		Cyprodanil	Not yet set	10	0.006	0.02
2004	8	Pirimiphos-methyl	5	25	0.05	0.38
		Cyprodanil	Not yet set	25	0.009	0.03

B. Growth regulators

Harvest year	Number of samples	Residues detected	MRL mg/kg	Incidence (%>LOQ)	Mean mg/kg	Max mg/kg
2002	26	Chlormequat	2	100	0.14	0.51
		Mepiquat	Not set	65	0.07	0.24
2003						
Barley	19	Chlormequat	2	100	0.12	0.33
Barley	19	Mepiquat	Not set	74	0.04	0.22
Malt	19	Chlormequat	2	100	0.09	0.25
Malt	19	Mepiquat	Not set	56	0.03	0.15
2004						
Barley		Chlormequat	2		Not done	
Barley		Mepiquat	Not set		Not done	
Malt	20	Chlormequat	2	95	0.07	0.23
Malt	20	Mepiquat	Not set	90	0.04	0.19

Residues of growth regulators were found in the majority of both barley and malt samples tested, but concentrations were very low. The highest concentration of chlormequat detected was approximately 25% of the EU MRL. No MRLs have yet been set for mepiquat in the EU or the UK. However, the highest concentration detected in this survey was well below the MRL for mepiquat in barley in Germany and France (1 mg/kg).

C. Glyphosate

Selected samples of malts from stored barleys from the 2002 harvest were tested for residues of glyphosate and its major metabolite AMPA. Not residues were detected above the limit of quantification of 0.5 mg/kg.

3.1.3 Heat-generated toxins

Evidence that chloropropanols, and in particular 3-monochloropropanediol (3-MCPD), could be formed when cereals were heated strongly first emerged in 1997. Since then detailed studies at BRi have established that 3-MCPD is formed during the production of speciality malts *(Reports to FAC, 1998, 1999).* Acrylamide in foods emerged as in issue in May 2002. Initial information suggested that formation occurred when starch-rich foods were heated above 110° C and research subsequently published by three separate groups *(Stadler et al, Mottram et al)* further indicated an association with Maillard reactions. This meant that it was highly likely that speciality malts could contain acrylamide.

As apart of the initial HGCA project (project 2279) speciality malts were tested for both acrylamide and 3-MCPD. The results indicated that heat treated cereals such as roasted products and speciality malts could contain high levels of both. Further investigations, funded by the UK's Food Standards Agency, has confirmed the occurrence of heat generated toxins in heat treated cereals and has established the factors which affect formation. The studies have also confirmed that levels of both 3-MCPD and acrylamide in final consumer products such as beer are low, partially due to dilution but also to losses during the brewing process. Consequently, beer is not regarded as a significant source of heat generated toxins in the diet. However, we consider it prudent to continue monitoring levels in brewing materials such as speciality malts and roasted cereals in order to confirm that acceptable levels in final products are not exceeded.

A set of commercial speciality products, representing all manufacturers in the UK, was collected in 2004 and tested for 3-MCPD and acrylamide. Some samples were also used to test for another Maillard-related heat generated toxin, furan, which was identified as a potential problem in some foods in 2004. Results are shown in Table 11.

Results for acrylamide and 3-MCPD are in line with those found earlier and remain generally within industry guidelines. The analyses also confirm that, as suspected, furan can be formed during the roasting stage of speciality product manufacture, and levels can be very high. Further studies outside the remit of this project indicate that levels in consumer products such as beer are low, probably due to significant losses during the brewing process.

Table 11. Heat generated toxins in speciality malts and roasted barley

Type	Colour Range EBC	Acrylamide Mean µg/kg	Acrylamide Range µg/kg	3-MCPD Mean µg/kg	3-MCPD Range µg/kg	Furan Range µg/kg
Cara malt	26 – 50	428	255 - 821	11	<10 - 21	100- 110
Crystal malts	26 – 400	542	229 - 905	24	<10 - 63	200 - 1200
Amber malt	16 – 100	600	152 - 956	66	<10 - 186	ND
Roasted malts and barley (brown, black, chocolate)	150 - 1520	68	12 - 243	284	197 - 408	2000 - 3000

ND = not done

3.1.4 Dioxins

Dioxins are highly toxic materials and can accumulate in the food chain consequently limits are set for dioxins in animal feeds. Dioxins can be formed in trace quantities during the combustion of organic materials, thus they could potentially be formed during the heating stages of malting (kilning and/or roasting for speciality products. Barley drying has also been suggested as a potential source. The previous project (*HGCA No 2279)* looked at the dioxin content of barleys and found that levels were too low to be significant. However, neither kilned or roasted products nor malting by-products such as malt culms were investigated. One of the major problems is the very high cost of dioxin analysis, especially since many of the products concerned (such as malt culms) do not have a high intrinsic value. An investigation into the feasibility of using cheaper screening kits was therefore included in the current project.

