Quality Assurance and Safety of Crops & Foods ISSN 1757-8361

ORIGINAL ARTICLE

Towards harmonized approaches for mycotoxin analyses: an assessment

Anton J. Alldrick[1], Hans P. van Egmond[2], Michele Solfrizzo[3], Hayrettin Ozer[4], Sébastien Gofflot[5], Angel Angelov[6], & Velitchka Gotcheva[6] and members of the MoniQA Mycotoxins & Phycotoxins Working Group

1 Campden BRI, Chipping Campden, UK
2 National Institute for Public Health and the Environment (RIVM), Postbus, Bilthoven, the Netherlands
3 Consiglio Nazionale delle Ricerche, Institute of Sciences of Food Production (CNR-ISPA), Via Amendola, Bari BA, Italy
4 Tubitak Marmara Research Center, Gebze/Kocaeli, Turkey
5 Division Du Laboratoire D' Hormonologie Animale.Centre d'Economie Rurale, Rue du Point du Jour, Marloie, Belgium
6 Department of Biotechnology, University of Food Technologies, Plovdiv, Bulgaria

Keywords
analysis; legislation; mycotoxin; sampling.

Correspondence
Anton J. Alldrick, Campden BRI, Chipping Campden GL55 6LD, U K
Email: a.alldrick@campden.co.uk

Received and accepted 15 December 2008.

doi:10.1111/j.1757-837X.2009.00008.x

Abstract

Mycotoxins (the poisonous metabolites of certain filamentous fungi) are potential contaminants of staple food commodities and, if uncontrolled, may present a significant public health hazard. In many jurisdictions, questions relating to mycotoxin contamination are addressed at both generic and specific levels by food-safety legislation. Key to the successful management of the mycotoxin question, both in terms of verifying food-safety measures by the agri-food businesses and ensuring compliance with statutory limits by enforcement agencies, is the use of reliable sampling and analytical methodology. Evidence from European Union Rapid Alert System for Food and Feed data suggest that harmonization of methodologies used to determine the mycotoxin content of foods would contribute to improved compliance at both regulatory and commercial levels.

Introduction

Mycotoxins are poisonous metabolites produced by certain filamentous fungi. They represent a group of compounds with diverse chemical structures and physiological effects. Depending on the mycotoxin of concern, the adverse effects cover a wide range of trauma including hepatotoxicity and carcinogenicity (e.g. aflatoxins), immuno-suppression (tricothecenes), nephro-toxicity (ochratoxin A) and neurological and psychological disorders (ergotamine and psilocybin). Depending on the mycotoxin, these effects can arise after either acute or chronic exposure. Mycotoxins present a significant challenge to the health of humans, crops and livestock. For example it has been estimated that over 25% of the world's crop production is affected by mycotoxin contamination to one degree or another (Charmley *et al.*, 1995).

It is therefore unsurprising that in order to protect public health, many jurisdictions have set legislative limits setting out the maximum limits of particular mycotoxins permitted in specific foods. Consequently, agri-food businesses have had to put in place appropriate food-safety management systems to ensure that mycotoxin contamination is minimized and does not exceed regulatory maxima. Central to the validation and verification of any mycotoxin management system and enforcement of mycotoxin control is the need for reliable analytical methods. For the present purposes, a reliable method is one that, for any particular sample, can give consistent results when repeated in a particular laboratory and reproduced in another.

This paper seeks to highlight some of the current issues in the practice of mycotoxin analyses faced in the European Union (EU) within a commercial/regulatory context and represents a 6-month review of the issues by the Mycotoxins and Phycotoxins Working Group of the European Union Network of Excellence Project, 'MoniQA' (MoniQA, 2007). The paper begins with an overview of the current commercial/regulatory environment within the EU and then moves

on to discuss issues relating to the sampling of material for analysis and finally analytical method performance.

The commercial/regulatory environment within the EU

General legislative considerations

Member States' food-safety legislation is harmonized through appropriate EU directives and regulations. In terms of mycotoxins, their regulation is addressed in both generic and specific legislation. The current basis of food law within the European Union lies in Regulation (EC) 178/2002. The basic food laws of all Member States must comply with the provisions of Regulation (EC) 178/2002 and in particular clauses 1 through 4 of Article 14 *(Food Safety Requirements)*. Clause 1 states that if food is unsafe it shall not be placed onto the market. Clause 2 defines unsafe food as food that is either injurious to health or is unfit for human consumption, while clause 3 requires any decision to bear in mind the conditions of use throughout the food chain and information provided to the consumer. Clause 4 takes this point further; requiring a decision that a food is injurious to health to be based on not only short/long-term effects on the consumer but also succeeding generations, the consequences of acute versus chronic effects and whether any subgroup of the population may be particularly at risk.

