



RESEARCH REVIEW No. 49

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

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MALTING BARLEY AND UK MALT**

by

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EXECUTIVE SUMMARY

This project has established a system by which 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, can be routinely scanned for items relevant to the UK malting industry. Information identified as relevant is communicated to the industry and is also used to guide a detailed surveillance programme for UK-grown malting barley and malt.

This surveillance programme deals only with food safety and health-related parameters. It is not concerned with quality –related parameters such as variety, nitrogen or Hot Water Extract. Representative sample sets of UK malting barley or UK- produced malts, as appropriate, have been collected for each year of the project, with the collaboration of the MAGB. These sample sets have been used for analysis of a wide range of safety-related parameters which are currently an issue in the UK, the EU or in the major export markets for UK malt. These parameters include mycotoxins, pesticides, heavy metals, dioxins, nitrosamines, radionuclides, chloropropanols and (in maltings byproducts destined for animal feed) *Salmonella*. New analytical methods suitable for cereal matrices have been developed as necessary.

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

Some samples have also been analysed for the processing contaminant acrylamide, which was only identified in foodstuffs as recently as May in the final year of this project. As a consequence, funding has now been obtained from the Food Standards Agency for further studies on the mechanism of formation of acrylamide in heated cereals.

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 mycotoxins such as ochratoxin A and deoxynivalenol, and pesticide residues, all of which are recognised as potential risks for cereals. In several cases the current surveillance programme has confirmed that concentrations of some contaminants (including arsenic and non-volatile nitrosamines) which were an issue in the past are now much lower than the values published in the literature.

Some issues arose during the duration of the project. Apart from acrylamide, these were mainly related to legislative priorities in the EU, and are described in the text of the report.

Certain other issues have been identified as a direct result of the surveillance carried out. These include potential trends (for example increases in deoxynivalenol, albeit from a very low baseline), analytical problems (variations in replicate analyses within and between different laboratories for lead) and changes in the priorities for pesticide testing.

It is therefore considered to be important for the malting industry that this type of surveillance is continued, and in particular that it continues to be linked to information gathering, so that it is able to adapt rapidly as new issues emerge or older ones gain new prominence.

Some malts have also been analysed for the vitamin folate, which is now thought to be important in protecting against cardiovascular disease and some cancers. These tests indicate that malted cereals could provide a significant dietary source of folate and separate funding has been obtained from the HGCA to explore this further.

1. BACKGROUND AND SCOPE OF PROJECT

Over the past decade, issues relating to food safety and the public's perception of wholesomeness have become increasingly important for all food products. As a consequence, the food safety and wholesomeness of food ingredients, including cereals, have become a major factor in the marketplace, both for entry into new markets, and also in existing markets, for maintaining a competitive advantage over similar products from different sources. At the same time, 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), means that trace 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.

Coincidentally, we have seen a number of widely publicised food-related incidents, including BSE in beef and benzene in carbonated drinks in the UK, dioxins in pork and milk products from Belgium, contamination of foods with pesticides in Japan and tainted coca-cola in Belgium and France. Such incidents, together with the continuing controversy about GM crops, have combined to leave the general public in many countries widely distrustful of their food supply. In an attempt to counter this suspicion, the governments of several countries have re-organised their management of food safety issues and, in many cases, have increased the amount of food safety-related legislation. This is especially true in Europe, where the number of food- and food crop- related directives and regulations has burgeoned in the past few years, and, with the setting up of a new European Food Safety Authority, is not likely to diminish in the short term.

Against this background, it becomes increasingly important for manufacturers even in the cereals sector, which has a long history of wholesome food use, to have access to up-to-date scientific, medical, agronomic and technical information which underlies food safety trends and emerging issues. Analytical information, including surveillance data for both recognised and newly identified contaminants, is also essential. This is especially important for sectors like the UK malting industry, which serves a substantial export market. The value of such surveillance data is very significantly enhanced if it can be co-ordinated centrally by an independent body such as the BRi. This allows individual results to be placed in context with those from the rest of the UK industry, and, where available, with published results from other studies both in the UK and elsewhere. Central co-ordination also facilitates the identification of possible implications for subsequent processing (for example, during brewing) and for consumers.

Another factor which should not be overlooked is the increasing public interest in healthy foods and in the potential for micro-nutrients in foods to protect against

common illnesses such as cancer and cardiovascular disease. The importance of cereal-based foods as a cheap and widely available source of both the major nutrients and of trace nutrients deserves to be more widely known.

Scope of this project.

The aim of the current project was four-fold;-

- to provide access to continuing and regularly updated 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 regular surveillance for a number of materials which were already recognised as potential contaminants of barley and malt, and to identify any issues, such as trends in the concentration of these contaminants
- 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
- to carry out initial exploratory work to identify components of barley or malt which might confer specific health benefits and to suggest where further research was required.

This project commenced on January 1st 2000 and finished on 31st December 2002.

2. METHODS

2.1 Information sources

BRi has an 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
- The Pesticides Monitor
- HMSO web site
- The Official Journal of the European Commission (L and C series)
- The EU's Scientific Committee for Foods, Opinions and Reports
- 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 a representative set of samples of barley malt was collected with the collaboration of the Maltsters Association of Great Britain (MAGB), whose members represent the majority of commercial maltsters in the UK. The sample set consisted of about 50 malts, 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. The malts, which included lager, ale and distilling types, were collected in March – April each year, and were prepared from barleys from the previous year's harvest. These barleys would therefore have been in store for approximately 6 months. The original barleys used were drawn from all the major malting barley growing areas of the UK, and included all IOB recognised malting varieties. A summary of malt sample sets is given in Table 1 (see Appendix).

Additional sample sets were also collected as described below.

- **Adjuncts.**

In year 1, a small set of adjuncts (that is, sources of carbohydrate which may be used by brewers as an alternative to or in addition to malted barley) were

collected for comparison of mycotoxin content. This set consisted of 2 samples of maize, 4 of wheat (3 raw and 1 malted) and one maize syrup. An adjunct set was also collected in year 2; this comprised 10 samples of maize (including 2 of maize starch), and one of torrefied wheat.

- **Raw barley.**

In year 1, a small set of barleys from the 1999 harvest was collected for dioxin analysis. These samples were composites of several bulks of commercial malting barleys, blended to give 3 mixtures, each representative of one of the main growing areas for malting barley in the UK (East Anglia, Borders and Central Scotland).

- **Speciality malts.**

In year 3, a set of 11 speciality malts (including roast barley) was collected for chloropropanol and acrylamide analysis.

- **Malting co-products.**

In year 3, a small set of 3 samples of malting co-products (2 malt pellets and 1 of malt rootlets) was collected for analysis of *Salmonella*.

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*). The mycotoxins were extracted using acetonitrile/water, partially purified using trichothecene clean-up columns, then derivatised and analysed by GC-mass spectrometry. BRi is currently engaged in obtaining UKAS accreditation for this method and 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. BRi participates in FAPAS proficiency tests for zearalenone and Z scores are available on request.

- **Fumonisin**

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

- **Ochratoxin A.**

This was analysed by HPLC with fluorescence detection, following extraction and clean-up with immuno-affinity columns (*Baxter, Slaiding and Kelly, 2001*). 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. BRi participates in FAPAS proficiency tests for aflatoxins and Z scores are available on request.

2.2.3 Pesticides

Multi-residue pesticide analysis was carried out by Eclipse laboratories, a UKAS accredited laboratory. A list of the residues sought and their limits of detection is given in Table 6. Analysis of chlormequat was carried out at CSL, York, using an LCMS method. 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 Heavy metals

Heavy metal analysis was carried out by Eclipse Laboratories, a UKAS accredited laboratory, and by CCFRA, which has UKAS accreditation for heavy metal analysis in cereal matrices.

2.2.5 Nitrosamines

Apparent total N-nitroso compounds (ATNC) were measured by a method developed and published by CSL (*Massey et al, 1990*). This depends upon chemiluminescence detection of nitric oxide released by chemical denitrication. BRi has UKAS accreditation for this method.

2.2.6 Chloropropanols

3-Mono-chloro-propanediol (3-MCPD) was analysed using a method developed and validated by CSL (*CSL Report FD 97/95*). 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.

2.2.7 Acrylamide

This was analysed using a GC-MS method based on that developed by Castle and others at CSL, York (*L. Castle et al*). 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. Performance of the modified method was acceptable but analytical error with duplicate samples was significant, and it is evident that further method development is still desirable.

2.2.8 Dioxins

Analysis for dioxins and dioxin-like PCBs was carried out by CSL, York.

2.2.9 Salmonella

Salmonella was measured in malting co-products by the standard BSI method (*BS EN ISO 6579:2002*). Samples (25g) were incubated in buffered peptone water at 37°C for 16-20 hours. Aliquots of the liquid were then transferred to nutrient broths (Selenite cysteine broth and Rappaport Vassiliadis broth) and incubated for 24 hours at 37°C and 42°C respectively for further enrichment. Aliquots of these growth media were then tested using Brilliant Green and XLD agar plates. If suspect colonies appeared, further confirmatory tests were applied, including growth on Triple Sugar Iron Agar and Lysine Iron Agar plates, API 20e identification strips and Oxoid latex agglutination tests.

