Session 4C

Mycotoxins

A Food Safety Issue

Chairman:	Dr Wendy Matthews *Food Standards Agency, London, UK*
Session Organiser:	Dr Simon Edwards *Harper Adams University College, Newport, UK*
Platform Papers:	4C-1 to 4C-3

Mycotoxin legislation applicable to UK agriculture

S Origgi
Food Standards Agency, Aviation House, 125 Kingsway, London WC2B 6NH, UK
Email: simona.origgi@foodstandards.gsi.gov.uk

Mycotoxins are toxic substances that are produced by moulds. They can occur in a diverse range of foodstuffs including cereals, nuts, spices, dried fruits, milk, apple juice and offal. Human exposure arises either from direct consumption of such foods or indirectly via animals that have ingested mycotoxin contaminated feed. Whilst there are over 300 known mycotoxins, only a few of them are of concern from a food safety perspective. These include the aflatoxins (B_1, B_2, G_1, G_2 and M_1), ochratoxin A, patulin and the toxins produced by fusarium moulds, that is, fumonisins (B_1, B_2 and B_3), trichothecenes (principally nivalenol, deoxynivalenol, T-2 and HT-2 toxin) and zearalenone. The toxic effect on humans of each type of mycotoxin is not always clearly defined but some of the most common mycotoxins are carcinogenic, genotoxic, or may target the kidney, liver or immune system.

In order to protect consumer safety, legislative limits for aflatoxins, ochratoxin A, fusarium toxins and patulin in certain foodstuffs are set in Europe by Commission Regulation (EC) No 1881/2006. These limits apply to the specified foods whether they are imported into the EU or produced in the EU.

Aflatoxins tend to be associated with products from sub-tropical climates and therefore are not expected to be present in food grown in the UK. However, the wet and warm UK summers do provide an ideal environment for the production of fusarium toxins, which are produced on cereal crops whilst in field, and ochratoxin A, which tends to occur during storage. Patulin can also be produced in apples in the UK.

Fusarium toxins

Fusarium ear blight (also referred to as fusarium head blight) of UK cereals may be caused by several fungal pathogens. Some of these fungi produce fusarium toxins whilst others do not. The most common fusarium toxin detected in small grain cereals is deoxynivalenol. Another fusarium toxin which is found less frequently is zearalenone. The major sources of dietary intake of fusarium toxins are products made from cereals, in particular wheat and maize.

Since fusarium toxins are stable during processing and, if present in the raw grain, may occur in finished food products, maximum limits for fusarium toxins intended for human consumption have been set for unprocessed cereals as well as for consumer products. The fusarium toxins covered by these limits are deoxynivalenol, zearalenone and fumonisins in unprocessed cereals, flour, finished products and infant food.

With the introduction of these limits in 2006, the European Commission produced a set of general principles to assist producers to minimise the amount of fusarium toxins in cereals. These were published as a Commission Recommendation addressed to Governments in EU Member States.

In the UK, fusarium toxin levels are generally low, although a small proportion of the UK wheat crop has exceeded these limits in recent seasons. To assist UK agriculture with meeting the new limits, the Food Standards Agency commissioned Harper Adams University College to develop a UK-specific Code of Practice for the reduction of fusarium toxins in cereals (www.food.gov.uk/foodindustry/farmingfood/fusariumadvice).

European limits for T-2 and HT-2 in cereals and products are expected to be set by 1 July 2008. Although high levels of HT-2 and T-2 have been detected in UK oats, all oats used for human consumption are de-hulled and current research suggests that de-hulling can reduce the mycotoxin content by more than ninety percent. The impact of de-hulling explains the difference in mycotoxin content of oats identified at harvest and the low concentrations of HT-2 and T-2 detected in retail oat products, as found in a 2003 Food Standards Agency Survey (www.food.gov.uk/science/surveillance/fsis2003/35cereal).

Ochratoxin A

Ochratoxin A can be produced by a number of fungi such as *Aspergillus* species and *Penicillium verrucosum* in a range of crop commodities worldwide. In UK cereal production, *P. verrucosum* is believed to be the sole species responsible for ochratoxin A production. *P. verrucosum* is only rarely found on cereals in the field and ochratoxin A is not found on crops of cereals in the field. However, this fungus is readily found in cereal grain stores and can accumulate on old grains and dust remaining in stores and machinery from the previous harvest. During harvesting, transportation and entry into store, freshly harvested grain can become contaminated with this fungus.

