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Acceptance of analytical methods for implementation of EU legislation with a focus on mycotoxins

Elke Anklam [a,*], Joerg Stroka [a], Achim Boenke [b]

[a] European Commission, Joint Research Centre, Institute for Health and Consumer Protection, Food Products Unit, I-21020 Ispra, Italy
[b] European Commission, Directorate General Research, Life Sciences, Health, Food and Environment, 200, Rue de la Loi, B-1049 Brussels, Italy

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Abstract

This paper gives an overview on current strategies and prospectives of analytical methods as a tool for implementation of regulatory limits and trade specifications. Examples are given on mycotoxins that have been selected on the basis of being currently relevant contaminants in food and animal feed. Further information is given on methodological demands and limits, legal and normative frameworks and commonly accepted procedures for the implementation and acceptance of analytical methods as 'confirmatory methods', 'screening methods', and strategies for measurement as well as for prevention.

1. Introduction

Analytical methods used by enforcement laboratories for the implementation of legislation must be subject to validation procedures, in order to show that the method produces reliable results. These methods need to provide accurate, repeatable and reproducible results within and between different laboratories. This is especially important in view of legal actions, trade specifications as well as monitoring or risk assessment studies. In addition to the analytical part of data generation, reliable sampling plans are also needed. If the quality of sampling is questionable, a not reliable statement can be made for the analytical data obtained, independently of the quality of the analytical method. It is crucial to make such decisions on the basis of reliable data, especially in the case of risk assessment, prevention strategies (e.g. HACCP approaches) and legislative actions (e.g. regarding maximum levels of a contaminant or additive), which may otherwise generate serious problems in political decision making processes.

A recent review on the occurrence of aflatoxins in food products has shown that aflatoxins can be found frequently in food products at levels that are of substantial concern (Pittet, 1998). Thus, systematic and comprehensive monitoring of aflatoxins for consumer protection is a big challenge especially in the future, since an increased production of food products can be estimated; e.g. the world peanut production doubled within the last 20 years to 29 million tonnes (FAO, 2000).

Since the discovery of mycotoxins, several methodologies for their determination have been developed. Methods routinely used nowadays are mainly based on either thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) or enzyme linked immuno sorbent assay (ELISA).

Due to the demand for reliable and comparable methods, performance requirements have been established at national and international level for implementation as official methods, e.g. by European regulations, the European Committee for Standardisation (CEN) or the Association of Official Analytical Chemists International (AOAC International).

Thus any method proposed to become official must be validated in a collaborative trial study, resulting in defined method performance characteristics (CEN, 1999), while the framework for the design and conduction of such collaborative trial studies as well as the statistical evaluation are also defined in appropriate protocols (Horwitz, 1995). Any method that has been successfully validated according to these protocols can be recognised as an official method for use in legal cases or for international trade purpose. In

* Corresponding author. Fax: +39-0332-78-5930.
E-mail address: elke.anklam@jrc.it (E. Anklam).

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addition to these performance criteria, economical and prevention strategy aspects have also lately become important in method development. Demands for fast and efficient procedures (consumption of chemicals and materials) and the ability for automatisation are highly desired features (van Rhijn, Viveen, & Tuinstra, 1992).

This paper is aimed at providing a brief description of the occurrence of mycotoxins in food and animal feed, legal limits and the impact on human and animal health; an overview on state-of-the-art analytical methods for various mycotoxins; principles of method validation, and the importance of proficiency testing and accreditation for the implementation of legislation, including prevention strategies such as the HACCP-approach. Hazard analysis of critical control points (HACCP) is a quality control tool in food processing, flexible enough to be applied by different companies, plants, processes or other. It has been widely used since about five year ago in the food industry to prevent food safety hazards or to ensure product quality (Unnevehr Laurian, 2000). The HACCP approach consists of seven basic principles, and is a scientific, rational and systematic approach used to ensure that food is safe to consume, by identifying, assessing and controlling any hazards encountered during the manufacturing, production, processing, preparation and use of food materials. The European Commission, Quality of Life and Management of Living Resources Programme (QoL), Key Action 1 on Food, Nutrition and Health is currently supporting a mycotoxin-cluster consisting of three projects aiming at the development of prevention strategies for mycotoxins (ochratoxin A and *Fusarium* mycotoxins) entering the human and animal food chain. The work within this cluster is being carried out in the framework of Hazard Analysis to identify critical control points at which these enter the food chain and how successful prevention strategies can be developed.