3.2.10 Folates in barley and malts.

Malting was carried out on a 300g scale, in a glass jar. Malting barley was steeped at 16°C using a programme of 8 hours wet, 16 hours air rest and 24 hours wet. It was then allowed to germinate at 16°C for 4 days, with samples of the green malt being withdrawn daily and dried in a forced draught oven for 8 hours at 45°C followed by 16 hours at 65°C.

Folates were analysed by a microbiological assay which detects total folate, that is, all the different naturally occurring forms of folate are included. An extract of the cereal was prepared using a trienzyme method based on that of Pfeiffer (*Pfeiffer et al, 1997*). An aliquot of the extract was inoculated with a standard amount of a *Lactobacillus* species which can only grow in the presence of folate. The mixture was incubated overnight, then the extent of cell growth was estimated by measuring the optical density, which is calibrated using samples grown in solutions of known folate concentration. This method has been validated internationally by the EU-funded project "Folate; from food to functionality and optimal health".

3.2.11. Radioactivity

Three composite samples from the 2000 harvest set were tested for gamma-ray emitting radionuclides. Each composite sample was obtained by mixing together equal quantities of four malts from the same geographical area, to give

three composite samples representing the main malting barley growing areas of East Anglia, Scotland and Yorkshire. The analysis was carried out by the National Radiological Protection Board of Scotland, using a high resolution gamma-ray spectrometer.

4. RESULTS AND DISCUSSION

4.1 Information utilisation

4.1.1. 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 levy payers directed to BRi.
- 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.

4.1.2. Dissemination of data to other organisations.

- Results of the monitoring for pesticide residues has been communicated to PSD for inclusion in their 2001 Annual Report.
- Results of tests for dioxins were communicated to FSA for use in negotiations with the European Commission (see 4.1.3. below).
- Data from the Ochratoxin A testing were presented to the European Commission at the meetings of the Mycotoxin Forum in 2001 and 2003.
- Data for *Fusarium* toxins were communicated to the FSA for use in their study of the prevalence of these toxins in the UK diet.
- Data on the preliminary studies into acrylamide formation in heated cereals have been communicated to the FSA and funding has been obtained from the FSA for a more detailed investigation in this area. This work is expected to commence early in 2003.

4.1.3 Issues which arose during the lifetime of this project

- **Dioxins;** In year 1(2000), following an incident in Belgium where animal feed became contaminated with dioxins and resulted in the contamination of pork and milk, the EU moved to introduce legislation on dioxins in both human food and animal feed. Little information was available on dioxin levels in UK cereals in general and in malting barley in particular. A set of three composite

samples of malting barley were therefore collected (see section 3.2.1) for dioxin analysis (see section 4.2.8) and the results communicated to the FSA for use in negotiations with the Commission. It was subsequently agreed that cereals did not make a significant contribution to the dietary intake of dioxins and the draft limit was withdrawn from the legislation. However, a limit for animal feeding-stuffs has been imposed (*Council Directive 2001/102/EC*, which may necessitate gathering data on dioxins in malting co-products in future).

- **Deoxynivalenol**; a number of sources, including:
 1. estimates of dietary intake in Nordic countries (*Eriksen & Alexander, 1998*)
 2. estimates of dietary intake in the Netherlands (*Pieters et al, 1998*) and
 3. the SCF's review of toxicity (*SCF Opinion, 1999*)

have suggested that in Europe levels of deoxynivalenol (DON) in the diet are close to exceeding the SCF's Tolerable Daily intake of 1 µg/kg body weight. Subsequently, the Commission has imposed "Action levels" of 750 µg/kg DON for raw cereals and 500 µg/kg for processed cereals, and it is very likely that these figures (or similar ones) will become legal limits in the relatively near future. In 1999 an HGCA project report indicated that the prevalence of *Fusarium* infections in UK wheat was increasing significantly (*HGCA project Report PRC207*). Current levels of trichothecenes are generally relatively low in the UK, but there is some concern that agronomic practices could affect this by changing the prevalence of toxigenic versus non-toxigenic *Fusaria* and related mould species. (*HGCA Sept 2001*). Subsequent HGCA projects have surveyed levels of *Fusarium* toxins in UK cereals, but these surveys have tended to concentrate on wheat, and for the barleys which were included, samples have been categorised as "malting barley" on variety alone, regardless of whether they were of the quality needed for malting. A priority for this project was therefore to collect a substantial data set relating to concentrations of the *Fusarium* toxins, particularly DON, in commercial malt. Malt was analysed rather than barley since it is recognised that there is a potential for additional mould growth during the malting process. 3-acetyl-DON, which is said to be prevalent in Europe (*SCF Opinion, 1999*) was also analysed from the start of the project. At a later stage, 15-acetyl-DON (prevalent in North America) was also included, since there is little information about the potential for inter-conversion of these related species. (See Section 4.2.1 for results).

- **Ochratoxin A**: after considerable discussion, legislation to control ochratoxin A (OA) in cereals was introduced by the EU in March 2002. This regulation sets a lower limit (3 µg/kg) for processed cereals than for raw cereals (5 µg/kg), which is a problem for the malting industry since processing to produce malt does not reduce levels of OA and indeed can increase them. More recently there has been a proposal by the Commission to introduce a lower limit of 0.2 µg/kg for OA in cereal-based foods for infants and young children (*Draft amendment to Regulation 466/2002*). This very low limit could

potentially pose a problem for the cereals and malting industry, and therefore surveillance of OA in malts was increased with the most recent malt set. The Commission is still considering whether further legislation is required.

- **Zearalenone;** this *Fusarium* mycotoxin is less acutely toxic than DON but there is some concern about its oestrogenic potential, and the SCF has advised a Tolerable Daily Intake of only 0.2 µg/kg body weight (*Opinion of the Scientific Committee, June 2000.*). There is as yet no EU limit for zearalenone in any foodstuffs, but limits of 50 µg/kg have been proposed by Germany for zearalenone in cereals for food use.
- **Fumonisin:** the fumonisin mycotoxins FB₁ and FB₂ are most commonly produced by the mould *Fusarium verticilloides* (formerly known as *Fusarium moniliforme*) which is widespread, but mainly infects maize. The EU's SCF has recently reviewed the toxicity data available for fumonisins and has allocated a Tolerable Daily Intake of 2µg/kg body weight for FB₁ (*SCF Opinion, Oct 2000*). In the USA, the FDA has recommended limits of between 2 and 4 mg/kg for total fumonisins in various maize products (*US Center for Food Safety and Applied Nutrition, June 6, 2000*). This compares with a TDI of 1µg/kg body weight for DON. There is as yet only limited data for dietary exposure to fumonisins in Europe, but maize-based food products have been found to be widely contaminated with these mycotoxins, and there have been some reports of detection in beers in north America (*Hlywka and Bullerman*). As a consequence the BBPA (British beer and Pubs, the UK Trade Association for the brewing sector) has recently commissioned a small survey of fumonisins and other mycotoxins in beers sold in the UK market. A selection of malts from the year 3 set (2001 harvest) were therefore tested for the presence of fumonisins.
- **Pesticides;** at the start of this project the pesticides considered to present the most risk for cereals were the insecticides which are authorised for use post-harvest and which can leave significant residues in the grain. Analysis of residues of these insecticides was included in the multi-residue screen of 112 chemicals utilised during this project. However, as a result of the Commission's review of existing pesticides, a large number of older pesticides are soon to be withdrawn in the EU, and very few post-harvest pesticides are still available for use. In 2000 the UK's Pesticides Safety Directorate published a survey of pesticide residues in foods, including beer. This survey drew attention to the potential for water-soluble pesticides to survive processing and persist into the final product. At the same time a number of pesticide contamination incidents in imported foodstuffs in Japan heightened the awareness of pesticide residues in this key export market. As a result, customers are increasingly becoming concerned with residues of pre- as well as post- harvest pesticides. Consequently the multi-residue screen has been extended to include specific water soluble chemicals such as chlormequat and glyphosate, which are more likely to survive processing

and lead to residues occurring in beer. It is also recommended that a new, risk-based approach to pesticide analysis should be adopted in the future.

- **Heavy metals**

Legislation was introduced in 2001 in the EU setting limits of 0.2 mg/kg and 0.1 mg/kg for lead and cadmium respectively in barley (which would include malted barley). (*Commission Regulation 466/2001*). Prior to the introduction of this legislation, lead and cadmium levels were surveyed over three years by the MAGB. With raw barley samples taken from the 1996 and 1997 harvests, a proportion of the sample set, (up to about 28%) exceeded 0.2 mg/kg. However, with the 1998 sample set, all samples were well below the proposed limit. This was in line with expectations, since environmental lead is predicted to fall following the introduction and of unleaded petrol. An HGCA-funded study carried out by Rothamsted also found that the concentration of lead in an overwhelming majority of barley samples grown at 2 sites in the UK were well below the EU limit, with less than 1% exceeding the new limit (*McGrath*). However, the MAGB surveillance experienced some problems with inter-laboratory variations. It is also necessary to eliminate the possibility of lead pick-up during commercial harvesting, handling and distribution, which would not be apparent with samples collected on the small scale directly from the field, as happened in the Rothamsted study. It was therefore considered necessary to continue surveillance during the three years of this project. At the same time, the testing was extended to include other metals for which legal limits have not yet been set (copper, zinc and the metalloid arsenic), in order to build up a database for future reference. Malted barley was tested rather than raw barley, since this is closer to the end product and thus more relevant for consumers, but processing is not likely to affect heavy metal content materially, other than removing any soil residues which might contaminate the grain. The moisture content of malt is lower than that of raw grain. The EU limit is set for wet weight; taking this to be 14.5% moisture, and that of malt to be 4%, on a dry weight basis the limits would be 0.225 mg/kg for lead and 0.11 mg/kg for cadmium.