Details of the kit and the principles upon which it was based are given in the Methods section. Calibration was carried out using α-naphthalene

We used the kit to analyse four samples which had received varying degrees of heat treatment during normal processing (a white malt, a roasted malt, malt culms and brewery spent grains) for dioxins. Results are obtained as the weight of 2,3,7,8-TCDD equivalents, in pg/g.

Figure 6. Standard curve obtained using alpha-naphthalene as the standard reagent:

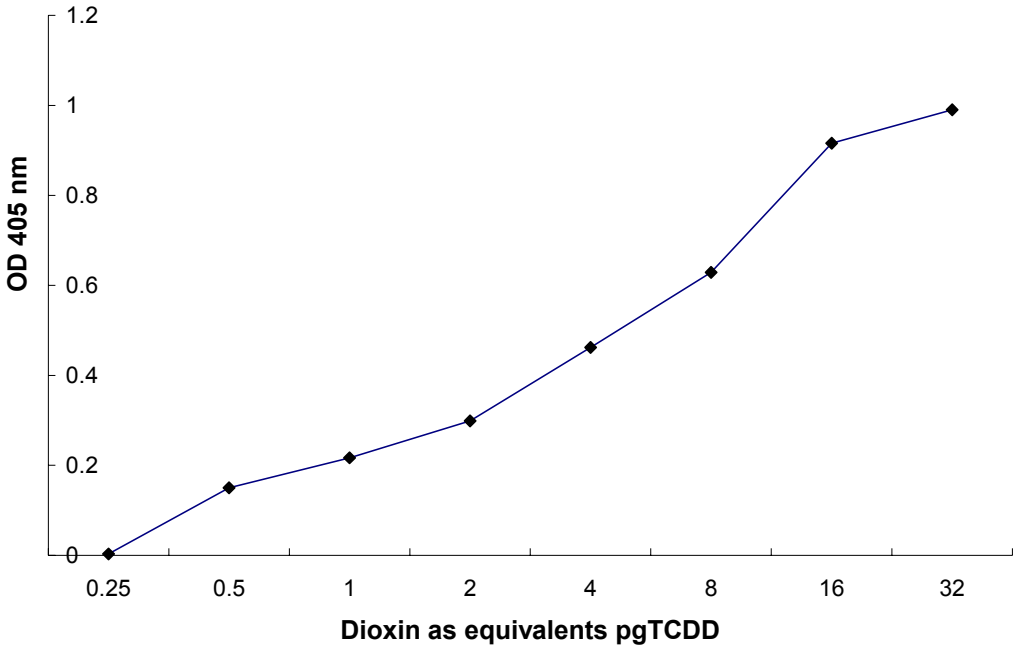


Table 12. Dioxins in some processed cereal samples

Sample	Dioxin content (pg equivalents of TCDD / g
Ale malt	37
Black malt	60
Malt culms	48
Brewery spent grains	42

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 the 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 screening 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.

3.2 Information utilisation

3.2.1 Communications to levy payers

The information gathered from the sources listed under "Methods" is stored in databases at BRi for ease of access. It has been made available to Maltsters, other levy payers, and their customers by a number of routes, which include:-

- Electronic databases on food safety issues, agrochemicals and EU/UK food safety and environmental law. These can be accessed by MAGB members via the BRi web site. Alternatively, information held on the databases can be accessed by BRi staff to answer any relevant queries from HGCA levy payers.
- Information on pesticides which are acceptable for use on malting barley for brewing is available to levy payers via a list produced by the BRi in collaboration with the British Beer and Pubs Association (BBPA). This list can be accessed by levy payers via the MAGB web site (www.ukmalt.com).
- An electronic alert network is being operated to advise maltsters and the HGCA of any incidents etc which could pose problems for the industry
- A regular monthly food safety bulletin, also electronic, has been instigated. This contains details of any new or draft food legislation or other food safety developments of interest to the malting and brewing industries
- Results of food safety surveillance, for example for mycotoxins and heavy metals, are being made available to levy payers through the MAGB web site.