2. Importance of mycotoxin control

Mycotoxins are secondary metabolites of fungi. Their presence in food and feed are a health risk for animals and humans. They can be found in a diverse range of food and feed due to invisible spoilage in the field during plant growth, harvesting, storage and processing. It can be assumed that about 20% of food products (mainly of plant origin) is substantially contaminated. Some of the most relevant mycotoxins (e.g. aflatoxins and ochratoxin A) can be detected in food products and animal feed deriving from developing countries. A total of about 300 different mycotoxins have been described (Cole & Cox, 1981) that are produced by about 200 different fungi. However, only a small fraction of approximately 20 out of these 300 mycotoxins are normally found in food and feed at levels that are considered to be a health risk for humans and animals. Among these, the aflatoxins represent the main worldwide threat, due to their occurrence and toxicity. Aflatoxins are produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* and were discovered in the early 1960s, when more than 100,000 turkeys died in the United Kingdom because of contaminated animal feed produced in Brazil. Aflatoxin B1 was found to be the cause of the so-called ''Turkey-X Disease'', and subsequent research has shown it to be the strongest natural carcinogen in animals. This is underlined by epidemiological studies that demonstrated a correlation between the content of aflatoxins in food and human primary liver cancer, especially in areas with a high incidence of hepatitis B. Aflatoxins are therefore classified as human

Table 1
Results of surveys on aflatoxin contamination (according to Pittet, 1998)

Commodity	Country (analysis)	Incidence (%)	Max value (ng/g)	Year of publication
Almonds	USA	1	372	1993
Brazil nuts	USA	17	619	1993
Chillies	Pakistan	66	80	1995
Corn	Argentina	20	560	1996
Corn	India	47	666	1997
Corn	Venezuela	14	50	2000
Copra meal	Philippines	100	186	1995
Cottonseed	UK	71	25	1997
Figs (dried)	Austria	13	350	1993
Nutmeg	Japan	43	666	1993
Peanuts	India	45	833	1996
Peanut meal	India	97	6280	1995
Pistachios	Qatar	48	274	2000
Pistachios	NL	59	165	1996
Rice	Ecuador	9	40	1997
Soy beans	Argentina	10	36	1991
Various nuts	Qatar	23	289	2000
Wheat	Uruguay	20	20	1996

carcinogens by the World Health Organisation, and a no lowest safe dose for aflatoxin B1 has been established (W.H.O., 1979). It is only recommended to reduce the intake to levels as low as reasonably possible.

The occurrence of aflatoxins is shown in Table 1.

Other commonly known and health relevant mycotoxins are fumonisins, ochratoxin A, trichothecenes (e.g. nivalenol, deoxynivalenol, T-2 toxin), zearalenone, and patulin. Worldwide legislation has been put in place in order to limit the intake of aflatoxins.

3. Legislative frameworks on analytical methods

Mycotoxins are regulated in more than 77 countries worldwide (FAO, 1995), while regulations vary from country to country on the type of mycotoxin, matrix (type of food or feed) as well as the maximum allowed level. As a result of the establishment of the European Union (EU) and the aim for harmonisation of the common market within the EU, regulations concerning certain contaminants (e.g. Regulation 466/2001) were drafted on the basis of articles 94 and 95 (former articles 100 and 100a) of the *Treaty establishing the European Community*. These articles state that the European Commission may propose directives for the drafting of such laws, regulations or administrative provisions of the Member States that affect the establishment or function of the common market and that scientific facts, consumer health and safety will be taken into account for the harmonisation of the common market. Regulation 466/2001/EEC sets limits in food products, while Directive 98/53/EEC defines acceptable sampling and analysis methods (European Commission, 1998a, 2001). Contamination limits of 2 ng/g aflatoxin B1 allowed in food products such as cereals, peanuts, pistachios and figs marketed in Europe are five times lower than those in the US.