Samples were analysed by the same contract laboratory as was used for the 1996 MAGB survey.

- **Beneficial ingredients in malt; folates**

During the late 1990s evidence emerged from clinical studies that ;

- (1) high levels of homocysteine in blood serum might be a risk factor for heart disease,
 - (2) dietary folates were associated with lower levels of serum homocysteine, and
 - (3) beer was a significant dietary source of folates for many people
- (*Ubbink et al, 1998; Mayer et al, 2001*).

A large EU-funded project involving 13 research groups from eight countries, including the IFR and BRi from the UK, was set up to investigate these observations further, and to examine the contribution of various foods,

including beer (the BRi input). It is recognised that cereals are a major dietary source of folates, and it was therefore likely that the folates in beer were derived from malt. The effect of the malting process was uncertain, although it was quite probable that it resulted in an increase in folate. It was therefore decided to carry out a preliminary study into the effect of malting on folate in barley as a part of the current (*HGCA project 2366*).

- **Acrylamide**

In the final year of this project, in May 2002, Swedish researchers published results which suggested that heating of starch-rich foods could give rise to relatively high levels of acrylamide, which is a recognised animal carcinogen (*Tareke, et al, 2002*). The seriousness of this potential threat to human health is apparent from the speed with which national and international bodies across the world have responded, setting up their own research programmes and food surveillance. So far most of the public attention has focussed on potato-derived foods such as chips and crisps. However, it is clear that there is also a risk of acrylamide formation in the manufacture of some cereal-based foods, which are also starch-rich and may be subjected to strong heating during processing. Steps were therefore taken at BRi to include within this project some preliminary work to establish the extent of the potential threat which acrylamide poses to malt based foods.

4.2. Surveillance

4.2.1. Mycotoxins

This project has covered surveillance for toxins from both field fungi (mainly *Fusarium spp.*) and storage fungi (*Penicillium verrucosum* and *Aspergillus flavus*). Analyses have been carried out on malts rather than barleys because it is recognised that there is a potential for mould growth during the malting, thus it is possible for malts to contain higher concentrations of mycotoxins than the original barleys.

- ***Fusarium* toxins: trichothecenes**

All of the malts from each of the 3 main sample sets were analysed for a range of trichothecenes, including DON, 3-acetyl-DON, NIV (Nivalenol), the more toxic T-2 and HT-2 toxins and (in the third year) 15-acetyl-DON. Results were compared with those for the adjunct samples (wheat and maize).

Full details of the results are displayed in Table 2 A and B, (see Appendix). These demonstrated that trichothecene levels in UK barley malts are currently relatively low. In particular, they were significantly lower than the levels found in a survey of grain from the 1999 UK harvest (*HGCA, July 2000*). In the 1999 HGCA study only 29% of barley samples and 23% of samples designated as intended for malting, contained no detectable deoxynivalenol (DON). This compares with the current project, which found that 91% of malts made with barleys from the same year contained no detectable DON. Again, the 1999 HGCA study found that approximately 25% of the barleys contained more than 50 µg/kg of DON, whilst in the current study, no malts from the same year did so. Although the HGCA –funded study looked at barleys, while the present study was concerned with malts, there is no evidence that the malting process is likely to reduce mycotoxin concentrations, and indeed is more likely to increase them, since the germination phase of malting can allow further mould growth. It is more probable that the malting industry's stringent intake checks for mould infection means that barleys accepted for commercial malting are likely to contain lower mycotoxin levels than barleys overall, even those of malting varieties.

The highest concentration of DON over the 3 years was only 53 µg/kg, about one tenth of the EU's recommended "action level" of 500µg/kg for processed cereals. Only a few samples contained detectable acetylated DON, and concentrations were low, confirming that there was no significant acetylation during processing, and no "hidden" reserves of DON. NIV (which is more toxic than DON) was detected less frequently than DON, but the concentrations, although still relatively low, tended to be slightly higher than those of DON. The most toxic trichothecenes, T-2 and HT-2, were not detected in any of the malts.

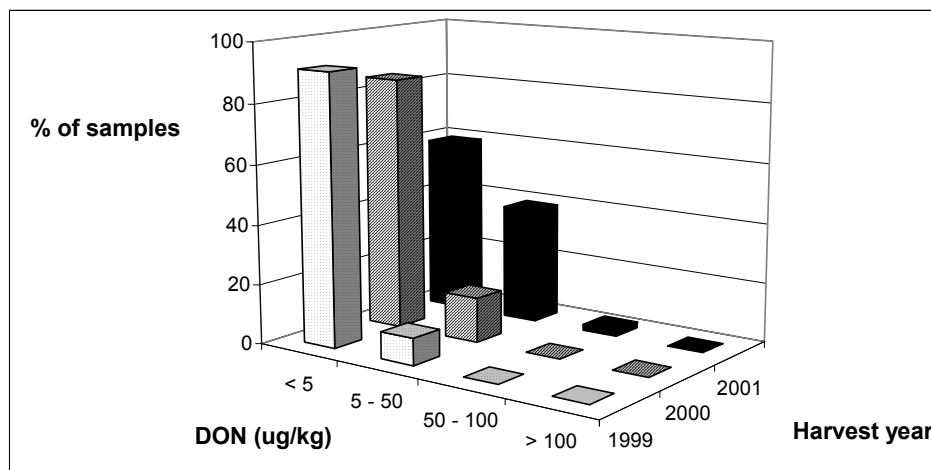
The adjuncts tended to be more frequently contaminated, and at higher concentrations, than did the malts, although only a few samples were analysed, particularly for wheat. All the maize samples contained high concentrations of

DON together with somewhat lower concentrations of NIV. Many also contained acetylated DON and a few also contained HT2 and T2 toxins. It is probable that these maize samples were not harvested in the UK, although they were obtained from UK brewing companies.

In spite of the low levels of contamination of the malts, there was a marked trend towards increased infection over the 3 harvests. The number of samples containing no detectable DON fell from 91% of samples in the 1999 harvest to only 59% in the 2001 harvest (see Figure 1).

Given that the 2001 harvest was very wet in the UK, it is not possible from 3 years' data to be certain whether the increases are the beginnings of a long term trend or whether they are an anomaly due to climatic conditions. No such trend was apparent with NIV.

Figure 1. Trends in the occurrence of DON from 1999 to 2001



- ***Fusarium* toxins: zearalenone.**

A sub-set of malts from each year's harvest was analysed for zearalenone. This mycotoxin was detected only sporadically; concentrations were generally low but on one occasion approached the limit proposed in Germany for cereals. No trend was apparent. Results are shown in Table 3.

After consultation with the MAGB and the HGCA, these results for all trichothecenes and zearalenone were made available to the UK's Food Standards Agency, for their survey of *Fusarium* mycotoxins in foodstuffs.

Table 3. Zearalenone in UK malted barleys

Harvest Year	Number of Samples	LOD (µg/kg)	Number of samples < LOD (%)	Zearalenone content (µg/kg)	
				Mean* (µg/kg)	Maximum (µg/kg)
1999	14	5	13 (93)	4.7	33
2000	9	5	8 (89)	6.6	40
2001	8	5	8 (100)	2.5	2.5

* Mean is calculated assuming that all samples <LOD contain half the LOD

- **Fusarium toxins: fumonisins**

In the third year of the project (2001 harvest) a subset of 6 malts was also analysed for fumonisins B₁ and B₂. No fumonisins were detected in any of the samples (limit of detection, 0.05 mg/kg).

- **Ochratoxin A**

The majority of the malts tested (around 80%) contained no detectable OA (Table 4). This is comparable with the findings for all cereals in the HGCA survey (HGCA, 2000).

Table 4. Ochratoxin A in malts

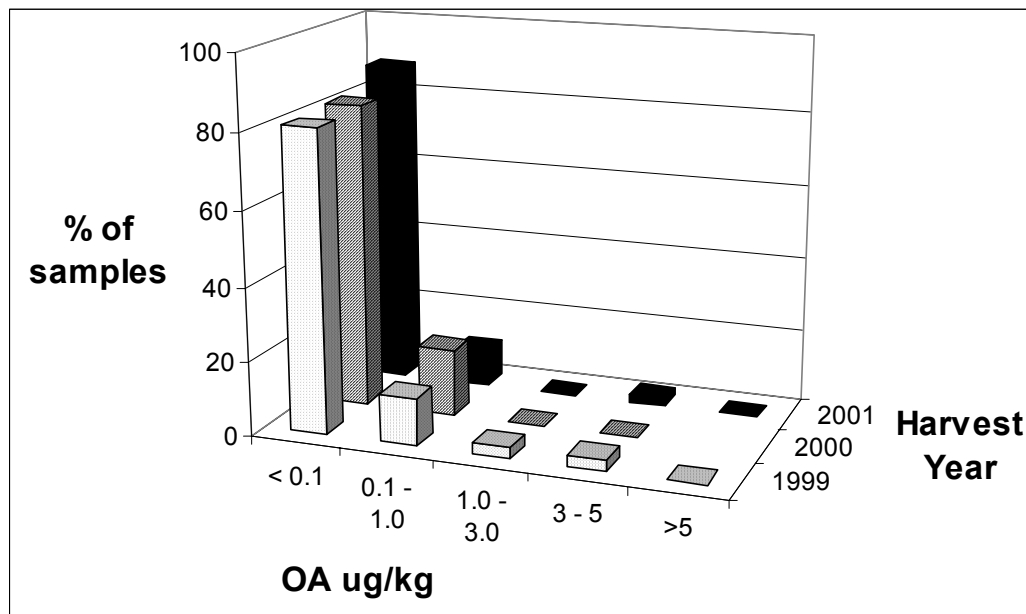
Harvest Year	Number of samples	Limit of detection (µg/kg)	Number of samples below limit of detection	Ochratoxin content (µg/kg)	
				Mean *	Maximum
1999	32	0.1	26 (81%)	0.270	3.91
2000	39	0.1	32 (82%)	0.067	0.27
2001	49	0.1	39 (80%)	0.152	4.28

* Mean is calculated assuming that all samples <LOD contain half the LOD

Levels in most of the positive samples were low, but in two out of the three years one sample contained enough OA to exceed the new limit, had it been in place at that time. This information was relayed via the MAGB to the manufacturer involved, together with advice on storage conditions.

