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Preface

Over the last few decades it has become increasingly clear that mycotoxins play a significant role in food and feed safety. Indeed, mycotoxins have been shown to be the principal threat regarding chronic toxicity. Legislative limits for a range of mycotoxins continue to develop worldwide resulting in an increased number of official controls deriving from national food safety plans and for food trade purposes. This book therefore focuses on recent developments in the determination of mycotoxins and mycotoxigenic fungi in food and feed.

A mycotoxin test procedure is a multi-stage process generally consisting of three steps: sampling, sample preparation and analytical determination. The sampling phase is the largest source of variability of the test procedure. The official sampling protocols are still complicated and very challenging in practical terms. Further extensive research on sampling plans is mandatory, taking into account the real risk to human health together with the economic perspective. New developments in sample preparation focus on faster, environmentally friendly, cost effective and fit-for-purpose extraction and clean-up methods in food, feed, biological tissue and bodily fluids. Screening immunochemical and confirmatory chromatographic analytical methods are widely used; a clear trend towards multi-mycotoxin analysis and more precisely towards LC–MS/MS has been noticed.

Quality assurance in mycotoxin analysis is of the utmost importance. Notwithstanding the general acceptance of the benefits of adopting a performance criteria-based approach, some countries have a regulatory framework which requires the publication of 'official methods' in their own regulations. Food control laboratories should continuously follow actual progress in analysis development and statistical method validation within an accredited quality environment such as prescribed in the ISO 17025 norm. Further attention towards a harmonized method validation procedure is necessary.

In order to understand possible links between mycotoxins and human disease or animal disease outbreaks, it is necessary to measure the exposure to the toxin in question. Advances in analytical techniques have resulted in the development and

use of various biological markers (biomarkers) which allow more accurate and objective assessment of exposure at the individual level. The development and determination of validated exposure as well as mechanism-based biomarkers is critical to reduce the existing uncertainty in the risk assessment of most mycotoxins.

Fungal isolates involved in mycotoxicoses are preferably identified by a polyphasic approach in order to avoid mistakes, starting at genus level and further to species level using a combination of morphological, physiological, nutritional and chemical data. The identification is validated by PCR-based molecular methods which can be considered under two main complementary approaches: by targeting conserved functional genes or regions of taxonomical interest, or by focusing on the mycotoxigenic genes. The possibility of using a highly standardized, rapid and practical DNA barcoding protocol that can be easily used both by researchers involved in species definition studies and by non-experts for practical uses is currently investigated. However, in order to assess the risks related to the presence of mycotoxigenic fungi in food and feedstuffs reliably, one should also investigate whether or not the mycotoxin genes are expressed. Further progress in transcriptomics, proteomics and metabolomics will continue to advance the understanding of fungal secondary metabolism, providing insight into how to reduce mycotoxin contamination of crop plants and the food/feed derived therefrom.

Fungal secondary metabolites, mycotoxins and food safety will continue to be of critical interest to a variety of researchers for years to come. Innovations take place at a rapid pace, for example through new nanotechnology-based biosensing techniques and non-destructive spectroscopic techniques. Furthermore, the discovery of masked mycotoxins and the inherent analytical challenges will be the subject of future research.

Sarah De Saeger

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Sampling strategies to control mycotoxins

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Abstract: Mycotoxins can have adverse effects on human and animal health, productivity, economics and trade. Efficient and cost-effective sampling protocols and analytical tools and methods are needed for the detection and control of mycotoxins worldwide. Effective testing schemes depend on sound analytical methods and on sampling plans that generate results that reflect the actual concentrations present in consignments or lots of produce. Test results can be used to implement regulatory decisions on the suitability of lots of food for consumption or trade. Several studies have been conducted to gain knowledge on the variability of mycotoxins, and enabling the establishment of sampling plans for the control of mycotoxins in several commodities. Some official sampling protocols for the control of mycotoxins in food and feed are discussed, including those formulated by the European Commission for several mycotoxins and by Codex Alimentarius for aflatoxins in peanuts, corn and treenuts. Even when using accepted methods or protocols, there are uncertainties associated with the mycotoxin test procedure. This chapter describes options to reduce the total variability associated with a mycotoxin test procedure, and discusses the design and the performance of sampling plans. Producing safe and good quality food is a prerequisite to ensuring consumer health and successful domestic and international trade, and a key to the sustainable development of national agricultural resources. Therefore, a holistic approach for the control of mycotoxins, which includes the adoption of the best agricultural practices in the field and throughout the whole farm-to-fork chain, the best sampling practices, the use of validated and fit-for-purpose methods, trained professionals, and participation in integrated food control systems is important.