In addition to the requirement that food businesses must not sell unsafe food, there is also a requirement that all parts of the food chain produce it in a hygienic manner. These requirements were recently consolidated in Regulation (EC) 852/2004. Among other things, this regulation defines hygiene as

> The measures and conditions necessary to control hazards and to ensure fitness for human consumption of a foodstuff taking into account its intended use.(Article 2.1 (a))

The regulation also requires food businesses to manage safety in line with the principles of Hazard Analysis Critical Control Points (HACCP) and good hygiene practice (Article 1) together with operating appropriate traceability systems. Of particular relevance to the management and control of mycotoxins is the requirement under Article 4 for the food business to undertake such sampling and testing as deemed appropriate. Appropriate laboratory testing is often an essential tool when validating or verifying processes concerning a food-safety management measure.

Legislation and Mycotoxins

Mycotoxins are considered to be contaminants. The term contaminant is defined in Regulation (EEC) 315/93 as:

> . . . Any substance not intentionally added to food which is present in such food as a result of the production (including operations carried out in crop husbandry, animal husbandry and veterinary medicine), manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food, or as a result of environmental contamination. Extraneous matter, such as, for example, insect fragments, animal hair, etc, is not covered by this definition.

At an absolute level, the presence of any contaminant in a food renders it unsafe (see 'General legislative considerations') and could be taken to imply that part(s) of the food chain has failed to operate in a hygienic manner. While this applies to many substances deliberately added to foods for nefarious reasons (e.g. Sudan dyes), for natural and technological (process) contaminants this is not always the case. In the case of mycotoxins, the maximum permitted levels of contamination for particular mycotoxin/food-stuff combinations have been detailed. These are set out in regulation EC 1881/2006 (as amended). How these limits are enforced is laid out in regulation EC 401/2006, which specifies the following criteria:

- The method of sampling to be undertaken by the enforcement agencies of Member States (Annex I of the regulation).
- The performance criteria for any analytical method on which Member State enforcement agencies base their decisions to accept/reject a lot at port of entry or to remove from sale (Annex II of the regulation).

There are two very important points, relevant to those operating within the agri-food business, that arise out of this legislation. These are:

- It is a requirement of Regulation (EC) 401/2006 to provide an estimation of the measurement of uncertainty and recovery. Enforcement officers may only seize material that is 'beyond all reasonable doubt' in excess of the statutory limits.
- These regulations apply to the enforcement of a *regulatory requirement* and not any commercial/contractual obligation. The methods initially used to determine the latter will, frequently not reach the same degree of rigour as required under the regulation.

In addition to legislation covering mycotoxin contamination in general; certain commodities imported from

Non-Member States (e.g. peanuts from China and hazelnuts from Turkey) and known to be at high risk of aflatoxin contamination are subject to further control (Commission Decision 2006/504/EC as amended). These control measures require that shipments must enter a designated port and be accompanied by a certificate of analysis from an authorised laboratory which details its aflatoxin content. It is a further requirement that a certain proportion of all of these shipments be subjected by the border authority of the importing Member State for clearance by analysis. Despite this regulation, significant numbers of consignments are still being rejected at port of entry (European Commission, 2007).

Consequences for the Agri-food business

Relevant authorities within Member States therefore have a duty to enforce the law, while food businesses have a legal duty to ensure that their products comply with it. In other words the food they sell must be 'safe' – both in terms of any direct effect on consumer health and also legal requirements regarding permitted maximum levels of contaminants. For both groups of stakeholders, reliable analytical tests are an essential tool in validating and verifying the efficacy of both enforcement systems as well as commercial operations and transactions. Irrespective of why the analysis is performed, an adverse result will have detrimental economic consequences. At a simplistic level, rejection at port of entry (and subsequent destruction) of a 10 tonne load of hazelnut kernels would incur a minimal cost of at least 80 000 Euros. In terms of foods already on sale to the general public, the commercial costs would be even greater due to the additional penalties incurred in undertaking product recalls and damage to brand image (discussed by Ramsey *et al*., 2001). Decisions relating to these sums of money must therefore be based on robust analytical methodology.

Consideration of Rapid Alert System for Food and Feed (RASFF) data published by the Commission and anecdotal evidence indicates that there is still considerable room for improvement in both sampling and analytical methodology. For example in the first 5 calendar months of 2008 (RASFF, 2008), the major source of notifications was as a result of border control rejections, predominantly as a consequence of enforcement of the requirements of Commission Decision 2006/504/EC (349, see Figure 1). This contrasted with 33 'Information' notifications – effectively non-conforming material removed from the environment before it had reached the consumer and 22 alerts (product recalls/withdrawals).

From analyses such as these, it is clear that there is a need to ensure that:

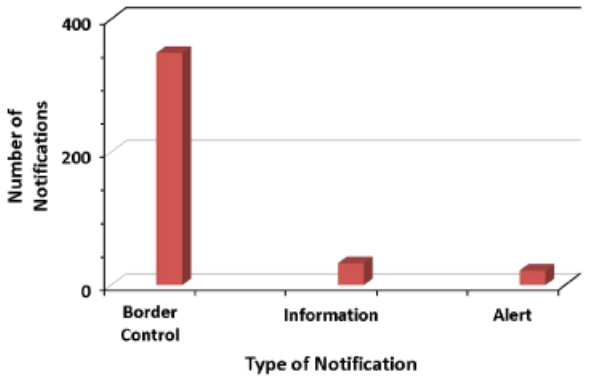

Figure 1 Mycotoxin-related notifications under the RASFF system within the EU for January–May 2008, analysed by type.