(It should be emphasised that the malts in this survey were not sampled according to the EU sampling protocol, which requires that a composite sample of at least 10 kg should be taken, and that the whole of this 10kg should be milled and mixed prior to a sample being taken for analysis. When this sampling regime is followed strictly small pockets of infection are less likely to be missed, but the overall concentration of OA is often found to be lower than when smaller samples are analysed (Wilson, P. et al, Oct 1999)).

Figure 2. Occurrence of Ochratoxin A in malts from 3 years' harvests



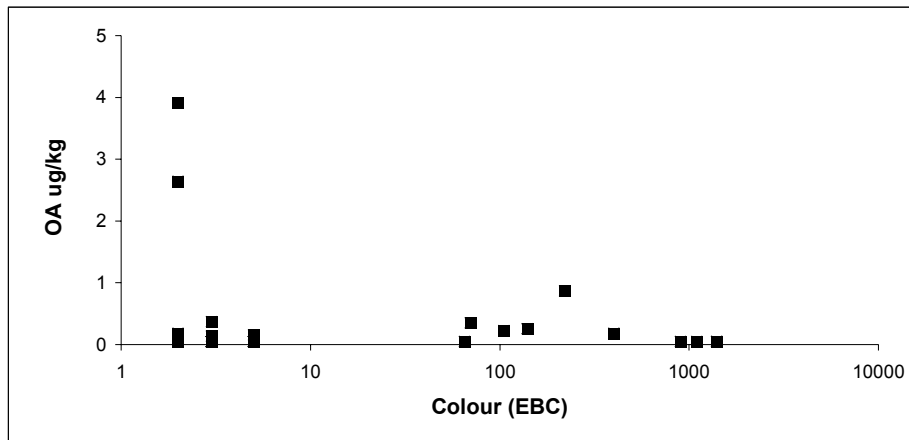
Unlike the situation with DON, there was no discernible trend in OA levels over the three years' harvests (Figure 2).

These results, together with others from BRi laboratories, were made available for presentation by the malting and brewing industry to the 2nd meeting of the European Commission's Ochratoxin Forum early in 2002. Subsequent to that meeting, the Commission has asked for assurance that different types of beer (such as ales and dark beers) are all acceptably low in OA, since most of the data available is for light coloured beers. Any perception that darker malts could pose an additional risk could be detrimental to the UK's speciality malts industry. The set of speciality malts collected for this project was therefore also analysed for ochratoxin A. The results are shown below in Table 5 and Figure 3, and were also provided to the Forum. These results indicated that, as expected, there was no significant association between malt colour and OA content. This is supported by an analysis of OA content of beers, which showed no significant difference between the mean OA content of lagers, ales and stouts.

Table 5. Ochratoxin A in speciality malts

Malt Type	Colour (°EBC)	Number of samples	Mean * (µg/kg)	Maximum (µg/kg)
Distilling, Pale ale, Lager	<5	32	0.27	3.91
Amber	< 100	2	0.20	0.35
Crystal	100-400	4	0.38	0.87
Chocolate, Roast	900 - 1400	4	0.05	0.05

Figure 3. Ochratoxin A content and malt colour.



- **Aflatoxins.**

The aflatoxins are potent liver carcinogens and EU legislation was introduced in 2001 setting limits of 4 µg/kg for total aflatoxins in cereals (2µg/kg for aflatoxin B₁) (*Commission Regulation 466/2001*). Samples of malts from the 2000 and 2001 harvests were therefore tested for these mycotoxins. The numbers of samples were limited because the moulds which produce aflatoxins (mainly *Aspergillus flavus* and *Aspergillus parasiticus*) flourish in conditions of relatively high humidity and temperature, thus the risk is low in UK grown grain. In 2000 six samples were tested and in 2001 ten samples. No aflatoxins were detected in any of the samples (limit of detection 0.1µg/kg).

4.2.2. Pesticides

A subset of malts was screened each year for pesticides, with 112 residues being sought (see Table 6 for details). This screen was supplemented with in-house tests for chlormequat and glyphosate, both of which may be legally used on barley in the UK, and, being water-soluble, are more likely to survive processing and persist into the beer in detectable (but not harmful) amounts. Results are shown in Table 6 (see Appendix) .

Most of the samples tested (13 samples over 2 years) contained detectable chlormequat (a growth regulator). However, the concentrations detected (0.02 – 0.5mg/kg) were well below the MRL of 2.0 mg/kg.

In year 3 (2001) harvest was wet, so some samples were tested for the herbicide glyphosate, which can be used on barley as a desiccant immediately prior to harvest. The MRL for this chemical is 20 mg/kg. No glyphosate was detected in any of the samples, using a method with a limit of detection of 0.5mg/kg.

The only residue which was detected in any of the three years using the multi-residue screen was pirimiphos-methyl, an insecticide which is registered in the UK for use post-harvest to prevent infestations in stored grain. The concentrations found (0.05-0.21 mg/kg) were well below the MRL, which is 5.0 mg/kg.

This multi-residue screen (see Table 7) is typical of that offered by contract laboratories for screening pesticide residues in a wide range of crops. However, it is apparent from Table 6 that most of the chemicals sought are not allowed on malting barley in the UK, and indeed are being withdrawn from use across the EU (*Commission Regulation No 2076/2002*). On the other hand, many of the newer chemicals which are widely used (for example the strobiluron fungicides) are not covered. Thus, although this type of screen will give indications of illegal use of products, it is less useful for monitoring residues likely to be present from the legitimate use of plant protection products, (although it does cover the post-harvest insecticides which are the most likely products to leave significant residues). It is suggested that a smaller screen, targeted at products likely both to be used and to leave residues, could be more useful.

Table 7. Pesticides sought in the annual multi-residue screening of representative malt samples

Residue	Limit of detection (mg/kg)	EU MRL for barley* (mg/kg)	Accepted by BBPA**
Acephate	0.02	0.02	no
Aldrin	0.02	0.01	no
Atrazine	0.05		no
Azinphos-ethyl	0.05	0.05	no
Azinphos-methyl	0.05		no
Benalaxyl	0.05	0.05	no
Bifenthrin	0.02	0.5	yes
Bromophos-ethyl	0.05		no
Bromophos	0.05		no
Bupirimate	0.05		no
Carbofuran	0.05	0.1	no
Carbophenothion	0.05		no
Carbosulfan	0.05	0.05	no
Captafol	0.02	0.05	no
Captan	0.05		no
Chlorfenvinphos	0.05		no
Chlordane	0.02	0.02	no
Chlorothalonil	0.01	0.1	yes
Chlorpropham	0.05		no
Chlorpyrifos	0.05	0.2	yes
Chlorpyrifos-methyl	0.05	3.0	yes
Chlorthal-dimethyl	0.05		no
Chlorthion	0.05		no
Cyanazine	0.05		yes
Cyfluthrin	0.02	0.02	no
Cyhalothrin	0.02	0.05	yes
Cypermethrin	0.05	0.2	yes
Cyproconazole	0.05		yes
DDT	0.02	0.05	no
Deltamethrin	0.05	1.0	yes
Demeton-S-methyl	0.05	0.1	no
Desmetryn	0.05		no
Diazinon	0.02	0.02	no
Dichlofluanid	0.05		no
Dichlorvos	0.05	2.0	no
Dicofol	0.02	0.02	no
Dieldrin	0.02	0.02	no
Dimethoate	0.05	0.02	yes
Diphenylamine	0.05		no
Disulfoton	0.02	0.2	no
Endosulfan	0.02	0.05	no
Endrin	0.01	0.01	no
Ethion	0.05		no
Ethoprophos	0.05		no
Etridiazole	0.05		no
Etrimfos	0.05	5.0	no
Fenarimol	0.02	0.02	no
Fenchlorphos	0.01		no