Key words: food control systems, operating characteristics curves, sampling plans, variability, uncertainty.

Objectives

The aim of this chapter is to introduce the reader to the basic issues related to sampling food for mycotoxins and the need for sound sampling plans. The reader is strongly advised to consult the reading guide at the end of this chapter to view the relevant available literature and selected web resources. The authors hope that the information provided in this chapter will stimulate further research into appropriate sampling methods and approaches.

1.1 Food safety and the requirements for international food trade

Agriculture is a dominant component of the global economy and pressure to produce abundant, available and safe high quality foods for the world's ever growing population has had a worldwide impact on agricultural practices. The ability both to detect contaminated food products and to trace their origin is of major concern to regulatory authorities, trading partners and the food industry owing to the rapid increase in cross-border trading of food commodities. The occurrence of mycotoxins in foods can have profound implications especially for developing countries, including health and economic impacts due to losses in grain and other staple foods and diminished animal production. The Food and Agriculture Organization of the United Nations (FAO) has estimated that 25% of crops worldwide are contaminated with mycotoxins and that the food losses amount to one billion tonnes per year. As a result, food security is challenged and trade is hampered. To ensure the safety of food many countries throughout the world have established effective food control systems (FAO, 2007). The problem of aflatoxin residues in food is mainly an issue for developing countries and can best be addressed by targeted international assistance, as advocated by Wagacha and Muthomi (2008) for mycotoxin problems in Africa. The socio-economic status of the majority of inhabitants of sub-Saharan Africa predisposes them to consume mycotoxin-contaminated products either directly, or in processed food at various points of the food chain. Mycotoxins in food affect human and animal health, productivity, the economy and trade. Clearly there is a need for efficient and cost-effective sampling protocols and analytical tools and methods that can be used for the detection and control of mycotoxins worldwide.

It is recognized that international trade in food plays an increasingly important role in achieving food security for many countries. One of the benefits is the availability of a broader choice of nutritious foods for the consumer. Food trade provides exporting countries with foreign exchange, contributing to economic development and an improvement in standards of living (WHO, 2002). FAO has worked for many years on capacity building of food control systems (FAO, 2006) as an effective way to demonstrate the equivalence of food safety systems and food quality, thereby promoting trade. Since much of the food for the developed world is produced by developing countries, it is important that the developed world shares the responsibility to ensure that effective farm to fork food safety systems are in place.

To prevent mycotoxins from entering the food production chain, controls should preferably be applied at the raw materials stage. It is of vital importance that consignments of food are tested to establish that they are 'fit for purpose'. Effective testing schemes depend both on sound analytical methods and on sampling plans designed in such a way that the results generated from analysis of the test samples reflect the actual concentrations present in consignments or lots of produce. The test results can then be used to implement regulatory decisions on the suitability of lots of food for consumption or trade (Whitaker *et al*., 2007b). The underpinning requirement is that the sampling plan adopted to acquire information about the mycotoxin contamination is truly reliable and representative.

1.2 Principles of food and feed sampling for mycotoxin analysis

1.2.1 Sampling food and feed for mycotoxin analysis

The impacts of mycotoxin contamination of agricultural commodities on human and animal health as well as on domestic and international trade are increasingly recognized in both developed and developing countries.

In general, developed countries have enacted regulatory limits to protect consumers from exposure to mycotoxins. In many developing countries, however, regulation is insufficient and certain agricultural commodities, including dietary staple foods, can contain unacceptably high levels of mycotoxins (Whitaker *et al*., 2009). In these countries, maximum limits should be set at a level which is reasonably achievable by following good agricultural and manufacturing practices and is consistent with consumer protection, considering the risk related to the consumption of the food (Cheli *et al*., 2009). Van Egmond provided an excellent compilation of the regulations worldwide relating to mycotoxins (2002, 2007).

