Quality assurance in mycotoxin analysis

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In recent years considerable attention has been directed towards improving and ensuring the quality of analytical data on contaminants in foods and animal feeds. Whether the data are used for assessing risk from exposure (food surveillance), for food control (regulatory monitoring) or for monitoring standards for trading purposes, it is critical that contaminants be identified correctly and that quantitative data be reliable. These requirements also apply to mycotoxins, which present unique analytical challenges in terms both of obtaining truly representative samples (Park, Njapau and Coker, 1998) and of undertaking analysis at the low regulatory limits of control (µg per kilogram and sub-µg per kilogram levels) required in many countries.

SAMPLING FOR MYCOTOXIN ANALYSIS

A number of papers (Park and Pohland, 1989; Gilbert, 1996; Park, Njapau and Coker, 1998) have identified the particular problems associated with the sampling of commodities for mycotoxin analysis and have reviewed the sampling schemes being used by various organizations. Unlike analytical methods, sampling schemes cannot be collaboratively tested; usually a particular sampling plan is proposed, based on statistical consideration of the measured toxin distribution, and thereafter adopted as an official procedure. It is important to understand that sampling plans have diverse objectives, and an acceptable sampling plan for quality control purposes may be very different from a plan intended for use in enforcement. In choosing to adopt a particular sampling plan, in addition to ensuring that it is based on sound statistical principles, practical considerations must be taken into account as there is little point in adopting a procedure that is so labour-intensive it becomes too costly to implement. General guidance is available from international organizations such as the Codex Alimentarius Commission (CAC, 1987) on the factors that should be taken into account when considering sampling schemes. In enforcement situations, there is some merit in specifying both the sampling scheme and the regulatory limit, so as to avoid dispute between parties on the level of mycotoxin contamination in a particular commodity when the individual samples have been taken in different ways.

The United States Food and Drug Administration (USDA) has well-defined sampling procedures for aflatoxins (Park and Pohland, 1989). These take account of the commodity type, whether samples are to be taken from retail or bulk commodities and, in the latter case, the lot size. For each circumstance the minimum number of subsamples to be taken is specified, as is the minimum unit size of each subsample. In addition, the equipment used for grinding and mixing of the bulk sample is specified, as is the subsample weight to be taken for analysis and the manner of its collection. In the United Kingdom, a study of aflatoxin contamination in a consignment of dried figs (Sharman *et al.*, 1994) led to a sampling plan based on the distribution of aflatoxin contamination as well as on practical considerations. Comparison of the United Kingdom, Netherlands and United States sampling plans (Whitaker *et al.*, 1995) gives an insight into sample sizes and the trade-off between consumer and producer risks.

VALIDATED ANALYTICAL METHODS

Validated analytical methods are those that have been subjected to collaborative trial assessment and for which performance characteristics such as recovery, repeatability (r) and reproducibility (R) have been determined. Such validation is intrinsically time-consuming as it takes several months to organize a trial and there are also lengthy procedural requirements, which have to be undertaken by official organizations (such as the European Standardization Organization, the European Committee for Standardization [CEN] and the Association of Official Analytical Chemists International [AOAC International]), that scrutinize the proposed methods. The *Official methods of analysis of AOAC International* have validated methods covering aflatoxins, deoxynivalenol, zearalenone, ochratoxin A, sterigmatocystin, patulin and fumonisins (AOAC International, 1995). A number of these methods date back to the 1970s and, although they can be reliable, when they are based on thin layer chromatography (TLC) they would not, today, be the favoured approach for many laboratories. In some instances, limits of detection are also inadequate to meet the increasingly stringent demands for measurement at low levels.

The collaborative testing of methodology requires considerable planning in terms of the design of the trial, the type of matrix or matrices to be analysed, the level of contamination of the mycotoxin of interest, and the numbers of samples that are to be included in the trial. Naturally contaminated materials are required for which homogeneity as well as stability of the mycotoxin during the period of the study have to be demonstrated. The requirement for blind duplicates, recovery experiments, supply of standards, minimum numbers of participants and the outline of the study protocol are set out in the International Organization for Standardization (ISO)/ International Union of Pure and Applied Chemistry (IUPAC)/AOAC harmonized protocol for collaborative studies (Horwitz, 1995).

After six to nine months' work in undertaking the trial and evaluating the data, assuming that the results are satisfactory in terms of acceptable r and R values, the report of the trial has to undergo extensive peer review before final adoption as an official method. The length and overall cost of this evaluation process, coupled with the fact that not all collaborative trials yield the desired results, help to explain the lag between methodological innovation and adoption as official methods.

PROFICIENCY TESTING

Proficiency testing is a means of continuous objective assessment of the ability of a laboratory to produce accurate and reliable results. In proficiency testing, laboratories receive samples for analysis at regular intervals, report the results to the scheme organizers, and are then given an assessment of their performance. The identities of the laboratories involved are kept confidential, although each laboratory receives a report with useful indications about the overall performance of all participants and information on performance related to the methods of analysis that have been employed.

Proficiency testing should be regarded as an integral part of accreditation and should be seen as providing valuable information for internal purposes, as well as being an indicator of laboratory performance for third parties. In proficiency testing (unlike collaborative trials) all participants use the methodologies to which they are accustomed and which are in everyday use in their laboratories. Ideally, both the matrix and the analyte should be routinely tested in the laboratory, and participants should be discouraged from undertaking proficiency testing with unfamiliar materials. Frequently, however, the desired matrix may not be available and the best approximation has to suffice.