In 2002 the European Commission introduced maximum legal limits for Ochratoxin A in cereals, cereal-based products and vine fruits. Maximum levels were set based on the current human exposure in relation to the tolerable intake of the toxin in question and which can be reasonably achieved following good practice at all stages of production. Limits for coffee, grape juice and wine have since been added and limits for spices and liquorice products are expected to come into force in 2008.

In parallel with the work on fusarium toxins above, the Food Standards Agency has also produced a code of practice for ochratoxin A reduction in cereal grain.

Patulin

Patulin is produced by fungi belonging to several genera including *Penicillium* and *Aspergillus* and *Byssochlamys*. Patulin can occur in many mouldy fruits, grains and other foods but the major source of patulin contamination is in apples with brown rot and in apple juice, particularly cloudy apple juice. In 2003 the European Commission set limits for patulin in apples and apple products.

Conclusion

The ideal position would be to prevent mycotoxin formation in foods completely. However, as naturally occurring toxins, this is not possible and therefore the intention of the legislative limits described above is to provide a balance between what is achievable by agriculture and industry and the protection of consumer health.

Impact of agronomy on the fusarium mycotoxin content of wheat

S G Edwards
Harper Adams University College, Newport, Shropshire, TF10 8NB, UK
Email: sedwards@harper-adams.ac.uk

Mycotoxins are natural toxic substances produced by fungi and exist in our diet as a result of the presence of specific fungi on food crops, either in the field or in store. Mycotoxins can be hazardous to the health of humans and animals even at low concentrations. The European Commission (EC) introduced legislative limits for the fusarium mycotoxins, deoxynivalenol (DON) and zearalenone in cereals and cereal products in 2006. A combined limit for HT2 toxin and T2 toxin (HT2+T2) will be introduced in the near future.

Fusarium mycotoxins are produced in cereal crops whilst in the field as a result of the disease fusarium head blight. The most important head blight pathogens are *F. graminearum* and *F. culmorum* which produce DON and zearalenone. It is known that weather conditions, particularly when the wheat crop is in flower in early summer, are critical for disease occurrence and severity. A five-year project started in 2001 to ascertain the effects of agronomic practices on concentrations of fusarium mycotoxins in UK wheat. The project involved the collection of three hundred samples of wheat per year from fields of known agronomy over a number of seasons, which were analysed for ten trichothecenes, including DON, and zearalenone. The mycotoxin content was modelled against the agronomic practices applied to each field to identify the impact of each agronomic factor (eg variety, cultivation and previous crop).

Of the eleven mycotoxins analysed from field samples of wheat only seven were detected, of these only four, DON, nivalenol, HT2 and zearalenone were detected above 100 ppb. DON was the most frequently detected fusarium mycotoxin, present in 86% of samples, and was usually present at the highest concentration. The concentration of DON and the incidence and concentration of positive samples of HT2+T2 and zearalenone were modelled against agronomic practices applied to each field.

Year, region, previous crop, cultivation, variety and fungicide application all had statistically significant effects on DON concentration. Statistical tests of the predictive quality of the model indicated it may be a good predictor of new observations. There was a significant interaction between year and region, which was probably due to fluctuations in weather between years and regions. Highest concentrations were found in the south and east of England; lowest concentrations occurred in Scotland. There was also a significant interaction between previous crop and cultivation. This was probably due to the importance of crop debris in the epidemiology of ear blight. Highest predicted DON concentration occurred in wheat following maize, which is a known alternate host for *Fusarium* species.

Ploughing generally reduced DON concentration; this reduction was greatest following maize, wheat and potatoes. Other recent studies in France and Germany have shown that the risk is greater after grain maize compared to forage maize, probably due to the greater amount of crop debris remaining. At the moment the acreage of grain maize in the UK is very low but it may increase in the future.