The analysis of mycotoxins requires not only validated, reliable analytical methods, and regulatory limits against which the analytical result is compared, but also validated sampling methods that are representative and practicable (easy to apply, quick and cost effective). This is especially important for trade goods or goods that are moved in large quantities (Stroka *et al*., 2004).

In regulatory control, it is important to be able to estimate as accurately as possible the true levels of a mycotoxin in a commodity so that correct decisions can be made about its suitability for consumption. This can only be achieved through the collection of truly representative samples, which requires carefully designed sampling plans. The consequences of using a poorly or inappropriately designed or implemented sampling plan can include health issues, trade rejections, false information for risk assessors and managers and litigation problems.

The adoption of well designed sampling plans and the early detection of mycotoxin-contaminated lots is essential in the food industry to ensure that mycotoxins are excluded from further processing/manufacturing stages. For most commodities, the production and marketing system acts as a mixer where many

different lots are blended together during handling, storage and processing (Pittet, 1995). Issues such as lot traceability, raw material specifications, quality control and quality assurance programmes and training are important in detecting and segregating mycotoxin-contaminated materials at the farm or at the first point of marketing.

The overall objective of sampling is to provide representative samples for analysis, the results of which can be used as a basis for 'fitness for purpose' investigations (Miraglia *et al*., 2005). It is important to understand that sampling plans may have different objectives. For example, an acceptable sampling plan for quality control purposes may be very different from a sampling plan for commodities at harvest. In general, sampling plans may be prepared for monitoring, which means conducting a planned sequence of observations or measurements with a view to obtaining an overview of the state of compliance with food law (EC 882/2004), for surveillance, which means a careful observation of one or more food businesses operators or their activities (EC 882/2004) and for targeted sampling. Monitoring is both a preliminary and a routinely performed activity and should be undertaken to protect the health of the population and to support trade. The number of samples to be collected for monitoring should be proportional to the food consumption rate and take into account the amount of domestic production and the amount of imports. Surveillance is undertaken whenever data from monitoring reveals that standard/legal values have been exceeded and it aims to provide a basis for centralized and qualified feedback (FAO, 2005). Targeted sampling is undertaken when there is a concrete suspicion that mycotoxins are present in excessive amounts, based on previous detection or a history of trade rejections. Targeted sampling focuses on specific sample populations which are either likely to be non-compliant, for example, goods produced or stored under bad conditions or food derived from animals showing clinical signs of intoxication, or are intended for more sensitive consumers such as babies or immunocompromized patients.

The Joint FAO/WHO Expert Committee on Food Additives has considered sampling requirements for the surveillance of mycotoxins (WHO, 2002). The committee noted that very little work has been done to address the need for specific sampling plans for surveys, which are the key to obtaining quality data for risk assessment studies. It was recommended that data for risk characterization should be obtained using effective and validated sampling protocols. The protocols should reflect the selection of the sampling sites within the food chain and geographically, also taking into consideration differences in agro-climatic conditions.

Even with well designed sampling plans, accurate estimation of the mycotoxin concentration in large quantities of bulk commodities is very difficult, owing to the large variability associated with the mycotoxin test procedure, which includes sampling, sample preparation, and analytical steps. Because of the inevitable errors associated with each step of the testing procedure, the mycotoxin concentration in a lot cannot be measured with absolute certainty and individual analytical results, as well as the estimated results for a lot, should always be reported with an estimate of the uncertainty.

1.2.2 Understanding variation

Fungal development and mycotoxin production are 'spot processes' and are significantly correlated to, for example, the type of crop, crop variety, agronomic practices, the weather conditions during growth and harvest, storage and processing conditions and the toxigenic potential of the different mould species.

The UK Home Grown Cereal Authority (HGCA, 2004) showed that grain quality can be extremely variable, not just between fields, but even within individual ears of grain. Grain quality will differ because of various factors, such as soil variations, geographical orientation of the field, sowing date, crop rotation, weather conditions at harvest, machinery used, moisture variation during the day and between trailer loads and variations between dried and undried grain.