- Procedures used by exporting countries under Commission Decision 2006/504/EC (as amended) are in harmony with those used by the competent authorities in Member States.
- The limitations inherent in analytical data and in particular concepts such as measurement uncertainty and limits of quantification (LOQ) are understood by all concerned.
- Development of mechanisms to assist in the preparation of appropriate commercial specifications as well as making certain that sampling and analytical methods used 'in house' (usually rapid methods) are fit for purpose – this involves taking into account not only the analyte but also the food matrix in which it is analysed.

From an analytical standpoint, these issues can only be addressed if steps are taken to reduce issues concerned with sampling, together with method performance and measurement uncertainty. These are discussed in greater detail below.

Sampling

Definition

For the purpose of this publication, *sampling* is taken to be that process whereby a portion of material is collected with the intent of subjecting all or part of it to analysis for one or more mycotoxins.

Regulatory and commercial implications of sampling

Under the general 'umbrella' of food safety, foods are analysed for mycotoxins for a variety of reasons, the two principal ones being:

- As part of an exercise to ensure compliance with regulatory limits by enforcement officers. Usually, this is at one of two levels

– Bulk lots of commodity (usually at the point of entry into the European Union),
– Samples intended for purchase by consumers and collected from food businesses or retailers;

• To verify compliance with commercial specifications for particular mycotoxins (e.g. to determine grain acceptability at mill intake).

Mycotoxins are generally heterogeneously distributed within a load; any sampling protocol therefore should be designed to take this into account. However, this raises a further point – any mycotoxin present within the sample collected will *a priori* also be heterogeneously distributed. Assuming that the extraction and analytical methods are fit for purpose, in order to obtain a reliable result, on which actions can be taken, there is a need to ensure that the sample itself is homogenized before any part of it is withdrawn, processed (extraction, etc.) and analysed. There are therefore two challenges that have to be met, sample collection and sample handling. These will be dealt with in turn below.

Sample collection

Bulk commodities and regulatory enforcement

Generally speaking, most of the work relating to mycotoxins and undertaken on bulk commodities imported into the EU will be in respect of Commission Decision 2006/504 (EU) – as amended. This designates certain commodities sourced from particular countries as requiring surveillance to ensure compliance with the set maximum permitted levels of aflatoxins. Within the EU, sampling of bulk commodities by enforcement agencies for the purpose of mycotoxin analyses is covered by Commission Regulation (EC) No. 401/2006. In the case of aflatoxin contamination this is supported by a 'Guidance Document' (European Commission – DG SANCO, 2008). Sampling, in particular that of commodities in sacks, brings its own logistical problems. A single container of peanuts imported into the EU might be expected to contain approximately 24 tonnes (400×60 kg sacks). Every sack would have to be off-loaded (at the importer's expense) and then $2 \times 100 \times 30$ g samples collected; effectively sampling from every other sack. As the size of the shipment increases, this becomes even more complicated. For example grain shipments of 30 000 tonnes are not unusual. Consideration of Commission Regulation (EC) No 401/2006 would require that the shipment be divided into six sublots, each of 500 tonnes. Each sublot would then have to be sampled 100 times to generate an aggregate sample of 10 kg. Each individual aggregate sample would then have to be submitted for analysis.

Generally speaking, border control agencies (e.g. Port Health Authorities) are not dealing with shipments of one container but many and, given the structure of the regulations, the sampling regime increases in complexity as the size of the shipment increases. Given the volume of material going through international ports such as Southampton or Rotterdam, it would not be unreasonable to assume that at peak periods, enforcement officers and those responsible for providing the labour necessary to off-load such commodities must be under considerable pressure. This relates to the dynamics of a modern port operation, because a maximum 'turn-around' time of 15 days is set out in Article 5 (3) of the Commission Decision referred to above; however, individual Member State legislation may require enforcement officers to complete the work in considerably less time.

Bulk commodities and commercial verification

Given the food-safety and/or regulatory implications of mycotoxins, purchasers are increasingly setting, in contract and/or specification, limits for mycotoxin contamination for the goods supplied to them. These will inevitably be lower than those set out in legislation. In the case of high-risk material, there might also be a requirement that the vendor supplies the purchaser with a certificate of analysis from a competent laboratory demonstrating compliance. However, it must be borne in mind that the purchaser is required by the principles of modern food-safety management techniques to *verify* the accuracy of the information supplied by the vendor on a periodic basis (the principle is very much the same as that enunciated in Article 5 of Commission Decision 2006/504). Given that EU legislation (Regulation 852/2004) also requires food businesses to operate food safety management systems in accordance with HACCP principles (one of which is verification); it could be argued that, where risk assessments indicated a significant possibility of mycotoxin contamination, analysis for these compounds would be expected within any verification programme.