Table 7 continued

Residue	Limit of detection (mg/kg)	EU MRL for barley* (mg/kg)	Accepted by BBPA**
Fenitrothion	0.05		no
Fenthion	0.05		no
Fenvalerate	0.05	0.2	yes
Fluazifop-P-butyl	0.05		no
Flucythrinate	0.05	0.05	no
Fonofos	0.05		no
Heptachlor	0.01	0.01	no
Heptenphos	0.05		no
Hexachlorobenzene	0.02	0.01	no
Hexachlorocyclohexane-alpha	0.02	0.02	no
Hexachlorocyclohexane-beta	0.02	"	no
Hexachlorocyclohexane-delta	0.02		no
Hexachlorocyclohexane-gamma	0.02	0.01	no
Hexaconazole	0.05	0.02	no
Imazalil	0.02	0.02	yes
Iprodione	0.02	1.0	yes
Iodofenphos	0.05		no
Malathion	0.05	8.0	no
Metalaxyl	0.05	0.05	no
Methacrifos	0.05	0.05	no
Methamidophos	0.01	0.01	no
Methamidathion	0.02	0.02	no
Methoxychlor	0.05	0.01	no
Metribuzin	0.05		no
Mevinphos	0.05		no
Omethoate	0.05		no
Oxadixyl	0.05		no
Parathion	0.05	0.05	no
Parathion-methyl	0.05		no
Penconazole	0.05	0.05	no
Pendimethalin	0.05		yes
Pentachloroaniline (PCA)	0.02		no
Permethrin	0.05	0.05	yes
Phorate	0.05	0.05	no
Phosalone	0.05		no
Phosmet	0.05		no
Phosphamidon	0.05	0.05	no
Pirimicarb	0.05		yes
Pirimiphos-methyl	0.05	5.0	yes
Procymidone	0.02	0.02	no
Prometryn	0.05		no
Propachlor	0.05		no
Propham	0.05	0.05	no
Propiconazole	0.05	0.05	yes
Propyzamide	0.02	0.02	no
Quinalphos	0.05		no
Quintozene	0.01	0.02	no
Simazine	0.05		yes
Sulfotep	0.05		no
Tebuconazole	0.05		yes

Table 7. continued

Residue	Limit of detection (mg/kg)	EU MRL for barley* (mg/kg)	Accepted by BBPA**
Tecnazene	0.02	0.05	no
Tefluthrin	0.05		yes
Terbutylazine	0.05		yes
Terbutryn	0.05		yes
Tetradifon	0.05		no
Thiabendazole	0.05	0.05	yes
Tolclofos-methyl	0.05		no
Tolyfluanid	0.05		no
Triadimefon	0.05	0.2	yes
Triadimenol	0.05	"	yes
Trifluralin	0.05		yes
Triazophos	0.02	0.02	no
Vamidotion	0.05		no
Vinclozolin	0.05	0.05	no

* for simplicity, the MRLs listed in the latest EU legislation as of November 2002 have been given. In some cases these will not yet have been implemented into UK legislation.

** listed in "Agrochemicals Accepted by the British Beer and Pubs Association and Brewing Research International for use on barley and hops". Technical Circular No 366, BBPA, April 2002.

4.2.3. Heavy metals

A subset of 8 samples was tested for a range of heavy metals and arsenic each year. Samples were selected so that all the main growing areas were covered. Results, quoted as mg/kg wet weight of malt (approximately 4% moisture) are shown in tabular form in Table 8 and are also displayed graphically in Figure 4. Mean values are calculated by assuming that a sample below the limit of detection contains that species at half the limit of detection.

- **Lead. (Figure 4A).**

In the first and second years of this project (that is malts from the 1999 and 2000 harvested barleys) all samples tested were below the laboratory's limit of detection for lead (0.05 mg/kg). However, in the third year, 7 of the 8 samples tested (by the same laboratory as in the previous years) were reported to contain > 0.2 mg/kg (the new legal limit). Repeat analysis of 4 of the same samples by the same laboratory gave 3 out of the 4 as below the limit of detection, while the 4th sample was slightly higher than when first analysed. This discrepancy was obviously unacceptable and finally, replicates of 7 of the 8 samples were sent for analysis by CCFRA, which uses a similar analytical procedure to Eclipse (the first laboratory used) but has UKAS accreditation specifically for lead in cereal matrices. CCFRA participates in FAPAS, and their Z scores indicated that they were within the acceptable limits for lead analysis. CCFRA found low levels of lead (less than 0.03 mg/kg) in all samples.

- **Cadmium (Figure 4B)**

All samples tested contained less than half the EU limit of 0.1 mg/kg wet weight (equivalent to 0.11mg/kg at the moisture content of malt), with 75% being less than one quarter of the limit (figure 3B). There was no evidence of any consistent trend across harvests. The samples from the 2000 harvest were all lower than those from 1999 and 2001, but given that all results were close to the limit of determination (0.01 mg/kg), these differences are unlikely to be significant.

- **Zinc (Figure 4C)**

Unlike cadmium and lead, zinc is an essential micronutrient for in humans and is also an essential yeast nutrient. Malt is an important source of zinc for many brewers, thus concentrations of zinc tolerated in malt are substantially higher than those set for toxic metals such as cadmium and lead. Brewing literature reports suggest a range of 0.1 - 1.0 mg/litre zinc as suitable for brewing wort (*Donhauser, 1981*). Allowing for 90% losses during brewing (*Leubolt, 1991*) and a dilution of 1-10 for conversion of malt to beer, this would indicate a range of 10 – 100 mg/kg as suitable for zinc in malt. All except one of the malts tested in these surveys were below 100 mg/kg, with most containing around 20 mg/kg. There were no clear differences between the three harvest years.

- **Copper (Figure 4D)**

Copper is also an essential trace nutrient for both humans and yeast. However, it is toxic at higher levels, and in beer can have deleterious effects on flavour

stability by promoting oxidation. Levels must therefore be controlled. There is little published data but the few studies which are available suggest that the average concentration in malt is around 5 mg/kg, although the range can be quite wide (2 – 15 mg/kg) (*Leubolt, 1992*). In the current study, all malts tested contained less than 5mg/kg of copper, with a mean of 2.55 mg/kg. There was no evidence of any consistent trend across harvests.

- **Arsenic (Figure 4E)**

Traditionally, malt has been considered to be a significant source of arsenic in beer, and levels of up to 2.4 mg/kg have been quoted in the literature for cereals (*Reilly, 1980*). However, the major sources were arsenic-containing coal used in directly fired kilns and arsenic-containing pesticides. Today coal is rarely used as a fuel source, and most kilns are indirectly fired. Arsenic is no longer used in pesticides in the UK. Consequently levels of arsenic in malts would now be expected to be very low, although there are few published studies and arsenic is still included in some brewers' specifications. In the current survey arsenic was measured in subset of malts from the 2000 and 2001 harvests. All samples contained less than one tenth of the UK legal limit of 1 mg/kg for arsenic in foods (*Arsenic in Food Regulations, 1959*). The mean concentration was 0.016 mg/kg, with a range of <0.01 to 0.08 mg/kg.

Table 8. Heavy metals and metalloids in malts

Species	Number of samples	Limit of detection (mg/kg)	Mean* (mg/kg)	Range (mg/kg)	Legal limit (mg/kg)
Lead	24	0.05	0.03	<0.05 – 0.08	0.2
Cadmium	24	0.01	0.018	<0.01 – 0.04	0.1
Zinc	24	1.00	23.6	2 - 105	none
Copper	24	1.00	2.55	<1 – 4.45	none
Arsenic	16	0.01	0.016	<0.01 – 0.08	1.0

* mean concentration is calculated by assuming that those samples below the limit of detection contain half that limit for that species

Figure 4. Heavy metal and arsenic residues in malted barleys.