Mycotoxins in cereals can originate either in the field during plant growth, or during storage. *Aspergillus* and *Penicillium* are mostly responsible for the production of aflatoxins (AF) and ochratoxin A (OTA), respectively, during storage. Mycotoxins produced under storage are often concentrated in 'hot spots' (Whitaker *et al.*, 1974; Whitaker, 2003; Whitaker and Johansson, 2005) as a result of a sudden fungal attack and can occur when the grain is stored for some time under optimal conditions for both the growth of the fungi and mycotoxin formation. For example, this could happen when moist grain is left for some time before it is passed through a hot-air dryer, with the highest risk of mycotoxin production being in the middle of the bulk lot, or when the grain is being dried from the bottom up; the highest mycotoxin risk in this case is in the undried grain on the top. This generally results in a heterogeneous distribution throughout a lot.

Fusarium species are mainly associated with mycotoxin production during plant growth in wet and cold weather conditions and can produce fumonisins and trichothecenes, for example, deoxynivalenol (DON) and/or nivalenol, and zearalenone. The distribution of *Fusarium* toxins is generally more homogeneous than the toxins produced during storage, being more likely to be attributable to mixing during handling and manipulation at harvest and further stratification during storage and transport. This is supported by the studies by Hart and Schabenberger (1998) and Biselli *et al*. (2008), which showed that DON was spread less heterogeneously than OTA in truckloads of wheat.

Knowledge of the variability of mycotoxins is essential for the design of effective sampling plans. A number of papers have been published on the variability of aflatoxins and other mycotoxins in various commodities, including peanuts (Whitaker and Wiser, 1969; Whitaker *et al.*,1994, 1999), raw shelled peanuts (Whitaker *et al*., 1970, 1972, 1974, 1979, 1994, 1995, 1996a; Vandeven *et al*., 2002), corn (Whitaker and Dickens, 1983; Whitaker *et al*., 1979 1998, 2001, 2007b; Shotwell *et al*., 1974; Johansson *et al*., 2000a), green coffee (Vargas *et al*., 2004, 2005; Whitaker *et al*., 2004; Whitaker and Johansson, 2005a), pistachios (Shatzki, 1995a, 1995b), almonds (Whitaker *et al*., 2006, 2007a), figs (Sharman *et al*., 1994), hazelnuts (Ozay *et al*., 2006) and ginger (Trucksess *et al*., 2009).

It was shown that there was a large variability among the aflatoxin results for ten replicate samples from each of six lots of shelled peanuts, with the maximum result being, for some lots, four to five times the average lot concentration. The

variability tended to decrease as the average lot concentration increased. The distribution of the ten laboratory sample results for each lot was positively skewed, meaning that more than half of the results were below the 'true' (mean) lot concentration. These observations are also generally true for other mycotoxins and other commodities (Whitaker and Park, 1993; Whitaker *et al*., 1998, 2000; Hart and Schabenberger, 1998; Johansson *et al*., 2000a; Cucullu *et al.*, 1986).

1.2.3 Theoretical distributions

In general, increasing the number of sample results improves the characterization of mycotoxin variability and facilitates more accurate mathematical modelling of contaminated food and feed. Several different mathematical models have been evaluated to describe the experimentally observed distributions of mycotoxins in contaminated corn and peanut lots (FAO, 1993). Four different theoretical distributions are generally considered; the lognormal, negative binomial, normal and compound gamma distributions. Except for the normal distribution, these theoretical distributions are positively skewed and have characteristics similar to the observed distributions of mycotoxins (Whitaker *et al.*, 1996b). The suitability of a theoretical distribution to accurately fit an observed distribution of sample test results is measured by a statistical goodness of fit (GOF) test, for example, the Kolmogorov–Smirnov (KS) test, the Chi-squared test or the power divergence (PD) test.

1.2.4 Sampling plan

A mycotoxin sampling plan may be defined as a mycotoxin test procedure that generates a test result, coupled to a defined acceptance/rejection limit, usually a regulatory limit, to which the test result is compared to check whether or not the lot meets the sanitary quality control criteria (Johansson *et al*., 2000a).

A mycotoxin test procedure is a multi-stage process generally consisting of three steps: sampling from the target population, sample preparation and analysis (quantification). Sampling consists of all operations which, applied to a lot of an agricultural product, lead to an aggregate sample/laboratory sample. The sampling step specifies how the sample will be selected or taken from the lot and the size of the sample. Sample preparation is the process of grinding, homogenizing and subsampling in order to obtain an analytical portion (test portion), which is solvent-extracted and analysed using an approved and validated analytical procedure to quantify the mycotoxin concentration.