However, not only legislative limits have been regulated, but also requirements for laboratories that are involved in the official control of foodstuffs as well as for sampling and analysis methods have been defined. The most relevant Directives in this sense are:

- Directive 85/591/EEC that lays down the framework for sampling and analysis methods on community level (European Council, 1985).
- Directive 89/591/EEC and Directive 93/99/EEC which define general principles for the official control of foodstuffs (European Council, 1989).
- Directive 98/53/EEC that defines sampling methods and the statistical requirements (method performance) for analysis methods (European Commission, 1998a).

It must be mentioned at this point that method requirements for the use in official control only make sense, when legislative limits exist for the specific mycotoxin. Currently such limits have only been drafted for aflatoxins at community level, while in the future legislative limits will be extended to other mycotoxins. Therefore the current regulation on aflatoxins will serve here as an example: with the introduction of Regulation 466/2001/EEC, legislative limits for aflatoxins were directly linked (with reference to Directive 98/53/EEC) to the sampling method and to requirements on analytical methods to be used for enforcement of food control (European Commission, 2001, 1998a).

Concerning the acceptance of analytical methods, several approaches exist at European Community level. One strategy is the draft of an explicitly defined method as a reference method. In this case the directive contains detailed information on the laboratory equipment and material to be used for analysis (method description), as it has been done in the past for other contaminants/ingredients and quality standards for food additives by Directive 93/28/EEC, Directive 71/393/EEC and Directive 81/712/EEC (European Commission, 1993, 1971, 1981).

Even though straightforward, one disadvantage of such kinds of regulations is the lack of flexibility to adjust, when further scientific and technical improvements are likely to be made. Thus a horizontal definition concerning the performance criteria of methods, as it has been done in Directive 98/53/EEC for aflatoxins, allows the use of different *state-of-the-art* methods (method principles).

As a result, Directive 98/53/EEC lists several performance parameters for methods, such as general requirements laid down in Directive 85/591/EEC, e.g. trueness, specificity, applicability, limit of detection and determination, as well as how these parameters have to be obtained, while additional specific requirements for repeatability (RSD_r), reproducibility (RSD_R) and analyte recovery.

In addition, with reference to Directive 85/591/EEC, the frameworks for conduction of collaborative trial studies for the elaboration of the method performance parameters have to be in compliance with internationally accepted protocols.

Further references are made in Directive 98/53/EEC on how certain method parameters (RSD_r and RSD_R) can be obtained according to the Horwitz equation (Horwitz, 1983). In addition, recovery values in Directive 98/53/EEC are listed in a table. These values are in accordance with the European Committee for Standardisation (CEN, 1999). Within CEN Technical Committee 257, working group 5 selects and elaborates methods of analysis for mycotoxins which become European standards. These standards can be used for monitoring according to legal limits. CEN Report 13505 drafted by the TC257/WG5 is a technical key document for *official control* that gives horizontal guidelines for minimum performance of analytical methods (CEN,

1999). Detailed performance criteria for monitoring the most currently relevant mycotoxins (aflatoxins, ochratoxin A, fumonisins, patulin, deoxynivalenol, nivalenol, zearalenone and several other *fusarium* toxins) are covered. Even though most of these mycotoxins are currently not regulated on a European Community level the report is an important up-to-date reference. The figures given in this report are the result of state-of-the-art knowledge and experience, which is supported by several collaborative studies that have been conducted during the last two decades according to internationally approved protocols.