As well as being made available to levy payers, the information gathered was also used to inform decisions on where to target analytical surveillance.

3.2.2 Dissemination of data to other organisations.

- Results of the monitoring for pesticide residues has been communicated to the FSA as apart of their pesticide residue minimisation initiative
- The mycotoxin monitoring data forms part of an EU-wide monitoring exercise for malt and malting barley co-ordinated by Euromalt (the Trade Association for malting companies in the EU) and has been used in negotiations with brewing industry customers and with the European Commission.
- The ochratoxin data has also been used in negotiations with the European Commission, to demonstrate the effectiveness of the controls on OA levels in barley and malt.

3.2.3 Issues which have arisen during the lifetime of this project

- **Fusarium toxins**

The European Commission set legal limits for DON in cereals for food use in 2005. All indications from the information obtained from the current project, and from the one preceding it, are that UK malting barleys and malts are well below this limit. Although the number of samples tested each year is relatively small, over three hundred samples have now been tested. The cumulative results for all samples (including both barleys and malts, freshly harvested and stored), shown in Figure 7, indicate that none of the samples contained more than 10% of the new limit, even during the wet years of 2001 and 2004. Although in 2001 it appeared that there might be a trend for increasing DON concentrations, this has not been substantiated, with DON contents in the 2005 being as low, if not low, than in 1999.

Figure 7. DON in malting barleys and malts; 1999-2005
samples below the limit of detection are regarded as containing half that limit)

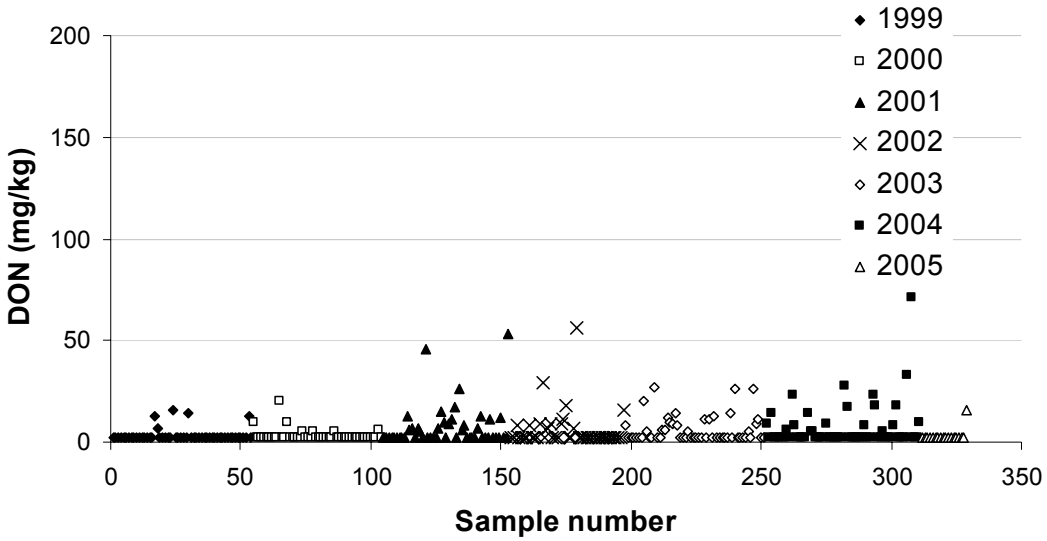
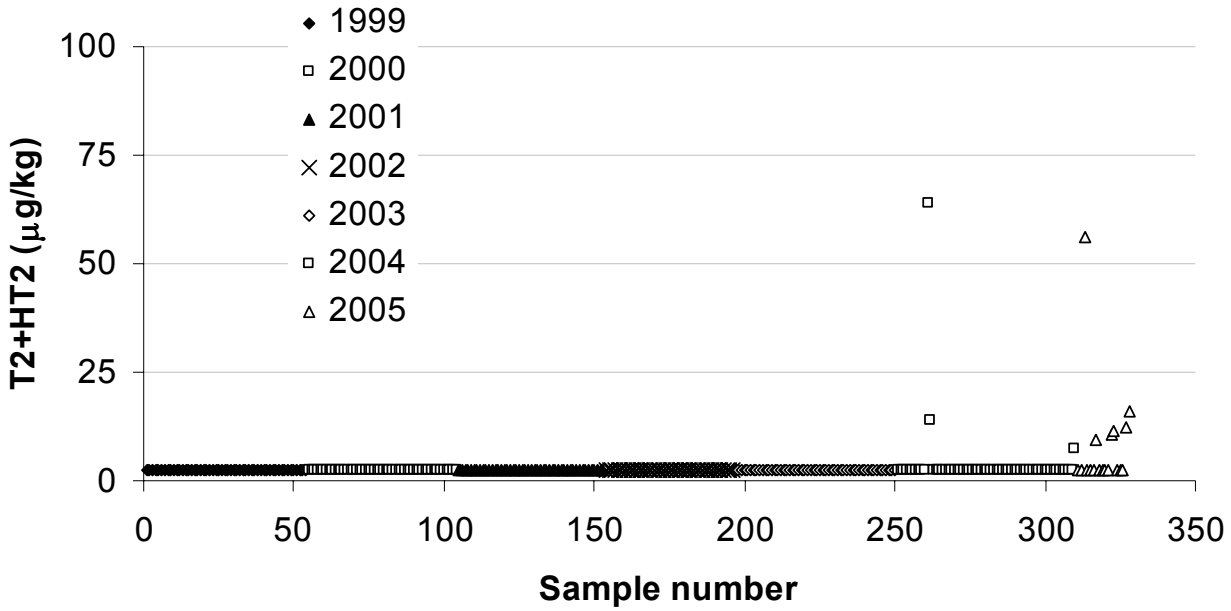