The mycotoxin concentration of a lot is usually estimated by measuring the mycotoxin concentration in a small representative sample taken from the lot (the laboratory sample). Based on the measured mycotoxin concentration in the laboratory sample, a decision is made about the quality of the lot. For example, in a regulatory environment, decisions will be made to classify the lot as acceptable or unacceptable based upon a comparison of the measured sample concentration to an accepted limit (for example, a legal limit, or a quality control level). If the

sample concentration does not accurately reflect the lot concentration, the lot may be misclassified and there may be undesirable economic and/or health consequences. Sampling plans should be designed to minimize this possibility.

1.2.5 Sample selection

To try to overcome the problem caused by the heterogeneous distribution of mycotoxins in food and feed, the sample selected for analysis should be an accumulation of many small portions, called incremental samples, taken randomly from many different locations throughout the lot (Whitaker *et al*., 1970; Whitaker and Dickens, 1983).

The incremental samples together form an aggregate sample. If the aggregate sample is larger than required for the laboratory sample, it should be blended and sub-divided until the desired laboratory sample size is achieved. This process of sub-sampling is critical and should be carefully conducted in order to ensure that the sample remains representative of the lot. Two frequent errors that can compromise the representativeness of the sample are taking too few incremental samples and taking incremental samples of inadequate mass (samples are too small).

Static sampling

When drawing an aggregate sample from a static container, a probing pattern should be developed so that product can be collected from different locations in the lot. An example of several probing patterns used by the United States Department of Agriculture (USDA) to collect aggregate samples from peanut lots is shown in Fig. 1.1. The ISO 24333:2009 standard (ISO, 2009) requires, in particular cases, eight incremental samples to be taken according to the pattern in Fig. 1.2.

According to Codex Alimentarius (FAO, 1993) the probes should be carefully selected on the basis of the type of container, since all the units should have the same chance of being selected. The ISO 24333:2009 standard gives examples, in Annex B, of the devices that can be used to sample static lots. Examples include manual concentric tapered sampling probes such as open shafts with several apertures, gravity type sampling probes with extension rods and T shaped handles, mechanical sampling devices such as suction or vacuum sampling devices and instruments used to take samples from sacks or bags, including Archimedes' screw sampling probes.

Dynamic sampling

Random sampling can be more nearly achieved when taking increments from a moving stream as the product is transferred from one location to another using, for example, a conveyor belt. The increments should be collected along the entire length and across the entire cross-section of the moving stream (see Fig. 1.3). At regular intervals the flowing stream of product can be diverted, for example from a hopper, into collection vessels. For very large lots this methodology is rather time consuming, since the procedure implies drawing samples at regular intervals of time which may be all night and day, with possible interruptions to the procedure.

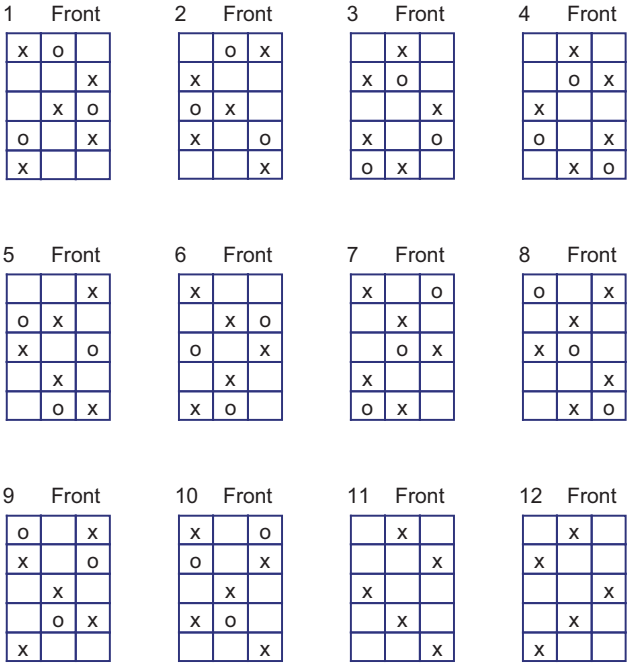

Fig. 1.1 Example of several five- and eight-sampling probe patterns used by the United States Department of Agriculture to sample peanuts for grade.