4. State-of-the-art analytical methods

Currently available TLC, HPLC or ELISA methods have been reviewed (Stroka & Anklam, 2002; Sydenham & Shephard, 1996; Stroka, van Otterdijk, & Anklam, 2000) and it was concluded that, for example, for aflatoxins, the determination of less than 1 ng/g aflatoxin B1 is no longer an analytical challenge (thus supporting European legislation and most of the other worldwide legislation). For example, a method for the determination of aflatoxins by HPLC in various food matrices at a level of 2 ng/g aflatoxin B1 and 4 ng/g total aflatoxins has recently been successfully validated and is presently in the process of being adopted as an official method (CEN and AOAC International) (Stroka, Anklam, Joerissen, & Gilbert, 2000). The successful validation of methods at such low contamination levels is mainly due to powerful analytical approaches, such as the availability of monoclonal and polyclonal antibodies for mycotoxins (e.g. aflatoxins, ochratoxin A, fumonisins, zearalenone and deoxynivalenol) for ELISA and immunoaffinity column based clean-up steps prior to HPLC or TLC (Scott & Trucksess, 1997; Betina, 1993), as well as improved and well-established detection systems, e.g. for specific derivatisation methods. With HPLC this can be achieved, e.g. for aflatoxins, by post column derivatisation such as bromination (Kok, van Neer, Traag, & Tuinstra, 1986) or irradiation by UV light (Joshua, 1993; Papadopoulou-Bouraoui, Stroka, & Anklam, 2002). As a result, excellent performance of methods using immunoaffinity column clean-up with subsequent HPLC-fluorescence determination of aflatoxins even at very low levels such as 0.1 ng/g has been recently demonstrated in a collaborative trial carried out at an international level (Stroka, Anklam, Joerissen, & Gilbert, 2001).

The immunoaffinity column based clean-up procedures, in particular, boosted not only the performance due to the provision of very clean extracts (absence of interfering substances) but are also applicable for automated sample clean-up (Sharman & Gilbert, 1991).

4.1. Screening methods

Screening detection techniques such as ELISA are currently available, being developed and/or used for major mycotoxins such as aflatoxins, fumonisins, ochratoxin A, zearalenone, and trichothecenes. Their detection limits are comparable with those of HPLC methods. However, a disadvantage is the possibility of false positive results due to cross-reactions and more important the possibility of false negative results. Confirmation by, for example, HPLC-type procedures of doubtful and/or positive ELISA results, is necessary. New approaches for rapid methods are currently made on the basis of biosensors, dip-stick like kits as well as other immunochemistry-based techniques such as surface plasmon resonance (SPR) (www.biocore.com) or the ORIGEN® Technology (www.igen.com), which are based on flow-cells and can be used for a high throughput analysis of samples and consequently successful screening of a large sample throughput in a small time interval, still maintaining reliability. All these different immunochemistry-based applications clearly show the direction of innovation that can be expected in the near future for screening tests as one of the two main pillars of a reliable and cost-effective measurement and prevention strategy.

Other promising approaches are based on non-destructive techniques, such as near- or mid-infrared spectroscopic methods (Kos, 2001; Pettersson, 2001), fourier transform infrared photo-acoustic spectroscopy (FTIR-PAS) (Greene & Gordon, 1999) as well as other methods which do not measure the toxin itself, but indicate that with a certain probability the material is contaminated (Stroka & Anklam, 2002).

4.2. Confirmatory (reference methods)

TLC was the first method principle used for the determination of aflatoxins as well as for some other mycotoxins. However, TLC was more and more replaced in industrial countries by HPLC or GC in the early 1980s, since these techniques offer several advantages compared to TLC such as automation, high performance separation and generally lower detection limits, while on the other hand the instrumental requirements increased as well. Both methods (TLC and HPLC) have in common the chromatographic retention, which is an important specific parameter for each mycotoxin for confirmation. Immunochemical based methods, however, provide high specificities in case monoclonal antibodies are being used.

With the availability of commercially available immunoaffinity columns (IACs) for several mycotoxins such as aflatoxins, ochratoxin A, fumonisins, zearalenone and deoxynivalenol, recent analytical methods combining the analytical advantages of both method-

ologies (immunoselectivity and chromatographic separation) have been developed and validated successfully for many applications (Burdaspal, Ma Legarda, & Gilbert, 2001; Dragacci, Grosso, & Gilbert, 2001; Entwisle et al., 2001; MacDonald, Long, Gilbert, & Felgueiras, 2000; Visconti, Solfrizzo, & De Girolamo, 2002; Visconti, Pascale, & Centonze, 2002). Even though these validated methods apply HPLC as a separation technique, it was shown that the use of TLC in combination with IACs is a promising application that can compare with HPLC concerning the performance requirements (Stroka & Anklam, 2002).