Figure 4A: Lead

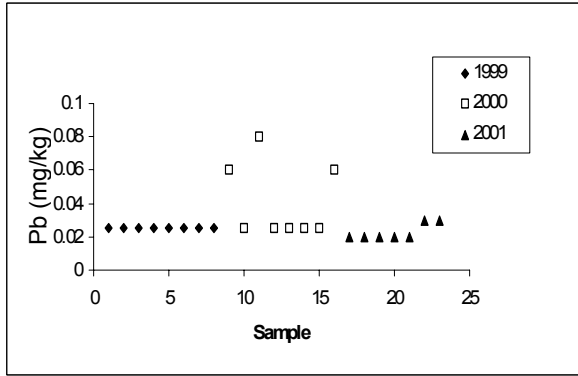


Figure 4B: Cadmium

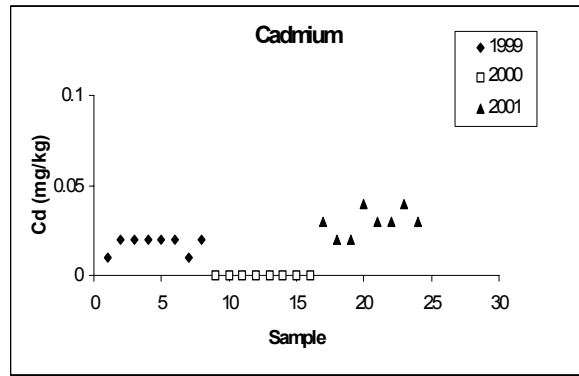


Figure 4C: Zinc

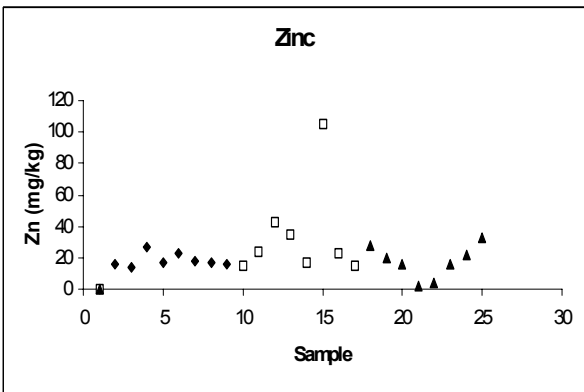


Figure 4D: Copper

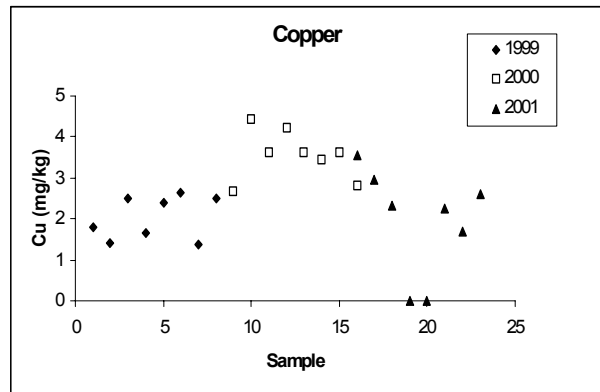
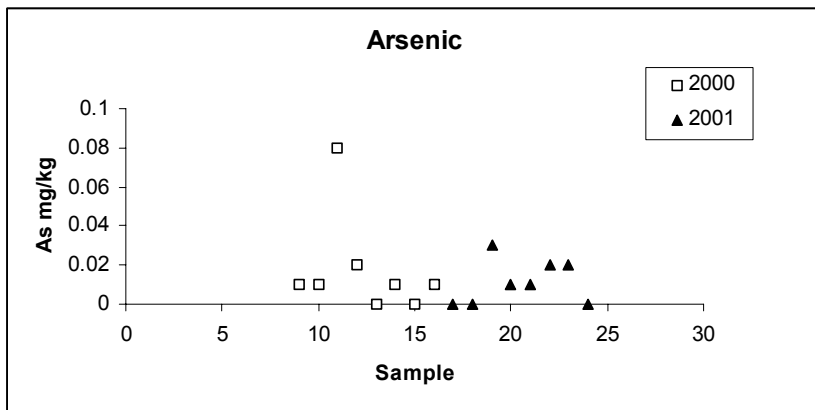


Figure 4E: Arsenic



4.2.4. Nitrosamines

The potential for volatile nitrosamines, particularly N-nitrosodimethylamine (NDMA), to form during malt kilning is well recognised, and is therefore routinely monitored by maltsters. NDMA was not therefore included in the current project, since there is already ample data available.

Non-volatile nitrosamines (ATNC) can also be formed during kilning, but are less regularly monitored. Initial studies by MAFF indicated that up to 200µg/kg ATNC could be formed in malt during kilning, contributing up to 20 µg ATNC /litre of beer (*Massey et al 1987*). The higher concentrations detected in some beers are triggered by reduction of wort nitrate, catalysed by bacterial infection in the brewery. At that time 200µg/kg was considered to be the lowest technically achievable level for ATNCs in malt, and a voluntary limit of 20 µg/litre in beer was therefore adopted by the UK brewing industry. Subsequently, the widespread introduction in UK maltings of indirectly fired kilns is expected to have significantly reduced the extent of ATNC formation during kilning. It was therefore considered timely to establish current ATNC levels in UK malts, especially in view of a scheduled survey of ATNCs in UK beers by the FSA in 2000/2001.

A subset of malts, including at least one sample from each malting company in the MAGB, was analysed for ATNC each year. The results are given in Table 9 and also displayed graphically in Figure 5. These show that the majority of UK malts (overall mean, 81%) now contain no detectable ATNC. The mean concentration, calculated by assuming that samples below the limit of detection contained half that limit, was 20 µg/kg. There was a noticeable downward trend in the range of ATNC values over the three years.

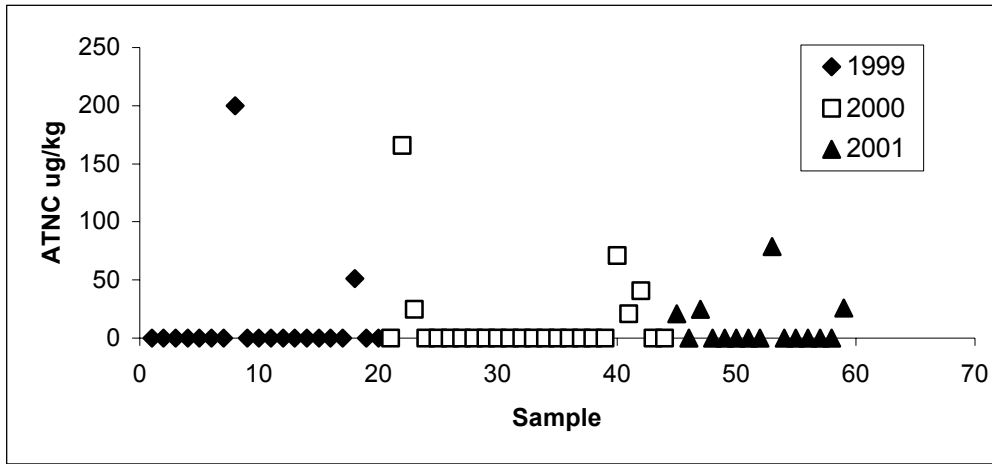
Table 9. ATNC in UK malts

Harvest	Limit of detection (LOD) µg/kg	Number of samples	Number (%) below LOD	Mean* µg/kg	Range µg/kg
Pre-1987**	20	54	Not available	62	<20 - 400
1999	20	20	18 (90%)	22	<20 – 200
2000	20	24	19 (79%)	21	<20 – 166
2001	20	15	11 (73%)	17	<20 – 79
Overall	20	59	48 (81%)	20	<20 - 200

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

** historic BRi data (*BRi manual of Good Practice*)

Figure 5. ATNC in UK malts over three years' harvests

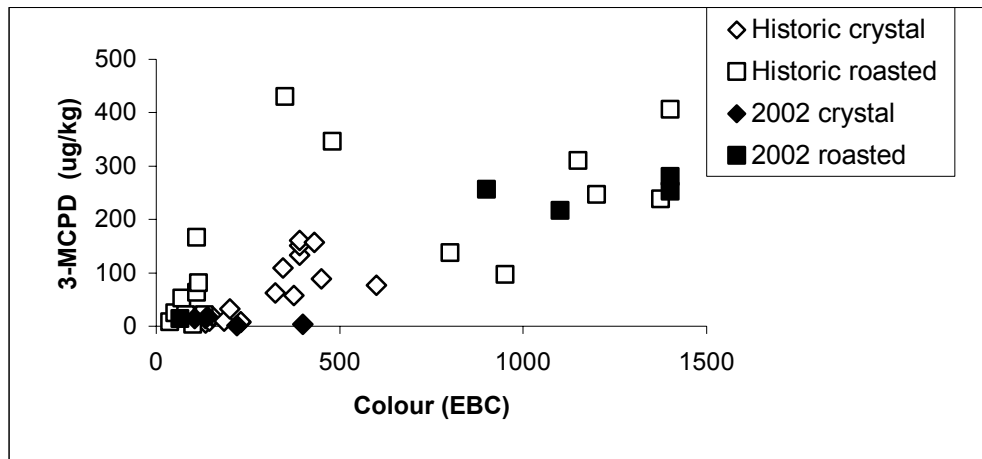


4.2.5. Chloropropanols

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*). The amount of 3-MCPD formed correlates with the amount of heating and thus very approximately with the colour. Crystal malts, however, generally contain less 3-MCPD per unit of colour since they are produced using higher moisture levels, thus Maillard reactions and colour formation occur at lower temperatures. The recommendation of the FSA is that levels of 3-MCPD in foods should be as low as is technologically achievable. In the case of speciality malts, the 3-MCPD is formed from endogenous precursors in the barley, thus there is little scope for the maltster to influence formation other than by limiting the extent of heating as much as possible consistent with achieving the desired colour and flavour properties. It was therefore considered important to monitor levels of 3-MCPD in malts currently on the market. Since relatively small quantities of speciality malts are required in a brew, the concentration of 3-MCPD in the final beer remains low.

In year 3 of the current project a full range of speciality malts was obtained from each of the two major UK manufacturers, and analysed for 3-MCPD. Results were compared with historic data obtained by BRi between 1997 and 1999. The data, shown in Figure 6, suggests that speciality malts currently being produced remain within the range previously established, possibly with a slight trend to lower levels. However, too few recent samples have been tested to be certain of this.

Figure 6. 3-MCPD in a range of speciality malts.