Currently, there are no harmonized methods for sampling for commercial purposes, still less for the purposes of collecting samples for mycotoxin analysis as part of necessary verification exercises. However, industry good practice does, to one degree or another cover this point. For example in the United Kingdom, *nabim*^{TM} (the National Association of British & Irish Millers) recommended code of practice for mill intake states:

> Sampling should observe a recognised system (e.g. ISO 13690:1999) to produce samples which are as fully representative as possible (National Association of British & Irish Millers, 2005).

Hook (2004) reviewed grain sampling procedures within the United Kingdom. In his review, the applicability of sampling in accordance with ISO 13690:1999 [International Organisation for Standardisation, (ISO, 1999)] for homogeneously distributed contaminants was acknowledged. However, his report went on to state that far more work had to be done in respect of heterogeneously distributed contaminants such as mycotoxins. There are therefore a number of potential sources of conflict, in particular given that the sample at mill intake is considered definitive, vendors may consider that the value of performing their own analyses in order to demonstrate compliance with commercial specification is diminished.

Given the above, unless sampling and analysis were undertaken as required by legislation, it is debatable whether non-conformance at intake would be accepted as a legal justification for condemning the delivery – given that the sampling method used has not been demonstrated as being 'fit for (regulatory) purpose.' Conversely, the use of such data to demonstrate the legal concept of a 'due diligence' defence in terms of demonstrating compliance with food-safety legislation has yet to be determined.

Retail samples

Within the EU, current legislation recognizes that the sampling methods applicable to bulk commodities may not be appropriate for enforcement purposes in connection with retail samples. It goes on to state that alternative methods may be used, providing that the aggregate sample is at least 1 kg. MacArthur *et al*. (2006) have recently published the results of statistical modelling exercises highlighting the limitations of such an approach and the need to take sufficient increments to produce the 1 kg aggregate sample. Using an ochratoxin A/dried fruit model, they demonstrated that aggregate samples made from 10 incremental samples exhibited a statistical variation greater than the analytical uncertainty; however, sampling variation became insignificant when measurements based on a 60-increment sample were made. The situation is very much dependent on the commodity–mycotoxin combination. The authors also considered aflatoxin B_1 in pistachio nuts and found that sampling variation was significant even when up to 200 increments were taken.

Sample handling in the laboratory

Once a sample has been collected, the same problem of heterogeneity arises. In the case of enforcement activities there is a requirement that any composite sample taken must be homogenized and divided into three before analysis. The three samples represent those for the enforcement agency, the owner of the shipment and a third referee sample in the event of a dispute. Laboratories undertaking work for enforcement agencies and dealing with bulk commodities therefore need to have facilities that can demonstrably homogenize any particular commodity for the mycotoxins for which the sample is to be analysed. In the case of processing laboratory samples for official control purposes Commission regulation 401/2006 states:

> TREATMENT OF THE SAMPLE AS RECEIVED IN THE LABORATORY
> Each laboratory sample shall be finely grinded and mixed thoroughly using a process that has been demonstrated to achieve complete homogenisation.

And

> REPLICATE SAMPLES
> The replicate samples for enforcement, trade (defence) and reference (referee) purposes shall be taken from the homogenised material unless such procedure conflicts with Member States' rules as regards the rights of the food business operator.

The last point is important. Early work by Whitaker and co-workers (reviewed in Whitaker & Johansson, 2005) working with bulk commodities and more recently that of MacArthur *et al*. (2006) working with retail samples have shown that the degree of heterogeneity of mycotoxin distribution is a function of both the commodity and the mycotoxin.

Given the bulk of the material, even within a homogenized sub-sample, there can be substantial differences. One example of this is work undertaken by Maestroni *et al*. (2005). In her study, 10×1 kg sub-samples from a 20 kg 'homogenized' parent sample of maize were derived. From each 1 kg sub-sample 5×150 g portions were derived and analysed in duplicate for the mycotoxin fumonisin B_1 (FMB_1). For the purposes of this paper, the data from that study were subjected to one-way analysis of variance – the results of which can be seen in Figure 2.

Two points of interest immediately arise:

- The mean values obtained for each 1 kg portion are different and in some cases significantly ($P < 0.001$) so. The widest difference is between portions 1 and 10 (2.12 versus 3.64 mg kg^{-1}).