4.3. Method application

Screening techniques (e.g. ELISA, Bio-sensors) allow a high sample throughput at relatively low costs, while in cases of positive or doubtful results, these need to be confirmed by techniques based on HPLC or GC. The following example can be given to illustrate this strategy: 3560 samples were measured for ochratoxin A in Germany from 1987 to 1995, revealing that 2763 were contaminated with ochratoxin A below the limit of detection (LOD), whereas 797 contained various levels of ochratoxin A (European Commission, 1998b). Where only confirmatory type analytical processes such as HPLC applied, the cost would amount to approximately 178,000 EURO. This sum would be significantly reduced if screening (e.g. ELISA) prior to confirmatory analysis were performed, leading to a total sum of about 44,000 EURO only.

However, it is essential that screening techniques are also sufficiently robust and reliable to make their usage attractive and sound, i.e. they need to generate a low percentage of '*suspected false negatives*' (e.g. less than 5%). This can be examined by measuring the relevant mycotoxin in a wide variety of matrices together with validated robust analytical processes. Also only a low number of '*suspected false positives*' is allowed in order to avoid repeating many analyses with confirmatory chemical techniques. This can be checked by analysing the reaction kinetics and/or by the specific blocking of immunoglobulins used by the assay. Specific blocking can, however, only be applied if the assay is carried out by using monoclonal antibodies. Experiments need to be designed where monoclonal antibodies would be used for coating the antibody in the ELISA. Consequently, specific fragments of these anti-idiotypical monoclonal antibodies would then be used to block the paratopes of the coated antibodies, specifically.

The World Health Organisation (WHO) provides a detailed description on important acceptance/validation criteria for kits for immunoassay and other protein binding systems such as: (a) analytical validity; (b) maintenance of assay to assay reproducibility; (c) assessment of random errors; (d) assessment of systematic errors; (e) specificity; (f) stability (WHO, 1980; Stroka et al., 2000; Stroka, Reutter, von Holst, & Anklam, 2002; Stroka et al., 2001). Specific additionally important acceptance/validation criteria can also be formulated for sensors such as: (a) robustness; (b) very little maintenance and calibration; (c) very long lifetimes; (d) stability at different temperatures; (e) selectivity and sensitivity; (f) low answering times; (g) low signal drift in relation to its zero point (Boenke, 1998a).

5. Method validation

In order to obtain reliable results, and hence give consumers and producers confidence in testing methods, there is an urgent need for internationally validated methods, which could serve as confirmatory methods and form the other main pillar in a reliable and cost-effective measurement and prevention strategy. Especially where legal proof may become necessary, analytical methods must be subject to validation procedures. The objective of the method validation is to demonstrate that the defined system (which may include various steps in the analytical procedure, and may be valid for a restricted matrix) produce acceptably accurate, repeatable and reproducible results for a given property. Depending upon the intended purpose of the analysis, i.e. qualitative and/or quantitative screening or quantification, different validation parameters have to be evaluated. As already discussed, a precise quantification of mycotoxins in food products is extremely important in order to demonstrate that the levels of contamination are below the legal limit(s).

However, large-scale collaborative studies require a considerable level of effort and resources and should be conducted only on those methods that have undergone appropriate pre-testing (Fig. 1).

Such pre-tests are designed to define the performance characteristics of a method, and to set target values for the parameters to be evaluated in the validation trials. These data provide information on the expected precision (within laboratory standard deviation), possible systematic error (bias), recovery values (on the basis of spiking measurements), applicability, interference with other compounds and/or matrix components during analysis and best calibration approaches (Table 2).

5.1. Tools for establishing and controlling robust analytical processes

In-house studies are mostly based on a detailed investigation and evaluation of one single analytical procedure by

- studying its applicability for a range of matrices by checking its compliance to various acceptance criteria