Figure 8. T-2 and HT-2 toxins in malting barleys and malts; 1999-2005
(samples below the limit of detection are regarded as containing half that limit)



The Commission has expressed its intention to set limits also for T-2 + HT-2 in cereals and is actively seeking data to help identify suitable levels. The accumulated results from these projects (shown in Figure 8) indicate that while these toxins have been rare or absent in UK grown barleys, incidence has increased sharply in 2005. This situation will need to be monitored closely to establish whether this is an isolated incident or whether it is the beginning of a trend.

- **Limits for animal feed materials**

Co-products from the cereal processing industries, including malting, milling and brewing, are frequently used as materials for animal feed. Legal limits are already set for aflatoxins and ochratoxin in such materials, and either limits or guideline values are being considered for the main *Fusarium* toxins, including DON and ZEA. The generally low levels of mycotoxins detected in malting barleys and malt suggest that co-products from the malting process should easily comply with any new limits for feed materials. However, substantially lower limits are likely to be recommended for particularly vulnerable species such as pigs. Additional monitoring would to advised since it is known that toxin levels are likely to be higher in fines and screenings.

- **“Emerging” mycotoxins**

The Commission has also expressed interest in the extent to which other mycotoxins produced by common moulds occurred in European cereals and could persist into food products. Toxins identified included neosolaniol, fusarenon-X and diacetoxyscirpenol, all of which are produced by *Fusaria* species, and citrinin, which is produced by *Penicillium verrucosum*. The behaviour of these moulds and/or toxins during malting and brewing is being investigated in a separate project (HGCA RD-2004-3005). In addition, analysis for these toxins has been included in the surveillance carried out within this current project and results to date suggest that these toxins are rare in UK grown cereals.

- **Cytochalasin**

There has been little concern in the food industry about the mycotoxin cytochalasin, produced by *Aspergillus clavatus*. However occasional literature reports have associated outbreaks of mycotoxicoses in domestic animals with consumption of visibly mouldy batches of cereal by-products, such as malt culms or brewery spent grains, and have sometimes cited the toxin cytochalasin as a cause. The current investigation was initiated in order to determine whether or not this mycotoxin did occur in UK barley and malts. Results indicate that the toxin is rare in raw barleys in the UK. However, in some circumstances detectable amounts of cytochalasin can be found in the grain after processing. One possibility is that inadequate temperature control during malting may be a relevant factor. There are no current legal limits for cytochalasin and it is unlikely that the amounts present in these malts were sufficient to have any toxic significance.