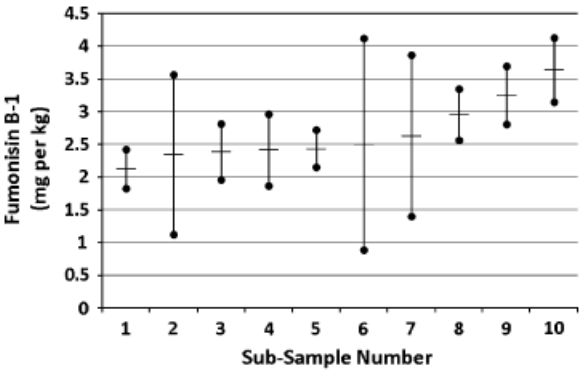

Figure 2 Summary statistical analysis (means ± 2 standard deviations) of the fumonisin B_1 contents of 10 × 1 kg sub-samples of maize using data generated by Maestroni *et al*. (2005).

• Standard deviations for individual lots are also highly variable, and the overall pooled standard deviation was determined to be 0.4144.

These data raise some interesting points from an enforcement perspective. Current EU legislation sets a limit of 2000 μg (i.e. 2 mg) total fumonisins (B_1 and B_2) per kg for unprocessed maize. For the sake of argument, let it be assumed that Maestroni and colleagues were measuring total fumonisins (i.e. FMB_1 & FMB_2); at first sight, the mean data presented in Figure 2 would suggest that the batch of maize from which the original sample was derived failed to conform with legislative requirements. However, consideration of Regulation 401/2006 reveals that a lot may only be rejected if:

> (one or more of) the laboratory sample(s) exceeds the maximum limit beyond reasonable doubt taking into account the correction for recovery and measurement uncertainty.

Measurement uncertainty is addressed elsewhere; however the standard deviation can be used as a crude estimate. Assuming that laboratory error equals 2 standard deviations, it could be argued that 60% of the 1 kg portions taken were actually compliant (i.e. it cannot be demonstrated 'beyond reasonable doubt' that those samples exceeded the statutory maximum). Thus, all other things being equal, it would be possible for the shipment to be accepted on the basis of results for one sub-sample analysed but rejected on the basis of data from another.

Method performance and measurement uncertainty

Introduction

In terms of determining compliance with a regulation or a commercial specification, any analytical method's fitness for purpose is determined by whether it can accurately measure the amount of analyte present within the boundaries set (accuracy criteria) and whether every result obtained for a particular sample can be repeated within the same laboratory and reproduced by others. In order to reduce barriers to trade, it is essential that the underlying principles of determining performance characteristics are operated in a uniform manner. Reference to data generated from a survey of the MoniQA Mycotoxin and Phycotoxin Working Group members (Tables 1 and 2, Solfrizzo *et al.* 2009), which included questions concerning how individual laboratories calculate limits of detection (LODs) and limits of quantification LOQs – indicates some of the differences in laboratory practices and highlights the need for harmonization of approaches.

These issues have been considered by reference to the current regulation concerning methods of sampling and analysis for mycotoxins with regard to their implications for good laboratory management.

Parameters for method validation

LOD (and its 'derivative': LOQ): As discussed elsewhere in this document, data generated from analyses have both economic and legal consequences. Thus, any analytical method must be 'fit for purpose'. A key element in establishing these criteria concerns the determination of the LOD. Annex II of Commission Regulation 401/2006 sets out the:

> criteria for sample preparation and for methods of analysis used for the official control of the levels of mycotoxins in foodstuffs.

One key criterion for mycotoxin analyses undertaken for regulatory control purposes (see Section 4.3.2 of the annex)

Table 1 Analysis of survey performed in January/February 2008 among MoniQA consortium laboratories concerning derivation of limit of detection (LOD) (after Solfrizzo, *et al*. 2009) for mycotoxins

Definition: LOD	No. of Laboratories
Signal/noise ≥ 3	6
Signal/noise 0.6 – 3	1
Signal/noise from 3 to 10	1
Noise of blank + 3 SD	2
The level below which the analyte cannot be measured	1
Confidence interval of calibration curve	1
The smallest amount selectively detected to which is associated a deviation > 40%	1
ISO 5725:1987	1
(3 × S)/b × f(conc.)	1

Table 2 Analysis of survey performed in January/February 2008 among MoniQA consortium laboratories concerning derivation of limit of quantification– (LOQ) (***after Solfrizzo,*** *et al*. 2009) for mycotoxins

Definition: LOQ	No. of laboratories
Signal/noise ≥ 10	3
Signal/noise from 6 to 100	2
Signal/noise = 500	1
Noise of blank + 10 SD	1
3 × LOD	1
Confidence interval of calibration curve	1
The smallest amount selectively detected to which is associated a deviation $> 35\%$	1
The lowest level measured with acceptable precision and accuracy	2
The lowest unambiguously determinable quantity of analyte	1
The lowest level measured with an accuracy of 20%	1
ISO 5725:1987	1

is the estimation of measurement uncertainty, a key parameter in determining whether, for potentially non-conforming shipments, an analytical result indicates 'beyond reasonable doubt' that the shipment truly fails to conform with regulations. The uncertainty value is calculated partially as a function of the LOD. Lack of harmony in how the LOD is determined will clearly influence the uncertainty values even for the same analytical method used in different laboratories. This has obvious potential consequences in terms of enforcement (see also 'Measurement uncertainty' below).