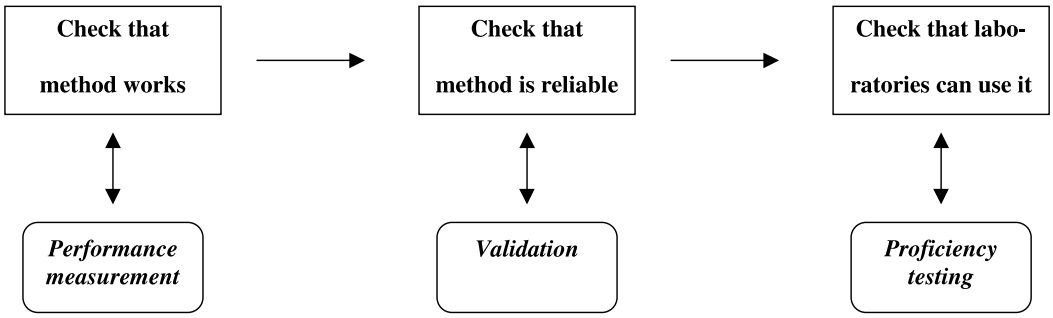

Fig. 1. Performance measurement, validation, and proficiency testing.

Table 2
Parameters determined through performance and validation studies

Term	Description
Specificity	The probability of obtaining a negative result, given that there is no analyte present
Linearity	Proportionality of the signal to the amount of reference material, demonstrated by the calculation of a regression line with the adequate statistical method
Range	Range of analyte concentrations over which the method is considered to perform in a linear manner
Accuracy	The closeness of agreement between a test result and the accepted reference value (ISO 3534-1)
Trueness	The closeness of agreement between the average value obtained from large series of test results and an accepted reference value (ISO 3534-1)
Detection limit	Minimum level from on the presence of an analyte can be measured with a given certainty (e.g. 95%) (DIN 32645)
Quantitation limit	Minimum level from on the analyte can be quantified with a given certainty (e.g. 95%) (DIN 32645)
Robustness	Stability of the method with respect to deliberate variations in the method parameters

(e.g. within-lab, within-day repeatability and within-lab, between-day reproducibility) for spiked and naturally contaminated materials (Luckas, Hummert, Thielert, Kirschbaum, & Boenke, 1994);
- studying its accuracy for a range of matrices by comparing it with an already validated and robust analytical procedure or a certified reference material (CRM).

Ruggedness-tests (Courtheyn, Moermans, Schilt, & Boenke, 1996; Visconti et al., 1999; Werimont, 1985) are a chemometrical approach and offer a cost-effective manner for generation of robust analytical processes. They can reduce the full factorial design of a series of test measurements by equally maintaining the required level of information. Both in-house and interlaboratory studies can make use of this test.

The output from in-house studies and ruggedness-tests is a detailed method description (standard operation procedure) to be distributed to the participants in the actual validation trial study as described below, along with a series of expected parameter values with which the full validation study results can be compared.

Interlaboratory studies (collaborative trial studies) can have different purposes:
- to validate one single analytical procedure or sampling plan applied by different laboratories (e.g. Stroka & Anklam, 1998) and to derive typical performance characteristics (e.g. repeatability, reproducibility, and accuracy);
- to compare different analytical procedures or sampling plans applied by different laboratories (Pettersson, 1998) to identify systematic errors or to certify a mycotoxin content in a given matrix in order to obtain a CRM (see also Section 4).
- Both of the above described types can be organised as the so-called '*step by step approach*'. This approach consists of a series of interlaboratory studies following the different steps of the analytical process. An overview of this approach in the mycotoxin area is presented in the literature (Boenke, 1998b). It is, in particular, preferred when the aim is to certify the content of mycotoxins in different food and feed matrices to obtain a CRM (Boenke, 1995a).

Different examples of protocols and reporting sheets for inter-laboratory studies can be found in the literature (for inter-laboratory studies: IUPAC, 1988, 1995; ISO, 1994; for end-determination step studies: Reynolds et al., 1997a,b; for studies involving the clean-up and the end-determination step based on a single and different analytical procedures Nicholls & Suett, 1994; Reynolds et al., 1998; Thompson & Ramsey, 1995; for sampling plan studies: Coker et al., 1998; for collaborative trials on sampling: Ramsey, Argyraki, & Thompson, 1995; for analytical procedures validation studies: AOAC International, 1995; for method performance needs and standardisation purposes: ISO, 1986).