- **Furan**

It has been recognised for some time that substituted furans (alkyl furans, di- and tetr-hydrofurans and furanones) occur in cooked foods, where they contribute to the overall flavour. However, in 2004, the US’s Food and Drugs Administration (FDA) detected furan at higher concentrations than expected in some bottled and jarred foods. Subsequent investigations revealed that furan, like chloropropanols and acrylamide, is a heat generated toxin formed in association with Maillard reactions when foodstuffs, particularly carbohydrate rich materials, are heated. Our studies have confirmed that furan is found at relatively high levels in roasted cereals, both raw and malted. However, the levels which survive into final products such as beer are very low, suggesting that significant losses may occur during brewing. This is being investigated further but is not within the remit of this project. The wider implications of furan in the diet are still unclear and are being considered by the World Health Organisation and the European Food Safety Authority.

- **Pesticide minimisation**

In 2003 the FSA announced its intention to implement a plan to minimise pesticide residues in certain food crops, including cereals. The FSA felt that awareness of pesticide residues was lower in the cereals sector than in other areas of the food industry, such as fresh produce. A meeting of cereal producer and processor groups, including representatives from the HGCA, was held in October 2003. During this meeting monitoring data from the different sectors, including the maltings sector, was presented.

4. CONCLUSIONS AND RECOMMENDATIONS

This project has continued the pattern set by Project 2279 for an ongoing identification of food safety and regulatory information relevant to the malting barley / malt industry, supported by the capability for a rapid and appropriate response to that information. Data collected through this project has continued to be submitted to regulatory bodies such as the Food Standards Agency, the Pesticides Safety Directorate and the European Commission. Together with the initiation of similar projects for the milling wheat and animal feed sectors, this provides evidence of an ongoing need for such targeted surveillance programmes for UK cereals and their products. Such programmes must be sufficiently flexible to respond rapidly to issues which emerge unexpectedly.

The three year surveillance programme has established a database of information relating to the status of UK-produced malted barley over a wide range of food safety-related parameters, particularly mycotoxins, but also including pesticides, dioxins and heat generated toxins

The data obtained during the initial project confirmed that UK malts are unlikely to be a significant dietary source of aflatoxins, heavy metals, arsenic or radionuclides, consequently these analytes have not been included in the current surveillance. The current project has focussed on the ongoing issues of mycotoxins, pesticide residues and heat generated toxins.

The surveillance has confirmed that UK mating barleys and malts are generally very low in these contaminants. However, a number of specific issues have also become apparent as a result of the surveillance exercise.

4.1. Mycotoxins

Cumulative data confirms that deoxynivalenol (DON) is generally low in UK malting barleys and UK malt, with concentrations being well below the new legal limits introduced in the EU. However, the increase in the incidence of the more toxic trichothecenes, T-2 and HT-2, observed in the last year observed over the three years' surveillance, it is recommended that monitoring should continue to order to establish whether this is the beginnings of a continuing trend and if so, what the causes might be. It is expected that the amalgamation of this project with the related projects in the animal feed and milling wheat sectors will facilitate a pooling of information from the different cereal types.

Ochratoxin A

Occurrence of ochratoxin in foodstuffs, and particularly in cereals and cereal products remains a major issue for the European Commission. In general levels of OA in UK malts are low, and compare favourably with average

for continental Europe. The monitoring within the current project has used samples collected according to the Official sampling and analysis protocols set by the Commission *(Directive 2002/26/EC)*. This has allowed the UK results to be incorporated into an EU-wide monitoring exercise co-ordinated by Euromalt (the Trade Association for malting companies in the EU). The results of this exercise have been presented to EU Commissioners with the aim of confirming the effectiveness of existing EU controls for OA in cereals, indicating that there is no need for further legislation. Given the generally favourable results obtained for OA in UK samples it may be possible to reduce the number of samples tested, within the restraints of the Euromalt collaborative tests.

Zearalenone

The surveillance carried out to date within this project suggests that zearalenone occurs sporadically in some malting barleys samples, although levels are generally low. It is **recommended** that surveillance for zearalenone should continue at the existing level and should be increased in animal feed materials in order to compile a more comprehensive data set to inform any future negotiations on regulation of this mycotoxin.

4.2 Pesticides

It is apparent that pesticide residues remain a key issue, both in home and overseas markets. This concern is not restricted merely to chemicals applied post-harvest. The risk assessment exercise carried out within this project has allowed residue monitoring to be carried out on a more focussed basis. It is recommended that attention should be directed towards continuing to develop residue monitoring screens which will allow monitoring for all the residues in the high priority group. When more incidence data is available, for example for the newer fungicides, it may be possible to reduce monitoring.