4.2.6 Acrylamide

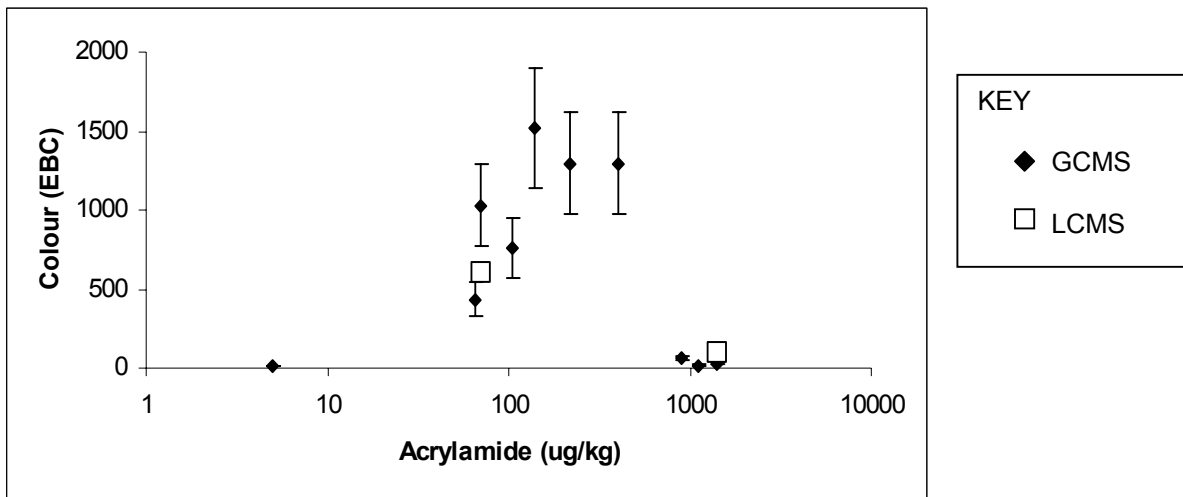
Acrylamide in foods emerged as an issue in May 2002. Initial information suggested that formation occurred when starch-rich foods were heated above 110° C and research subsequently published by three separate groups (*Stadler et al*, *Mottram et al*, *Sanders et al*) further indicated an association with Maillard reactions. This meant that it was highly likely that speciality malts could contain acrylamide. The set of speciality malts obtained for 3-MCPD analysis was therefore also used to check for acrylamide.

The results are shown in Table 10 and Figure 7. There was a marked increase in acrylamide content with colour up to about 200 °EBC, then little further increase between 200 and 400 °EBC. Levels of acrylamide in the darkest (most highly processed) samples was consistently low, suggesting that acrylamide has been degraded or possibly lost by evaporation. There is therefore a real possibility that acrylamide levels in speciality malts could be reduced by manipulation of roasting temperatures and /or times.

Table 10. Acrylamide in a range of speciality malts and roast barley

Type	Colour (°EBC)	Acrylamide (µg/kg)
White malt	3 – 5	14
Amber	65 – 70	508 - 1095
Light crystal	105 – 140	921 – 1169
Dark crystal	220 – 400	1292 – 1293
Chocolate	900 – 1400	21 – 63
Roast barley	1100 -1400	5 - 17

Figure 7. Acrylamide in speciality malts and roasted barley as a function of colour



There was substantial interference in the assay with many of these samples (see Methods), but satisfactory recovery of internal standards has been obtained using a modified method. Two samples (an amber malt and a chocolate malt) were by cross checked by RHM Technology, using an LC-MS method, also developed by Castle at CSL (data points shown as open squares in Figure 7). The values obtained were comparable with those obtained using the GCMS method. However, the analytical error obtained in replicate analyses by both methods was around 25%, indicating that considerable method development is still required particularly for highly processed samples. More research is also needed, in particular with samples in the colour range 400 - 800°EBC, which are uncommon in commercial practice.

The UK's Food Standards Agency (FSA) has now agreed to fund further work into acrylamide formation in cereals. This is expected to commence early in 2003.

4.2.7. Dioxins

Results were obtained for dioxins, *ortho*- and non-*ortho*- PCBs, and are given in Table 11 as WHO Toxicity Equivalents (TEQs) ng/kg whole barley (upper bound).

Table 11. Dioxins and dioxin-like PCBs in barleys from the 1999 harvest.

Sample	TEQ(Toxicity equivalents) (upper limit)* ng/kg whole grain			
	Dioxins	Non- <i>ortho</i> - PCBs	<i>Ortho</i> - PCBs	Total TEQs
Fanfare/Optic/ Chariot- (East Anglia)	0.16	0.01	0.04	0.21 (7)**
Chariot (Scotland)	0.11	<0.01	0.04	0.15 (5)**
Optic (N.Yorks/Borders)	0.11	<0.01	0.04	0.15 (5)**

* There are a large number of PCBs and dioxins, some of which are significantly more toxic than others. TEQs are the internationally agreed unit which takes into account the available toxicity data to generate a "Toxic Equivalency Factor" or TEF which expresses the toxicity in terms of TCDD. This is then multiplied by the concentration to give the TEQ. The figures given are the estimated *upper bound* concentration, which is calculated assuming that any cogener below the limit of detection is present at that concentration.

** The figures in brackets are expressed per kg of fat, assuming that barley contains 3% fat.

There is little data available for dioxins in whole cereals with which these results can be compared. No legal limit for cereals has been set by the EU, since cereals are not considered to be a major source of dioxins in the diet. These values are well below the limit of 0.75 ng WHO-TEQs/kg for dioxins, set for animal feeding-stuffs of plant origin (*Council Directive 2001/102/EC*). It should be noted that these values are significantly higher than those quoted for “miscellaneous cereals” (0.43 and 0.38 ng WHO-TEQ/kg fat for dioxins and dioxin-like PCBs respectively) in the UK Total Diet survey published by the Food Standards Agency in September 2000 (*Food Surveillance Information Sheet No. 4/00*). This is almost certainly because the FSA figures relate to processed cereal products such as those found in breakfast cereals, which may also contain added fat, while our figures are for whole, husked barley grains. The outer layers (husk) are more likely to be contaminated with atmospheric dioxins than are processed, bran-free milling fractions. In addition, when expressed on a fat basis, concentrations of dioxins and dioxin-like PCBs often appear to be relatively high in foodstuffs which are naturally low in fat (such as whole cereals). These discrepancies confirm that whole cereals and processed cereal products are not comparable with respect to dioxin content and illustrate the importance of collecting accurate figures for defined food groups. This is especially important in view of the Commission’s call for national monitoring programmes to be set up for dioxins and dioxin-like PCBs with the aim of generating data which can inform discussions about regulatory limits for these contaminants (*Commission Recommendation 2002/201/EC*).

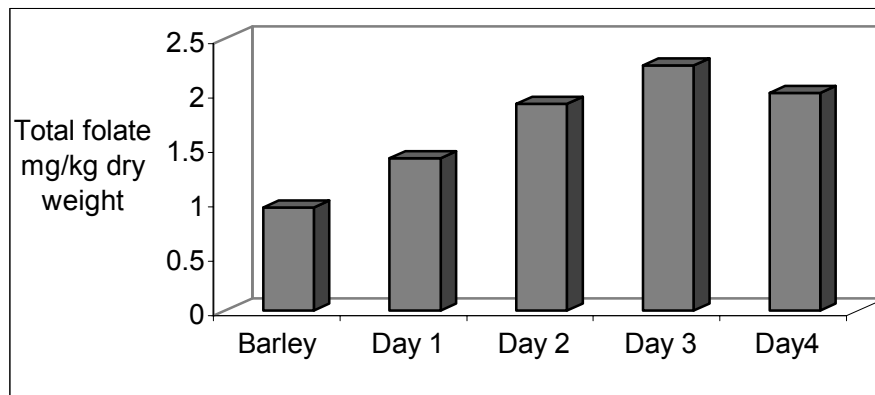
4.2.8. *Salmonella*

No *Salmonella* species could be detected in any of the three samples of maltings co-products. This is in-line with industry experience.

4.2.9. Folates

Levels of total folates were measured in raw barley and daily during germination.

Figure 8. Folate content of barley during malting



The results, shown in Figure 8, show that in this experiment total folates approximately doubled during germination. This suggests that there is a substantial increase in folate content during the malting process, thus malted cereals could be a significantly better dietary source of folate than are raw cereals.

The effects of the malting process on folate in cereals is now being investigated in more detail under the HGCA-funded project 2366 "The folate content of malted products; strategies for improvement."

4.2.10 Radioactivity

Possible contamination with radioactivity is important for some export markets, especially since the Chernobyl accident, and very little up-to-date data is available for malting barley or for malts. It was therefore decided to include some limited analysis for the radio-nucleides Cs 134 and Cs 137, both of which are indicators of contamination from industrial nuclear installations.

No Cs 134 or Cs 137 above the limit of detection (1 Bq/kg) was detected in any of the three composite samples tested from the 2000 harvest. These results compare favourably with the mean levels of 24 Bq/kg for Cs 137 and 18 Bq/kg for Cs 134 in barley, found by MAFF in their monitoring of foodstuffs in 1986, soon after the Chernobyl incident. More recent testing by some malting companies (1999) has found levels of gamma-ray emissions which are similar to those obtained during the current project.

5. CONCLUSIONS AND RECOMMENDATIONS

This project **has** laid a framework, not only for the ongoing identification of food safety and regulatory information relevant to the malting barley / malt industry, but also for a rapid and appropriate response to that information. The number of times data collected through this project was required to be submitted to regulatory bodies such as the Food Standards Agency, the Pesticides Safety Directorate and the European Commission is evidence of an ongoing need for a targeted surveillance programme for malt and malting barley. Such a programme must be sufficiently flexible to respond rapidly to issues which emerge unexpectedly, such as acrylamide.

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. These included mycotoxins, heavy metals, pesticides, dioxins, radionucleides, nitrosamines, chloropropanols and acrylamide.

The data obtained during the project confirms that UK malts are largely free from materials which could pose a risk to health. Where residues were detected, they were present only in trace quantities, below any legal limits or guideline values. For some parameters, such as arsenic, non-volatile nitrosamines and radionucleides, this project has confirmed that levels in UK malts are now much lower than historic literature values.