The requirements for establishing and running proficiency testing schemes are stipulated in an ISO/IUPAC/AOAC International Harmonized Protocol (Thompson and Wood, 1993) and in ISO Guide No. 43. A number of national and international commercial schemes are run on a regular and systematic basis. For example, in the United Kingdom, a proficiency testing scheme called the Food Analysis Performance Assessment Scheme (FAPAS) has been operating since 1990, and has included mycotoxins among the analytes that it tests. FAPAS has expanded rapidly since its inception and, as of mid-1998, had some 600 participating laboratories in 50 countries worldwide (Key *et al.*, 1997). Proficiency testing has also been organized by the World Health Organization (WHO) under the Global Environmental Monitoring System (GEMS) programme, but sample distribution has been more sporadic under this than under commercial schemes (Weigert *et al.*, 1997).

Proficiency testing schemes require procurement or production of materials that closely resemble those experienced in practice. After blending or preparation of these materials into a suitable form for distribution, homogeneity testing needs to be carried out to ensure uniformity of samples. Test materials are distributed to participants, analysis is undertaken using the normal method of analysis and results are reported back to a Secretariat within one month. The results must be processed rapidly for maximum benefit to participants. In FAPAS, a report is issued within one month, giving all results for the round and individual performance scores (known as z-scores) for each analyte. The z-scores are calculated on the basis of the "true" value of the analyte and the standard deviation expected at that concentration (from collaborative trial data or the Horwitz curve). A z-score of + 2 is deemed satisfactory, a z-score between - 2 and - 3 or - 2 and + 3 is deemed questionable. Z-scores outside this range are unsatisfactory.

CERTIFIED REFERENCE MATERIALS

Although they cannot substitute interlaboratory comparisons, certified reference materials do offer the possibility of demonstrating both the accuracy and the precision of a new method using naturally contaminated materials (Boenke, 1997; Gilbert, 1988). Two powdered-milk reference materials, which are available from the European Community Bureau of Reference Materials (BCR), have certified low level contents of aflatoxin M_1 (van Egmond and Wagstaffe, 1987). Peanut butters containing certified levels of individual aflatoxins are also available from BCR (Gilbert *et al.*, 1991), as are wheat and maize samples naturally contaminated with deoxynivalenol (Gilbert, 1995),

animal feedstuffs containing certified levels of aflatoxins (van Egmond *et al.*, 1994) and wheat containing ochratoxin A. The production of pig kidneys containing certified levels of ochratoxin A has proved more difficult to prepare and certify (Wood *et al.*, 1997; Williams *et al.*, 1998). Projects are currently under way on the development of cereals containing certified contents of fumonisins (Visconti *et al.*, 1996) and zearalenone. This increasing range of certified mycotoxin reference materials should, in future, offer greater possibilities for rapid assessment of new methodology, for both different toxins and different matrices.

CONCLUSIONS

The analysis of mycotoxins in foods and feeds is now far less "hit and miss" than was once the case and there are now adequate quality assurance means in place, both to assist laboratories to get accurate and reliable results and to check and demonstrate consistent satisfactory performance. The adoption of best practices in sampling and the use of validated methods, together with accreditation and participation in proficiency testing, are recommended means of ensuring the recognition of mycotoxin results worldwide.

Assistance by international organizations such as FAO may be necessary, particularly in developing countries, to stimulate and implement the necessary infrastructure. ◆

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Summary/Résumé/Resumen

Quality assurance in mycotoxin analysis

To ensure that representative samples of foods and feeds are taken for analysis, it is important to follow the stipulations set out in sampling plans – many of these plans are now encompassed as part of the regulations for controlling mycotoxin contamination. Validated analytical methods are those for which performance characteristics have been established by interlaboratory collaborative trials and these are now widely accepted as being essential for monitoring and regulatory purposes. In addition to employing validated methods, internal quality control procedures need to be implemented in chemical laboratories – this normally implies accreditation, participation in proficiency testing and the proper use of control and reference materials. This paper presents an overview of all these important aspects of quality assurance for mycotoxin analysis.

Assurance de qualité dans l'analyse des mycotoxines

Pour s'assurer que les échantillons de produits alimentaires et fourragers représentatifs soient prélevés pour être analysés, il importe de suivre les stipulations des plans d'échantillonnage, dont beaucoup font à présent partie des réglementations de contrôle de la contamination par les mycotoxines. Les méthodes analytiques validées sont celles pour lesquelles des caractéristiques d'efficacité ont été établies lors d'essais interlaboratoires et il est à présent largement reconnu qu'elles sont essentielles aux fins de surveillance et de réglementation. Outre l'emploi de méthodes validées, des procédures de contrôle de qualité internes doivent être appliquées dans les laboratoires chimiques – ce qui implique habituellement leur accréditation, leur participation à des essais d'aptitude et l'utilisation appropriée du matériel de contrôle et de référence. Le présent document expose tous ces aspects importants de l'assurance de qualité pour l'analyse des mycotoxines.

Garantía de la calidad en el análisis de las micotoxinas

Para garantizar que se tomen muestras representativas de alimentos y piensos a fines de análisis, es importante seguir las normas establecidas en los planes de toma de muestras: muchos de ellos se hallan ahora incorporados como parte de la reglamentación para el control de la contaminación por micotoxinas. Por métodos de análisis validados se entienden aquellos cuyas características de eficacia se han determinado de acuerdo con ensayos colaborativos entre laboratorios y que ahora son generalmente aceptados como indispensables a efectos de control y reglamentación. Además de emplear métodos validados, en los laboratorios químicos es menester aplicar procedimientos internos de control de la calidad: esto supone normalmente la acreditación, la participación en ensayos de aptitud y el buen empleo de materiales de control y de referencia. En este artículo se ofrece una descripción general de todos esos aspectos, tan importantes para garantizar la calidad en el análisis de micotoxinas. ♦