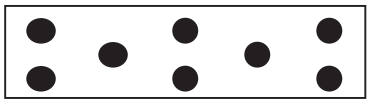

Fig. 1.2 Example of the eight-point probing pattern used for lots from 15t up to 30t according to ISO 24333:2009.

The deployment of automated sampling equipment, such as cross-cut samplers, can greatly assist the process (Codex Alimentarius, 2004).

Whether using automatic or manual methods, small increments of product should be collected and composited at frequent and uniform intervals throughout the entire time that the product flows past the sampling point. According to Pierre Gy (Pitard, 1993) it is important when sampling from a conveyor belt that the sides of the cutting device are strictly parallel and that the cutter traverses the entire stream at uniform speed, resulting in an equal representation of the entire width of the belt in the final sample. When sampling from a conveyor belt it is also important to respect the centre of gravity rule; that is, any particle having its centre of gravity inside the delimited incremental sample should be included in the increment. When using a cross-stream sampler, the top edges must be such that a

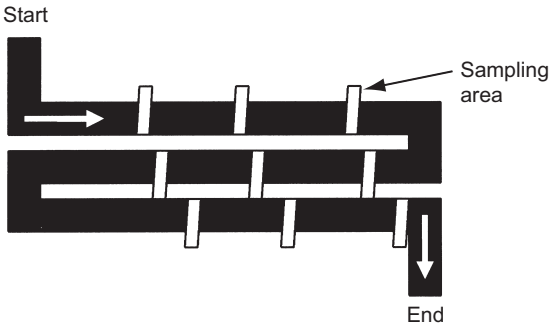

Fig. 1.3 Example of dynamic sampling from a conveyor belt.

particle hitting the device will either fall inside or outside depending on their centre of gravity (Petersen *et al.*, 2005).

When food lots are stored in bins/containers/silos where the access is limited, dynamic sampling as the product is either being put into or removed from the container is the recommended sampling method (FAO, 2001). The control of ambient conditions, in this case, is of utmost importance to avoid possible fungal attack and the production of mycotoxins.

1.2.6 Examples of established sampling plans

Examples of sampling protocols for the control of mycotoxins in food and feed are those formulated by the EU for several mycotoxins (EC 401/2006 and EC 152/2009), by Codex Alimentarius for aflatoxins in peanuts, corn and treenuts (Codex Alimentarius Commision,1995, revision, 2009), by ISO 24333:2009, and by the USDA for aflatoxins in several commodities (FDA, 2009). Some of these protocols are discussed below.

For mycotoxins other than aflatoxins, general principles, schemes and sampling plans adopted so far are mainly based on those for aflatoxins. More work is needed in this area, especially in the refinement of sampling plans according to the variability of certain mycotoxins in food commodities under specific agro-ecological production conditions and taking into account the real risk to human health and the economic perspective.

1.3 International guidance on sampling food and feed for mycotoxin analysis

1.3.1 European legislation

European Community regulations and decisions are directly applicable in the member states of the European Union. These legislative texts set limits for certain contaminants in foodstuffs, as well as detailing the official methods of control and

Table 1.1 Example of the sampling plan for cereals and cereal products

Lot weight T (tons)	Weight or number of sublots	Number of incremental samples	Aggregate sample weight (kg)
$T \geq 1500$	500 tons	100	10
$300 < T < 1500$	3 sublots	100	10
$50 < T \leq 300$	100 tons	100	10
$20 < T \leq 50$		100	10
$10 < T \leq 20$		60	6
$3 < T \leq 10$		40	4
$1 < T \leq 3$		20	2
$0.5 < T \leq 1$		10	1
$0.05 < T \leq 0.5$		5	1
$T \leq 0.05$		3	1

requirements for complying with food laws. The text with direct relevance to sampling is European Commission Regulation (EC) No 401/2006 (2006), laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. In this regulation, the European Commission has brought together, the sampling protocols and the performance criteria for the methods of analysis to be used for the official control of mycotoxins in foodstuffs, mainly for official local authorities and port health authorities. The official sampling protocols are very challenging in practical terms. The regulation states that an alternative sampling plan may be used, provided that it can be shown to be 'as representative as possible' and equivalent to the official plan. However, the directive lacks guidance about what is considered to be 'as representative as possible' (Van Egmond *et al*., 2007).