4.3 Dioxins

Cereals have not been identified as a high risk for dioxins in human or animal diets. However, if further monitoring is required, the kit evaluated within this project could potentially be used as a screening test. Further work would be necessary in order to calibrate the kit in terms of WHO TEQ units or to determine an acceptable threshold.

4.4 Heat-generated toxins

In view of the relatively high levels of heat generated toxins found in heated cereals it is recommended that annual monitoring should continue. However, it is unlikely that legal limits will be set in the foreseeable future for acrylamide or furan in foods. Legal limits already exist on the EU and some other countries for chloropropanols in certain foods, but these do not apply to cereals. The possibility of extending controls to other foodstuffs has been raised at EU and Codex level but no decisions have yet been taken.

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APPENDIX 1

Details of main annual barley and malt sample sets collected for surveillance

Harvest year	Date collected	Number of samples	Number of companies	Total tonnage represented	Varieties included	Growing areas covered
2002	Jan 2003	17 barleys	10	78K tonnes	Decanter, Golden Promise, Maris Otter, Optic, Pearl	England: Dorset, East Anglia, Hampshire, Norfolk, South East, Yorkshire Scotland: Highlands, Lothians, Borders.
2002	May 2003	26 malts	12	Minimum of 12K tonnes	Decanter, Diamond, Chalice, Fanfare, Gleam, Halcyon, Maris Otter, Optic, Pearl	England: Essex, Hampshire, Lincs, Norfolk, Suffolk, South West, Yorks. Scotland: Aberdeen, Tayside, Borders
2003	Oct 2003	17 barleys	11	88K tonnes	Cellar, Chalice, Maris Otter, Optic, Pearl	England: East Anglia, Norfolk, South West, Yorks. Scotland: Black Isle, Borders, Lothians,
	April 2004	19 barleys 19 malts	10 10	No data	Cellar, Chalice, Decanter, Fanfare, Golden Promise, Optic, Pearl	England: East Anglia, Hampshire, Yorks. Scotland: Aberdeen, Fife
2004	Oct 2004	20 barleys	9	96K tonnes	Cocktail, Decanter, Maris Otter, Optic, Pearl	England: Dorset, East Anglia, Norfolk, Shropshire, Yorks. Scotland: Borders, Fife, Lothians, North East.
2004	April 2005	20 barleys 20 malts	10	No data	Decanter, Fanfare, Maris Otter, Optic, Pearl	England: East Anglia, South, Yorks. Scotland: Aberdeen, Borders, Fife, Lothians, Moray
2005	Oct 2005	18 barleys	9	50 K tonnes	Cocktail, Decanter, Optic, Pearl, Troon	England: East Anglia, Oxford, Norfolk, Shrop., Yorks. Scotland: Borders, Lothians, North East.

APPENDIX 2 Risk Assessment of pesticides used on barley

BBPA / BRi List 2005

Assessment of risk of residues occurring in raw barley/malted barley

Chemical	Extent of use on UK barley	Latest time of use	Solubility	Hydrophobicity	Residues found in barley?	Analysis of barley/malt needed	EU MRL mg/kg
Post harvest							
Al phosphide	occasional	post harvest	moderate	low	not found	no	0.1
Bifenthrin	new introduction	post harvest	very low	high	yes	yes	0.5
Chlorpyrifos-methyl	occasional	post harvest	low	high	yes	yes	3
Diatomaceous earth	new introduction	post harvest	no	not applic	no	no	
Mg phosphide	rare	post harvest	moderate	low	not found	no	0.1
Malathion	new introduction	post harvest	slightly	moderate	yes	yes	8
Pirimiphos-methyl	frequent	post harvest	slightly	high	yes	yes	5
Field insecticides							
Alpha-cypermethrin	occasional	late	very low	v high	possible	yes	0.2
Bifenthrin	rare	early	very low	v high	yes	yes	0.5
Chlorpyrifos	rare		low	high	possible	some	0.2
Cypermethrin	frequent	late	very low	v high	possible	yes	0.2