Repeatability and reproducibility: Within Commission Regulation (EC) No. 401/2006, various performance criteria are defined in terms of repeatability and reproducibility. These include:

- RSD_r: Relative standard deviation, calculated from results generated under repeatability conditions and
- RSD_R: Relative standard deviation calculated from results generated under (between-laboratory) reproducibility conditions;

and are specified for different mycotoxins at different levels of mycotoxin contents. However, no requirements as to the conditions under which these values should be derived are provided within relevant legislation. Examples of factors to be considered include for example, the numbers of materials, duplicates and days to be taken into account for RSD_r determination. A similar position exists with regard to the conditions under which reproducibility values have to be established (e.g. number of laboratories, number of materials, duplicates, operators, instruments and methods of calculation). In terms of interlaboratory validation studies the AOAC/ISO/IUPAC harmonized protocol is normally used (IUPAC, 1995) and this is also normally needed for European Committee for Standardisation (CEN) methods; however, there is no regulatory requirement within the EU that it should be used. CEN has been mandated by the European Commission to update the original criteria document (CEN, 1999), which was the basis for these performance criteria within EU legislation. The activities started in April 2008 (van Egmond, personal communication), and need to be finalized by mid-2010. The update involves both data for toxins not dealt with in the original document (CEN, 1999), as well as a critical assessment of the currently existing data.

Recovery As stated in Commission Regulation (EC) No. 401/2006:

> The analytical result must be reported corrected or uncorrected for recovery. The manner of reporting and the level of recovery must be reported. The analytical result corrected for recovery shall be used for controlling compliance.

The regulation goes on to mention both ranges of acceptability of the recovery for mycotoxins by content level; however, it goes into no further detail. In terms of harmonization of approaches, therefore, one further pertinent question arises: is the reported recovery reported in routine analysis determined during the validation process or the one obtained in the routine analysis batch? A further point to consider is that from an analytical point of view, it is always better to perform the determination of the recovery with a certified reference material (CRM) in an incurred sample. Unfortunately, only a few CRMs (combinations of matrices and mycotoxins) are available. Consequently, it is rarely possible for a CRM to be available for the recovery determination. In this event, following Commission Decision 2002/657 EC, if there is no CRM available, the recovery should be calculated by using a fortified blank matrix within a defined scheme. In the case of mycotoxins such a scheme does not exist. It should also be remembered that CRMs have their limitations. If they are produced on an incurred basis (naturally contaminated), nobody knows the true content. The certified value is usually established by correcting the consensus value found, with the recovery factor (obtained with a spiked blank – hence the same problem). A further factor to consider is cost. CRMs are relatively expensive and there use would be seen as to be restricted to periodic checks on the analytical system rather than as a control sample in a regular series of samples for routine analysis.

In terms of recovery, therefore, a number of areas are in need of harmonization; these include:

- A common route to determine recovery, taking into account the level(s) at which it should be determined. This (these) level(s) should be established for both regulated and unregulated toxins with priority being made for the regulated toxins. A simple approach would be to determine the recovery at the regulatory limit or the limit specified in a contract.
- How recovery in routine analysis should be reported, e.g. determined during the validation process or in the batch undergoing analysis?
- There is a need for a harmonized way to prepare spiked samples and also in their frequency of use. It has to be decided whether every test sample (e.g. every 50 g) has to be spiked at various levels or a spiked bulk sample (e.g. 10 kg) has to be spiked and then every test sample has to be sampled from this bulk sample. In terms of laboratory practice, the latter option would be undesirable. It would lead to the need to thoroughly homogenize an additional 10 kg sample, after spiking, which would be cumbersome.
- Continuing on the question of spiked samples, what might also be important is how to spike dry materials, how long to dry and how to ascertain their homogeneity after spiking. Practice shows that these issues differ from one toxin to the other (e.g. for fumonisins, the drying time is quite critical for some maize-based foods). Experimental work may be needed here before recommending a practice.

Calibration: Commission Regulation (EC) No. 401/2006 does not specify any particular calibration method that should be used. Thus, with the exception of official methodologies (e.g. those governed by a particular norm), each laboratory is legitimately able to adopt its own calibration methods. A number of approaches are available; these include: matrix-assisted calibration, a calibration curve in a solvent or a matrix-matched calibration.

The advantages and drawbacks of each of these methods of calibration require discussion and should be taken into account before any defined harmonized approach to calibration is undertaken. The actual technique used for mycotoxin determination (HPLC-UV, HPLC-FLD, LC-MS/MS, immuno-assay, etc.) must also be taken into account in this exercise, since matrix effects can be more severe in some cases than in others. It should also be noted that CEN methods are usually quite specific in this respect. EU legislation does not prescribe the use of CEN methods, however Article 11 Council Regulation 882/2004 (as amended) states that if no method of analysis or sampling is set out in legislation, preference should first be given to,

> internationally recognised rules or protocols, for example those that the European Committee for Standardisation (CEN) has accepted or those agreed in national legislation.