Varieties of UK winter wheat are assessed for head blight resistance as part of the HGCA Recommended List trials. Results showed that varieties with a higher resistance had a lower predicted DON concentration. However, the current UK Recommended List has a limited range of resistance and would be classed as moderately susceptible compared to wheat varieties worldwide. There was no significant difference in the predicted DON concentration between organic and conventional samples. Within conventional samples, those which received an azole fungicide head spray (Growth Stage 59-69) had significantly lower DON than those which received no head spray.

The effect of agronomy on zearalenone is likely to be similar to that for DON; however, owing to the low incidence of zearalenone this could not be analysed with the same statistical robustness. One difference that was identified was the significantly higher zearalenone concentration in samples of spring compared to winter wheat. This may be because spring wheat ripens slightly later in the season and zearalenone is known to be produced once the crop ripens, and therefore conditions may be more conducive to zearalenone production later in the summer.

The effect of agronomy on HT2 and T2 appeared to be different to that for DON and zearalenone. This is understandable as HT2 and T2 are produced by different *Fusarium* species than those which produce DON and zearalenone. One important difference was that high levels of HT2 and T2 occurred all over the UK with no decline towards the north, indicating that temperature is not a critical factor in HT2 and T2 production in the UK.

The percentage of samples which would have exceeded the newly-introduced legal limits varied between 0.4% and 11.3% over the five-year period. There was a good correlation between DON and zearalenone concentrations although the relative concentration of DON and zearalenone fluctuated between years, consequently more samples would have exceeded the zearalenone legal limit than the DON limit in some years but not in others. This is probably due to the fact that DON is primarily produced in early summer whereas zearalenone is produced in late summer. The wet weather in late summer of 2004 resulted in the highest relative zearalenone-to-DON ratio and the highest percentage of samples which would have exceeded both the DON and zearalenone limits.

Overall, the risk of UK wheat intended for human consumption exceeding the newly introduced legal limits is low, but the percentage of samples above these limits will fluctuate each season depending on the weather conditions during the summer months. Results from this and other relevant studies have been used to inform the UK Code of Good Agricultural Practice to reduce fusarium mycotoxin in cereals issued by the Food Standards Agency. The agronomic advice is summarised as:

a) avoid maize as previous crop;
b) minimise previous crop residue on soil surface;
c) select resistant varieties;
d) consider a fungicide spray to control head blight;
e) timely harvest.

Acknowledgement

This research was funded by the UK Food Standards Agency and Home-Grown Cereals Authority.

Prevention of ochratoxin A in cereals

P Johnsson, M Olsen
National Food Administration, PO Box 622, SE-751 26 Uppsala, Sweden
Email: mool@slv.se

Mycotoxins are toxic substances produced by fungi. Ochratoxin A is a mycotoxin found in a number of foodstuffs and can be produced in cereals under adverse storage conditions. The overall objective of this project was the protection of consumer health by describing measures for decreasing the amount of ochratoxin A in cereals that are produced in Europe. The project included the whole food chain from primary production to the final processed product. The objectives and expected achievements were divided into four different tasks:

1. Identification of the critical control points (CCP);
2. Establishment of critical limits for the CCP;
3. Development of rapid monitoring methods:
4. Establishment of corrective actions.

These are all important steps in a Hazard Analysis and Critical Control Points (HACCP) management program for ochratoxin A in cereals. The outcome aimed to provide a pool of knowledge for HACCP-based management programs, which will increase food safety and support the EU cereal industry.

Task 1. Investigation of grain samples revealed that *Penicillium verrucosum* is the main, if not the only, producer of ochratoxin A in European cereals. It was concluded that *P. verrucosum* infection was best detected on DYSG media after seven days at 20ºC. Numbers of *P. verrucosum* found on DYSG and ochratoxin A content in cereals were correlated. More than 7% kernels infected with *P. verrucosum* indicated ochratoxin A contamination. The sources of infection of the grain were concluded to be the contaminated environments of combines, dryers, and silos. Prompt and effective drying of cereals at harvest is the major CCP for preventing the formation of ochratoxin A. In regions of Europe where the risk of wet weather at cereal harvest is usually great, measures to avoid mould and toxin problems are often effective. However, in areas which are normally at a lower risk, a wet harvest may be more difficult to handle. A significant problem arises where conditions at harvest are unpredictable. It may not be economic in some years to have expensive drying machinery idle while in other years the supply of damp grain may exceed the available drying capacity. Delays in drying may then put the grain at risk. Another problem arises when the infrastructure is such that sufficient funds and expertise are unavailable to advise on and ensure the best storage practice.