Chemical	Extent of use on UK barley	Latest time of use	Solubility	Hydrophobicity	Residues found in barley?	Analysis of barley/malt needed	EU MRL mg/kg
Deltamethrin	frequent	late	very low	high	possible	yes	1
Esfenvalerate	frequent	late	very low	v high	possible	some	0.2
Imidacloprid	rare	seed treatment only	high	low	not reported	no	
Lambda-cyhalothrin	frequent	late	very low	v high	possible	yes	0.05
Methiocarb	rare	early	moderate	moderate	not found	no	
Pirimicarb	rare	late	high	low	not reported	some	
Tau-fluvalinate	rare	late	very low	high	possible	some	
Tefluthrin	rare	seed treatment only	very low	v high	not found	no	
zeta-cypermethrin	frequent	late	very low	low	possible	yes	0.2
Fungicides							
Azoxystrobin	very frequent	late	moderate	moderate	possible	some	0.3
Boscalid	new introduction	pre ear emergence only	low	moderate	unlikely	limited	UK 0.02
Bromuconazole	frequent	late	high	moderate	possible	some	
Carbendazim	frequent	generally early	moderate	low	not found	no	0.1
Carboxin	frequent	seed treatment only	high	moderate	very unlikely	no	
Chlorothalonil	occasionally	before earing	low	moderate		no	0.1
Cyproconazole	v frequent	Earing	high	moderate		some	
Cyprodinil	v frequent	before earing	moderate	moderate/high	yes	yes	
Epoxiconazole	v frequent	pre ear emergence only	low	moderate	possible	some	

Chemical	Extent of use on UK barley	Latest time of use	Solubility	Hydrophobicity	Residues found in barley?	Analysis of barley/malt needed	EU MRL mg/kg
Famoxadone	frequent	Earing	very low	high	possible	yes	0.2
Fenbuconazole	rare	Earing	very low	moderate		no	
Fenpropidin	occasionally	Earing	moderate	moderate		no	
Fenpropimorph	v frequent	Earing	low	moderate	possible	some	0.5
Fludioxonil	frequent	seed treatment only,	low	high		no	
Fluoxastrobin	new introduction	start of earing	very low	moderate	close to LOD	limited	UK 0.5
Fluquinconazole	rare	Earing	low	moderate	possible xs treatment	some	
Flusilazole	frequent	late	moderate	moderate/high	only	some	
Flutriafol	rare	late	high	moderare		no	
Fuberidazole	frequent	seed treatment only,	high	moderate		no	
Guazatine	frequent	seed treatment only,	high	low		no	
Imazalil	rare	seed treatment only,	high	moderate		no	0.02
Iprodione	not used	late	low	moderate		no	1
Kresoxim-methyl	frequent	earing	low	moderate	not found	some	0.05
Mancozeb	rare	late	low	low		total EBDC	2
Maneb	rare	late	low	low		total EBDC	2
Metconazole	frequent	late	low	moderate	not found	no	
Metrafenone	new introduction		very low	high	possible	some	UK 0.5

Chemical	Extent of use on UK barley		Latest time of use	Solubility	Hydrophobicity	Residues found in barley?	Analysis of barley/malt needed
Picoxystrobin	v frequent	earing	low	moderate/high	possible	some	
Prochloraz	rare	late	moderate	high		some	1
Propiconazole	rare	late	high	moderate/high		no	0.05
Prothioconazole	new introduction	start of earing	low/moderate	moerate/high	not found	no	UK 0.05
Pyraclostrobin	frequent	pre ear emergence	low	moderate/high	possible	some	UK 0.2
Quinoxyfen	frequent	Earing	very low	high	close to LOD	no	
Silthiofam	rare	seed treatment	very low	moderate	not found	no	
Spiroxamine	frequent	Earing	high	moderate	unlikely	no	0.3
Sulphur	occasionally	late	low	low	not found	no	
Tebuconazole	mainly as seed treatment	seed treatment	moderate	moderate/high	unlikely	no	
Thiabendazole	rare	seed treatment	high	moderate	unlikely	no	0.05
Thiram	frequent	seed treatment	low	moderate	unlikely	no	
Triadimenol	rare	late	moderate	moderate	unlikely	no	0.2
Triazoxide	frequent	seed treatment	moderate	moderate	unlikely	no	
Trifloxystrobin	v frequent	late	very low	high	possible	some	UK 0.5
Triticonazole	rare	seed treatment	low	moderate	unlikely	no	
Growth Regulators							
Chlormequat	v frequent	early	high	low	yes	yes	2
Ethephon	v frequent	early	high	low	possible	yes	0.5