The surveillance programme also indicated that malted barley could be an important source of folate vitamins, and more detailed studies have been initiated as a result of this project.

However, a number of specific issues have also become apparent as a result of the surveillance exercise.

5.1. Mycotoxins

The three years surveillance indicated that UK malts are free from aflatoxins, T-2 and HT-2 toxins, which are among the most potent of cereal mycotoxins. It is increasingly likely that the European Commission will introduce legal limits for *Fusarium* toxins in foodstuffs, with deoxynivalenol being the first target. The levels of deoxynivalenol reported in this project would certainly be well within any limits likely to be set for barley and its products. However, in view of the increase in concentrations observed over the three years' surveillance, it is **recommended** that this monitoring should continue to order to establish whether this is merely a reflection of climatic conditions during the survey or the beginnings of a continuing trend possibly resulting from changes in economic practices. It is also important to note that the overall incidence of mycotoxins in barley or cereals in the UK is generally higher than in malting quality grain. This is likely to be due to the higher quality specifications applied to commercial

malting barleys, and can only emphasise the importance of initiatives such as the HGCA's Grain Sampling and Analysis Project, which should heighten the awareness of such specifications amongst growers.

These findings also underline the necessity for targeted surveillance of commercial malting barley and malt samples, as has been carried out under the current project, since more general surveys can give a misleading impression of the incidence of mycotoxins in commercial samples. This is a crucial quality specification for key export markets.

Fumonisin

It also seems probable that limits for fumonisins will be introduced in the near future. This project has confirmed that barley-based products such as malt are free from fumonisins, and compare favourably with imported maize, which may be used by some brewers as an alternative to malted barley. It might be possible in some circumstances to exploit this situation to the advantage of the UK malt industry.

Ochratoxin A

Occurrence of ochratoxin in foodstuffs, and particularly in cereals and cereal products is currently 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. These findings justify the emphasis placed in the UK on drying and storage, and are likely to improve further as the recently published HGCA Grain Storage Guide becomes more widely distributed. It is **recommended** however that intensive surveillance of malting barley and /or malts should be continued for the immediate future in order to inform negotiations with the Commission concerning the possible extension of existing legislation. In view of the recognised heterogeneity of storage mould infections, at least some of this monitoring should employ the Official sampling and analysis protocols set by the Commission (*Directive 2002/26/EC*).

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 in order to compile a more comprehensive data set to inform any future negotiations on regulation of this mycotoxin.

5.2 Pesticides

It is apparent that there is a high and increasing concern with pesticide residues in certain key overseas markets. This concern is not restricted merely to chemicals applied post-harvest. The forthcoming withdrawal of over 200 older pesticides in the EU means that the existing multi-residue screens offered by many pesticide testing laboratories are becoming increasingly irrelevant for malting barley and malt. Results from this project suggest that overall pesticide residues in UK malts are low, but there is still insufficient data for some of the

newer pre-harvest products, in particularly many of the new fungicides. It is **recommended** that attention should be given to developing a targeted risk-based pesticide screen for cereals and cereal products.

5.3 Heavy metals.

This project has highlighted ongoing problems with lead analysis by commercial laboratories. The discrepancies in lead concentrations reported, both within and between accredited laboratories, illustrates the uncertainties in measuring lead accurately at these low concentrations and highlights the difficulties likely to be encountered by commercial companies endeavouring to demonstrate compliance with the law. It is recommended that companies commissioning such analyses should be vigilant in checking both the accreditation and the performance of contract laboratories.

In other respects, however, the monitoring has indicated that UK malts are within both legal and industry guideline limits for heavy metals.

5.4 Dioxins

The data obtained confirms that cereals such as malting barley are not a major source of dioxins in the diet. However, dietary dioxins are currently a major issue for the Commission. Legislation limiting dioxins in plant-based animal feeds has already been introduced. It is probable that current EU limits will be extended and lowered in attempts to reduce the dietary intake of dioxins across the EU. Indeed, consideration is already being given to extending legislation to cover dioxin-like PCBs. Given the importance of cereals and co-products from the malting and other cereal-based industries for the animal feed industry, it is recommended that monitoring of malt and malting co-products for dioxins and PCBs should continue. This data will then be available for use in negotiations with the Commission, should this be necessary. Attention should be given to identifying a simpler and cheaper screening method which can be used on cereals.

5.5 Chloropropanols

The results obtained in this project indicate that levels of chloropropanols in UK-produced speciality malts and roasted barley are as low as is technologically achievable with currently available technology. However, it is indisputable that these products do contain significant quantities of chloropropanols and the situation should continue to be monitored.

5.6 Acrylamide

It is evident from the preliminary studies carried out for this project that heating of cereals, as in the manufacture of speciality malts, can potentially give rise to acrylamide. At present there is insufficient understanding of the mechanisms involved to be able to identify modifications which could be implemented in order to reduce acrylamide levels. This information may become available as a result of research to be funded by the Food Standards Agency in the UK. It is recommended that in the meantime monitoring of speciality malts for acrylamide should continue.

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APPENDIX

Table 1. Details of main annual malt sample sets collected for surveillance

Sample set	Harvest year	Number of samples	Number of companies included	Total tonnage represented	Varieties included	Growing areas covered
1	1999	54	12	155K	Chariot, Clarity, Cooper, Decanter, Derkardo, Fanfare, Golden Promise, Halcyon, Maresi, Maris Otter, Maud, Optic, Pearl, Pipkin, Prisma, Regina	Morayshire, Central Scotland, Angus, Tayside, Fife, Lothians, Borders, Northumberland, Yorkshire, Lincolnshire, Norfolk, Suffolk, Southwest England
2	2000	50	13	91K	Barke, Chariot, Decanter, Derkardo, Fanfare, Halcyon, Maresi, Maris Otter, Optic, Pipkin, Pearl, Prisma, Regina,	Northern Scotland, Inverness, Tayside, Angus, Fife, Lothians, Borders, Yorkshire, Lincolnshire, East Anglia, Essex, Gloucester, Wales,
3	2001	49	13	96K	Chalice, Chariot, Decanter, Fanfare, Golden Promise, Gleam, Halcyon, Leonie, Maris Otter, Maresi, Optic, Pearl, Regina,	Northern Scotland, Morayshire, Angus, Perth, Inverness, Lothians, Borders, Yorkshire, Lincolnshire, East Anglia, Cambridgeshire, Essex, Hertfordshire, Gloucester, Wiltshire, Hampshire, Dorset,

**Table 2A. Trichothecenes in UK malted barleys and some other cereal-based brewing raw materials:
Deoxynivalenol, acetylated species and Nivalenol**

Sample	Number of samples	LOD $\mu\text{g/kg}$	DON			3-Ac-DON			15-Ac-DON			NIV		
			% <LOD	Mean* $\mu\text{g/kg}$	Maximum $\mu\text{g/kg}$	% <LOD	Mean* $\mu\text{g/kg}$	Maximum $\mu\text{g/kg}$	% <LOD	Mean* $\mu\text{g/kg}$	Maximum $\mu\text{g/kg}$	% <LOD	Mean* $\mu\text{g/kg}$	Maximum $\mu\text{g/kg}$
Malts Year 1	54	5	91	3.4	16	94	2.8	8	<i>Not done</i>			94	3.4	24
Malts Year 2	50	5	86	3.4	20	98	2.6	5	<i>Not done</i>			92	5.7	109
Malts Year 3	49	5	59	7.4	53	92	3.5	37	98	2.6	6	94	3.2	18
Maize	10	5	0	304	533	20	7.0	17	<i>Not done</i>			0	15.1	27
Wheat	5	5	20	11.7	24	100	2.5	2.5	<i>Not done</i>			40	5.6	23

Mean is calculated by assuming that all samples below the limit of determination (< LOD) contain the contaminant at half that limit

**Table 2B. Trichothecenes in UK malted barleys and some other cereal-based brewing raw materials:
T-2 and HT-2 toxins.**

Sample	Number of samples	Limit of Detection $\mu\text{g}/\text{kg}$	T-2			HT-2		
			% < LOD Maximum	Mean*	Maximum	% < LOD	Mean*	Maximum
Malts Year 1	54	5	100	2.5	2.5	100	2.5	2.5
Malts Year 2	50	5	100	2.5	2.5	100	2.5	2.5
Malts Year 3	49	5	100	2.5	2.5	100	2.5	2.5
Maize	10	5	70	4.2	10	70	12.7	54
Wheat	5	5	100	2.5	2.5	100	2.5	2.5

* Mean is calculated by assuming that all samples below the limit of determination (< LOD) contain the contaminant at half that limit

Table 6. Results of pesticide analyses

Harvest Year	Multiresidue Screen			Chlormequat				Glyphosate and AMPA		
	Number of samples	Number with no detectable residues	Positive samples	Number of samples	Number with no detectable residues	Positive samples Mean (mg/kg)	Range	Number of samples	Number with no detectable residues	Limit of Detection (mg/kg)
1999	10	9	Pirimiphos-methyl, 0.21 mg/kg	6	2	0.32	<0.02 to 0.5	Not done		
2000	7	7	-	7	0	0.16	0.03 to 0.4	Not done		
2001	7	6	Pirimiphos-methyl 0.05 mg/kg	Not done				9	9	< 0.05