Task 2. The studies of the effect of temporal environmental factors on fungal growth, colonisation patterns and ochratoxin A production revealed interesting results which may explain why *P. verrucosum* is the main ochratoxin A producer in cereal grain in Europe. Generally, *P. verrucosum* was more dominant at lower water activity and 15ºC, whereas *A. ochraceus* was more dominant at higher water activity at 25ºC. Furthermore, results indicated that *P. verrucosum* was less sensitive to high concentrations of CO_2 than *A. ochraceus*, which may be a competitive advantage during storage. A mathematical model for safe storage time before the onset of significant growth of *P. verrucosum* and ochratoxin A production was developed. This model describes the effect of water activity and temperature on the growth rate of *P. verrucosum* in cereal grain. The model is valid for aerobic conditions, comparable to drying grain in near-ambient dryers or cooling grain by aeration prior to high-temperature drying. The probability of ochratoxin A levels above the EC maximum limit of 5 µg/kg at different levels of *P. verrucosum* in grain clearly increased when the concentration of *P. verrucosum* was above 1000 colony forming units/gram. A mathematical model was developed, which describes the risk for condensation in the headspace of a silo during storage of cereal grain. The model has been used to identify the conditions that cause moistening of the grain which increases the risk of mould growth and ochratoxin A production, and to develop control strategies to reduce this risk. Essential oils, resveratrol and lactic acid bacteria (LAB) can control growth and ochratoxin A production by *P. verrucosum* and *A. ochraceus* on grain. However, in small-scale storage experiments and experimental maltings, the inhibitory effect of the selected LAB strain could not be clearly shown. Out of twenty-four essential oils tested the most effective was found to be thyme, cinnamon leaf and clove bud.

Task 3. New diagnostic tools have become available that will provide means for rapid determination of ochratoxin A in cereals. This will enable an effective implementation of the European legislation and facilitate future in-house monitoring and scientific studies. Immunoassays in ELISA format, sensitive enough to meet the EU legislation for ochratoxin A, have been developed where large numbers can be analysed in a few hours. In addition a Lateral Flow Device taking less than five minutes to perform, which can be used on-site, has also been developed. A number of genes have been cloned, among them a polyketide synthase gene, which is involved in ochratoxin A biosynthesis. PCR primer pairs have been developed which appear to be highly specific for *A. ochraceus* and *P. verrucosum*. The primers may find a use in the development of rapid identification protocols for ochratoxigenic fungi. Several advances have been made towards molecular imprinted polymers (MIP) specific for ochratoxin A and their integration into solid phase extraction (SPE) and sensor systems. The materials demonstrate a high affinity and specificity for the target molecule in aqueous model samples. However, integration in real samples with complex biological matrices such as grain samples has proved to be difficult as interfering compounds affect the binding and measurements of ochratoxin A. Attempts to isolate and remove these interfering materials were unsuccessful and consequently the detection limits were not at the level required to meet the legislative requirements.

Task 4. This project has contributed with tools and recommendations for the cereal processing industry. These will facilitate decisions to be made to enable the maximum levels for ochratoxin A in foodstuffs to be followed. Examining the fate of ochratoxin A during milling revealed white flour as giving the most significant reduction of ochratoxin A (about 50%). Cleaning and scouring (1-2%) prior to milling, removed small amounts of ochratoxin A. Baking resulted in only a small fall in concentration. However, an overall reduction of about 80% is achievable for white bread and up to 35% for wholemeal bread with scouring included. The increase of ochratoxin A concentration during malting was two to four-fold in 75% of the samples studied and the process temperature had a pronounced effect. At the higher temperatures of 16-18°C, ochratoxin A formation was 20-fold compared to five-fold at 12-14°C. During the brewing process approximately 20% of the original ochratoxin A from the malt remained in the beer.

Acknowledgement

The authors acknowledge funding of the European Commission and all partners of the Quality of Life and Management of Living Resources Project No. QLK1-CT-1999-00433.