Chemical	Extent of use on UK barley	Latest time of use	Solubility	Hydrophobicity	Residues found in barley?	Analysis of barley/malt needed	
Imazaquin	rare	early	high	low		some	
Mepiquat chloride	v frequent	early	high	low	yes	yes	
Trinexapac-ethyl	v frequent	early	high	low	close to LOD	no	
Dessicants							
Diquat	no	before harvest	high	low		yes	10
Glufosinate-ammonium	rare	before harvest	high	low	possible	some weather	
Glyphosate	occasionally	before harvest	high	low	yes	depending	20
Herbicides							
Amidosulfuron	frequent	Earing	low	low		yes	
Bifenox	rare	early	very low	high		no	
Bromoxynil	frequent	early	high	moderate	not found	no	
Carfentrazone-ethyl	rare	early	very low	moderate	not found	no	
Chlorotoluron	winter only	early	moderate	moderate		no	
Clopyralid	rare	early	high	low	possible	some	
2,4-D	rare	early	high	moderate		no	0.05
2,4-DB	rare	early	moderate	moderate		no	0.05

Chemical	Extent of use on UK barley	Latest time of use	Solubility	Hydrophobicity	Residues found in barley?	Analysis of barley/malt needed	Chemical
Dicamba	frequent	early	high	low	no data	some?	
Dichlorprop-P	rare	early	high	low		no	
Diclofop-methyl	frequent	early	very low	high		no	
Diflufenican	winter only	early	very low	high	not found	no	
Fenoxaprop-P-ethyl	frequent	early	very low	high		no	
Flamprop-M-isopropyl	rare	early	low	moderate/high		no	
Florasulam	rare	early	high	low		no	0.01
Flufenacet	winter only	early	moderate	moderate	not found	no	UK 0.05
Fluroxypyr	v frequent	early	high	low	possible	some	0.1
Flurtamone	winter only	early	low	high	not found	no	
Glufosinate-ammonium	rare	see dessicants	high	low	possible	some	
Glyphosate	v frequent	see dessicants	high	low	yes	yes	20
Imazamethabenz-methyl	rare	early	high	low		no	
Iodosulfuron-methyl	rare	early	high	low	not found	no	UK 0.05
Ioxynil	v frequent	early	high	low	not found	no	
Isoproturon	v frequent	early	moderate	moderate	not found	no	0.05
Linuron	rare	very early	moderate	moderate		no	0.05
MCPA	frequent	early	high	moderate		no	

Chemical	Extent of use on UK barley	Latest time of use	Solubility	Hydrophobicity	Residues found in barley?	Analysis of barley/malt needed	
MCPB	rare	early	high	moderate			
Mecoprop-P	v frequent	early	high	moderate		no	
Methabenzthiazuron	rare	early	moderate	moderate		no	
Metosulam	rare	early	high	moderate		no	
Metsulfuron-methyl	v frequent	early	high	low		no	0.05
Pendimethalin	winter only	early	very low	v high		no	0.05
Picolinafen	rare	early	very low	v high		no	0.05
Thifensulfuron-methyl	v frequent	early	high	low		no	0.05
Tralkoxydim	v frequent	early	low	moderate		no	
Tri-allate	frequent	early	low	high		no	
Triasulfuron	rare	early	moderate	low/moderate		no	0.05
Tribenuron-methyl	frequent	early	moderate	low		no	
Trifluralin	winter only	early	very low	high		no	
Miscellaneous						no	
Metaldehyde	yes	not applied to crop,	high	low		no	no
Thiodicarb	yes	not applied to crop,	moderate			no	no

KEY

Parameter	Source	Interpretation
Extent of use in UK;	CSL survey 2002	v frequent = >100,000 ha: rare= < 1000ha
Timing of use	PSD pesticide database	v early= before emergence; early =before flowering; earing =up umtil end of ear emergence; late = after anthesis
Solubility	BCPC Pesticide Manual	very low= <1mg/l; low=<10mg/l: moderate <100mg/l: high=>100mg/l
Hydrophobicity	BCPC Pesticide Manual	based on partition coefficient (Kow)
Residues	BRi confidential data; published residue surveys	*Not found* means no residues in supervised trials, generally using an excess application rate *Possible* means residues just above LOD in supervised trials; *Yes* means residues found in BRi or published surveys *Not reported* means no supervised trial data available, but residues have not been reported in any surveys *No* means timing or mode of application means residues very unlikely