5.2. Use of certified reference materials (CRMs)

Certified reference materials (CRMs) are the cost effective tools for obtaining and maintaining robust analytical processes. The Bureau Communautaire de Référence (BCR)-, Measurements and Testing (M&T)- and the Standards, Measurements and Testing (SMT)-Programmes have produced substantially more CRMs in both the area of food as a whole as well as for mycotoxin analyses, in particular, than any other major CRM-producer (Maier, Boenke, & Mériguet, 1997). CRMs contribute to the validation of analytical procedures and sampling plans, and also satisfy a number of other needs such as making knowledge available for the preparation of inter-laboratory and proficiency scheme test-materials, and in-house quality control materials. Furthermore, they form tools for and help during the implementation of written standards/norms, EU-regulations, laboratory accreditation, laboratory total quality management (TQM), etc. They can also be employed for calibration purposes and for checking the performance of analytical procedures, sensors needed by the different end-users to show that the repeatability of the analytical process is robust and compatible with the repeatability of those of the certifying laboratories. CRMs are currently available for a number of mycotoxins (Boenke, 1997). CRMs also provide an important milestone in the development of robust analytical processes which are composed of screening and confirmatory procedures. They are a cost-attractive tool for validation and quality control of test kits and sensors. Also taking into account the rapid growth of automation in analytical laboratories, CRMs can substantially contribute to quality control of the accurate and precise (i.e. repeatable and reproducible) work of robots used for extraction and clean-up steps. Different examples of protocols for certification exercises can also be found in the literature (European Commission, 1994; ISO, 1985; Taylor, 1985; Wood, Entwisle, Farnell, Patel, & Boenke, 1995).

6. Proficiency testing/accreditation

Proficiency testing schemes are a special form of inter-laboratory studies aiming at the comparison of a laboratory's performance against that of similar laboratories and at the evaluation of the implementation of analytical procedures by analysts in different or in the same laboratories (Maier, Quevauviller, & Griepink, 1993). These schemes are also closely linked and sometimes directly part of the formal accreditation process of analytical laboratories (Esser, 1995; Kohl, 1996). Information on the feasibility of proficiency tests in sampling is discussed by Argyraki, Ramsey, and Thompson (1995).

The so-called '*New Approach and the Global Approach Directives*' in Europe were responsible for the generalised development of accreditation systems. These systems were based on the EN 45001 written standard. This written standard was derived from the existing ISO/IEC Guide 25 (ISO/IEC, 1985), which was used up to this time. Laboratory accreditation can be defined as a formal recognition by an authoritative body of the technical competence of a laboratory to perform tests or calibrations (ISO/IEC, 1996). This accreditation body provides the recognition and acts as third party between the laboratory and its clients. The aim is to establish confidence between these two parties. The overall main objective of accreditation can be summarised as '*once tested, everywhere accepted*' in order to avoid trade barriers. Today, accreditation is developing towards a competitive factor as part of a commercial and survival strategy of laboratories. It has also become mandatory for official food control laboratories in Europe.

Participation in proficiency testing has become systematic for laboratories (especially official control laboratories) over the past few years. The various proficiency schemes are designed to assess qualitative results as well as quantitative analysis capabilities. Several proficiency-testing programmes have been established throughout the world, which aim to identify problem areas within a laboratory, such as outdated instrumentation, provide experience for the participating analysts and help with the implementation of quality control practices. In contrast to a validation study, a proficiency testing programme does not specify which method will be used to determine the result: this is left open to the laboratory to choose. Nevertheless, the use of validated methods is an obligation for accredited laboratories. These tests are valuable tools for assessing the laboratory's analytical performance against a "best practice" benchmark. The organisers of the proficiency programme prepare only the test materials, making sure that the latter are homogeneous and stable (at least during the time the tests are being performed by the participating laboratories). Materials could be spiked with the compound to be analysed in order to provide participating laboratories with a stable and homogeneous material of known composition and contamination, thus assuring that the results can be reliably compared to a reference value. Obviously, naturally contaminated material would be ideal to use.

The results of such tests are returned by the participating laboratories, together with information about the method used, the calibration approach etc., to the proficiency test co-ordinator. The assessment of the laboratories' performance is then performed by comparing the results with the "true" value (e.g. spiked material) or to the combined results of all other laboratories (relative approach). In the case of investigation of qualitative testing capabilities, the number of false positives and

false negatives can serve as a basis for performance assessment.