Alternative methods (including rapid methods) could therefore follow this standardized approach.

In terms of other issues relating to calibration, two further items that merit consideration for harmonization concern:

- The process by which calibrants (e.g. reference solutions of particular analytes) are considered suitable, in particular with regard to use of recognized certification bodies.
- 'Goodness of fit' (r^2) – values should be harmonized for each mycotoxin according to the type of instrument and the *type of calibration curve* (What is to be considered satisfactory: 0.9999 or 0.999 or 0.99?).

Accreditation Bodies

Laboratories providing information for regulatory purposes or in connection with 'analyses critical to food safety' are expected and/or required to be accredited to an international standard (usually ISO 17025:2005; ISO, 2005). Discussions within the working group have revealed that different national accreditation bodies appear to interpret the standard in different ways – placing different requirements on laboratories to demonstrate compliance. Examples included:

- routine recovery checking at each batch of analysis or following a rolling programme,
- control charts, – number of quality controls that should be introduced in routine analysis,
- what type of quality control charts, use of quality control charts, definition of an out of control situation, and
- number of proficiency tests that should be performed by year,

It would be of benefit to all concerned if these aspects could be harmonized.

Measurement uncertainty

Reference has already been made to uncertainty of measurement (or measurement uncertainty). The uncertainty of measurement is defined according to IUPAC and ISO (ISO, 1993; IUPAC, 1997) as the parameter:

> Associated with the results of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand.

This implies that the elements of uncertainty must include all effects that can be attributed to random events which cause the dispersion of results, the 'precision', and those attributing to systematic effects, the 'bias'. Thus, it includes effects ranging from the preparation of standards (e.g. weighing, volumetric glassware and temperature) to the drift of a detector signal during an analysis sequence and signal integration (Stroka & van tgqcia, 2006). This is an important topic for a number of reasons, including:

- EU legislation on mycotoxins requires an uncertainty statement for official food control purposes.
- It is a requirement for the testing laboratory to be accredited for a particular method.
- Measurement uncertainty can be used as a tool to assess the suitability (the fitness-for-purpose) of a particular method.
- The measurement uncertainty can be used both to compare results and to assess their reliability.

Measurement uncertainty has been defined in the 'GUM' guide (ISO, 1995) and this definition should be followed to determine the uncertainty value associated with an analysis. In some aspects the current guide lacks defined procedures (e.g. number of samples to be taken). Given differing laboratory practices, the contribution of in-house practices, and whether or not to include them should also be considered. Furthermore, the ISO 1995 guide works on the principle of a 'bottom-up' approach. Uncertainties from all relevant steps of the procedure are combined to calculate the total uncertainty. In reality, this is not practical, and a 'top-down' approach is preferred (use of method validation data).

Conclusions

Sampling

For many mycotoxin/commodity combinations, maximum levels of permitted contamination are set out in law. Correct enforcement of the law or of material specifications relies both on the appropriate analytical methodology and on the securing of a representative sample from any lot of material subject to inspection. The distribution of mycotoxins within a commodity is known to be heterogeneous. Furthermore, there is increasing evidence to show that the degree of heterogeneity is a function both of the commodity and of the mycotoxin of concern.

Substantial differences in sampling methods exist between those used by enforcement agencies and food businesses; these differences extend between different food businesses within the food chain. In themselves these differences have the potential to confound any analytically based regime not only for securing compliance with either legal or commercial requirements but also for food business to demonstrate due diligence within their food-safety management systems.

A further area of concern is the need for laboratories to demonstrably show that their methods for homogenizing samples both in terms of producing sub-samples for regulatory enforcement and before analysis are robust. The question of sample communition as part of the sample preparation process has been discussed by others, including Spanjer *et al*. (2006) who compared dry milling to slurry mixing. This requirement is critical in assuring that truly representative data are obtained. Given what is already in the public domain, this needs to be done on a commodity/mycotoxin basis. One aspect to be considered would be to define and harmonize criteria used to assess (e.g. by granulometry of the ground sample) the homogeneity of a laboratory sample.

Method performance and measurement uncertainty

Together with sampling, method performance and measurement uncertainty is the second key factor to obtain reliable analytical data on which to base both regulatory/enforcement and commercial decisions. As outlined above, there are a large number of parameters which need to be harmonized not only for a better and efficient way to validate the methods of mycotoxin detection (screening and confirmation) but also to enable a better comparison of methods' performance. Harmonization in these areas will provide stakeholders with tools based on a greater fitness for purpose. Any harmonized validation scheme must also take into account the cost of the validation in order to provide efficient, relevant and cheap methods for routine analysis.

Acknowledgement

The authors were supported by funds provided through the EU Sixth Framework Network of Excellence Project 'MoniQA' (Contract Number Food-CT-2006-036337).