It is to be kept in mind that accreditation is different from the certification of quality systems, as it is related to the evaluation of quality systems (e.g. quality control and quality assurance schemes) and to technical competence (e.g. to perform reliable analyses). As explained above (see Section 2) proficiency testing schemes are an integral part of accreditation and ensure that technical competence is also maintained in the accredited laboratory. It has recently been reported that the percentage of unsatisfactory results in an accredited laboratory (13%) is lower than that in a non-accredited one (41%) (Cortez, 1999). In addition, a lower percentage of major faults (deviations greater than 100% from the reference value, i.e., more than double, or less than half the reference value) was obtained for results obtain by an accredited laboratory (Cortez, 1999).

7. Early detection and prevention

The early detection of mycotoxins, or the moulds that produce toxins, is a crucial factor for countries where contamination mainly occurs, in order to comply with trade requirements and keep reliable and cost-effective measurement and prevention strategies in place. However, it can be estimated that food authorities and food traders will possibly not be able to control a sufficient fraction of suspicious food products. In fact, it is known that many food authorities currently have a capacity to test approximately only 5–25% of imported food products for mycotoxins.

Especially in the view of current European legislation set for methods of analysis as well as for appropriate sampling (European Commission, 1998a), the major question is the technical feasibility of mycotoxin monitoring, unless new strategies in screening goods prior to analysis, or, even better, if prevention strategies such as those based on the HACCP-approach are put in place or become available. On the one hand these strategies will most likely be based on simple and rapid methods for the identification of suspect goods to minimise and focus on further analytical work. On the other hand simple and exact methods for the determination of aflatoxins are required that can also be applied under simple conditions and would make it possible to minimise economical losses due to import bans or health related costs. However, the question of appropriate and effective consumer protection can not only be answered with the availability of state-of-the-art methods, but must also take into consideration the costs of analysis, the frequency in which an analysis can be applied from the technological as well as economical aspects. Even more important is at which stage of the food chain the control mechanisms can be applied. Every step of the measurement strategies needs to be fully under control in order to make the assessment reliable. All steps must be accurate, repeatable and reproducible in order to generate results that are comparable with those obtained by different analytical processes and lead to a successful and highly cost-effective prevention with no or at least minimum losses of food raw materials for future processes. Any error in any of the individual steps may lead to a large uncertainty and will make the answer less correct, reliable, and less applicable by equally generating high raw material losses and eventual unwanted crises situations.

8. Conclusions

It is important to note that the robustness of the analytical process implies stability over several dimensions; e.g. time, chemical/biological/separative/sensing approach, and including calibration. On-going control steps/mechanisms/programmes often specified as quality control and quality assurance systems/schemes are established to guarantee the robustness of analytical processes in different dimensions. Such systems/schemes make use of general and sometimes very specific criteria related to: e.g. minimum sampling variability (i.e. coefficient of variation (CV) for the sampling step), representative sampling, recovery rates, minimum number of '*suspected false negatives and positives*' (i.e. samples detected by the applied test as being negative or positive but which are either positive instead of negative or negative instead of positive in reality). Further examples for such criteria are: minimum cross-reactivity of immunotechnology based procedures, linearity of the calibration curve, within- and between-day variability of the injection of calibrants, as well as within- and between-laboratory repeatability and reproducibility of results, minimum degree of chromatographic peak separation, etc. (Boenke, 1995b, 1998b; Heitzman, 1994). These criteria are either specified in or related to regulations, by international bodies (e.g. Codex Alimentarius), in norms/written standards, in industrial product requirements, and/or consumer health needs (CEN, 1999; European Council, 1985, 1989, 1993; Pittet, 1995; Verardi & De Froidmont-Görtz, 1995; Wood, 1997). The analyst has various tools at hand which help to ensure that each individual step of the analytical process is in conformity with its specific criteria and is consequently robust.

It has to be assured that the analytical methods used by official food control laboratories produce reliable results. In-house methods can be used as long they are regularly controlled by means of certified reference materials (CRMs) and/or appropriately validated con-

firmatory methods in case screening methods are applied as part of a cost-effective measurement and prevention strategy. This will also finally ensure that reliable data are generated for a robust risk assessment.

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