References

CEN (1999). *Food Analysis-Biotoxins – criteria of analytical methods of mycotoxins* CEN Report CR 13505, Brussels, Belgium.

Charmley L.L., Trenholm H.L, Prelusky D.B., Rosenberg A. (1995) Economic losses and decontamination. *Natural Toxins*, **3**, 199–203.

Commission Decision 2002/657 EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Official Journal of the European Union L125, 10-32

Commission Decision 2006/504/EC of 12 July 2006 on special conditions governing certain foodstuffs imported from certain third countries due to contamination risks of these products by aflatoxins Official Journal of the European Union L199, 21-32

Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Official Journal of the European Union L70, 12-34

Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union L364, 5-24

Council Regulation (EEC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food. Official Journal of the European Communities L37, 1-3

Council Regulation (EC) No 882/2004 (as amended by Commission Regulation 776/2006) on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. Available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2004R0882:20060525:EN:PDF Last accessed 10 June 2008

European Commission (2007) *The Rapid Alert System for Food and Feed (RASFF). Annual* Report 2006. Available at http://ec.europa.eu/food/food/rapidalert/report2006_en.pdf Last accessed 10 June 2008

European Commission – DG SANCO (2008) Guidance document for competent authorities for the control of compliance with eu legislation on aflatoxins. Available at http://ec.europa.eu/food/food/chemicalsafety/contaminants/aflatoxin_guidance_en.pdf Last accessed 10 June 2008

Hook S.C.W. (2004) Project Report 349: A national grain sampling and analysis system for improved food marketing and safety,Home Grown Cereals Authority, London, 92pp.

International Organisation for Standardisation – (ISO) (1993). International vocabulary of basic and general terms in metrology (ISO VIM DGUIDE 99999). Available at http:/www.iso.org Last accessed 10 June 2008

International Organisation for Standardisation (ISO) (1995). *Guide to the expression of uncertainty in measurement (GUM), ISO/IEC Guide 98:1995.*. ISO , Geneva.

International Organisation for Standardisation – (ISO) (1999). *Cereals, pulses and milled products – Sampling of static batches, ISO 13690:1999.*. ISO , Geneva.

International Organisation for Standardisation – (ISO) (2005). *General requirements for the competence of testing and calibration laboratories. ISO/IEC 17025:2005.*. ISO, Geneva.

International Union of Pure and Applied Chemistry – IUPAC (1995) Protocol for the design, conduct and interpretation of method performance studies. *Pure and Applied Chemistry*, **67**, 331–343.

International Union of Pure and Applied Chemistry – (IUPAC) (1997) Compendium of Chemical Terminology. Available at http:/www.iupac.org/goldbook/U06553.pdf (accessed 25. June 2006)

MacArthur R., MacDonald S., Bereton P., Murray A. (2006) Statistical modelling as an aid to the design of retail sampling plans for mycotoxins in food. *Food Additives and Contaminants*, **23**, 84–92.

Maestroni B., Bettaglio M., Ambrus A., Rathor M.N. (2005) Estimation of the uncertainty of sample processing for the analysis of fumonisin FB_1 in maize. *Proceedings of the BCPC International Congress: Crop Science and Technology*, **1**, 411–416.

MoniQA (2007) www.moniqa.org

National Association of British and Irish Millers (nabim ^{TM}) (2005) Recommended code of practice for mill intake Available at http://www.nabim.org.uk/images/pdf/Recommended%20Code%20of%20Practice%20for%20Mill%20Intake%20-%20Feb%202005.pdf Last accessed 10 June 2008

Ramsey M.H., Lyn J., Wood R. (2001) Optimised uncertainty at minimum overall cost to achieve fitness-for-purpose in food analysis. *Analyst*, **126**, 1777–1783.

Rapid Alert System for Food and Feed – RASFF (2008) Available at http://ec.europa.eu/food/food/rapidalert/index_en.htm Last accessed 10 June 2008

Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal of the European Communities L31, 1-24

Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs. Official Journal of the European Union L226, 3-21

Solfrizzo M., Alldrick A.J., van Egmond H.P. (2009) The use of mycotoxin methodology in practice: a need for harmonization. *Quality Assurance and Safety in Crops & Foods*, **1**, 120–131.

Spanjer M.C., Scholten J.M., Kastrup S., Jorissen U., Schatzki T.F., Toyofuku N. Sample communition for mycotoxin analysis: dry milling or slurry mixing? *Food Additives and Contaminants*, **23**, 73–83.

Stroka J., van Egmond H.P. (2006) How to deal with measurement uncertainty in routine mycotoxin determination. In: *The Mycotoxin Factbook* eds Barug D., Bhatnagar D., van Egmond H.P., van der Kamp. J.W., van Osenbruggen W.A., Visconti A. Wageningen Academic Publishers, Wageningen, the Netherlands. pp. 295–310.

Whitaker T.B., Johansson A.S. (2005) Sampling uncertainties for the detection of chemical agents in complex food matrices. *Journal Food Protection*, **68**, 1306–1313.