Since there are significant differences in the distributions of mycotoxins in different commodities, the Regulation provides for different sampling plans according to the type of food product. An example of a sampling plan for cereals and cereal products is shown in Table 1.1. According to the Regulation, 100 incremental samples of 100 g should be taken from any lot of cereals exceeding 20 tonnes. In the case of pistachios, the aggregate sample weight is 30 kg, which represents a challenge because it is neither easy nor economical to transport aggregate samples of this size to the laboratory. Significant financial investments in terms of large-volume grinders or 'slurry' processing equipment are also required at the laboratory in order to handle such samples.

EC 401/2006 provides the first published guidance for sampling at retail, in terms of the number of incremental samples and the weight of the aggregate sample. The European sampling plan regulations have been shown to be impractical in some instances. Spanjer (2006, 2007) summarized some of the practical difficulties encountered; for example in the import control of treenuts packed in sacks, two food inspectors would need half a working day to sample only one container on just one ship.

1.3.2 The ISO 24333:2009 standard: cereals and cereal products: sampling

This standard specifies requirements for the dynamic or static sampling, by manual or mechanical means, of cereals and cereal products, to assess their quality and condition. The standard is applicable to sampling for the determination of heterogeneously distributed contaminants, for example, mycotoxins.

Annex A of the standard describes the general types of mechanical sampling devices used for dynamic lots. Annex B gives examples of the devices used to sample static lots and of sample dividers. It also contains a guide for the selection of the proper sampling tools for cereals and cereal products. The standard states that sampling should be carried out under dynamic conditions using mechanical devices. Only when this is not possible should a manual sampling plan be implemented. Dynamic sampling methods should be adapted to the speed at which the products are flowing. The standard also gives rules concerning the patterns to be followed when sampling from static lots that have a depth less than 9 m (rail or road wagons, lorries, bulk tankers or ships). An example of the distribution of the sampling points for eight points is shown in Fig. 1.2. The standard provides sampling procedures for obtaining the minimum mass of laboratory sample for dynamic and static lots.

1.3.3 Codex general standard for contaminants and toxins in food and feed (Codex Stan 193-1995, revision 2009)

Codex Stan 193-1995, revision 2009, contains the main principles recommended for dealing with contaminants and toxins and lists the maximum levels for contaminants and associated sampling plans for commodities moving in international trade, such as peanuts and treenuts. According to this standard, contaminant levels in food and feed shall be as low as reasonably achievable through best practices such as good agricultural practice (GAP) and good manufacturing practice (GMP). National measures regarding food and feed contamination should avoid the creation of unnecessary barriers to international trade.

Annex 1 of Codex Stan 193-1995 contains the sampling plan for total aflatoxins in peanuts intended for further processing. The sampling plan calls for a single 20 kg laboratory sample of shelled peanuts (27 kg of unshelled peanuts) to be taken from a peanut lot and tested against a maximum level of total aflatoxins in μg kg^{-1}. The number of incremental samples to be taken from different locations throughout the lot, depends on the weight of the lot, with a minimum of 10 and a maximum of 100, as shown in Table 1.2. The weight of the incremental samples should be a minimum of 200 g. The standard also provides criteria for the sample preparation and the performance of analytical methods used in the analysis of the aflatoxins.

Annex 2 of the standard contains the sampling plan for aflatoxin contamination in ready-to-eat treenuts (almonds, hazelnuts and pistachios) and treenuts destined for further processing. The sampling plan also specifies a 20 kg aggregate sample, to be tested against maximum levels for total aflatoxins in 'ready-to-eat' and 'destined for further processing' treenuts of 10 and 15 μg kg^{-1}, respectively.

Table 1.2 Number of incremental samples to be taken depending on the weight of the lot according to the sampling plan for total aflatoxins in peanuts intended for further processing (Codex Standard 193-1995)

Lot weight T (tons)	Number of incremental samples
$T \geq 15$	100
$10 < T < 15$	80
$5 < T \leq 10$	60
$1 < T \leq 5$	40
$T \leq 1$	10

Table 1.3 Maximum levels, required number and laboratory sample size for total aflatoxins in treenuts (almonds, hazelnuts, and pistachios) 'ready-to-eat' and 'destined for further processing' (Codex Standard 193-1995)

	Ready-to-eat	Destined for further processing
Maximum level (μg kg^{-1})	10	15
Number of laboratory samples	2	1
Laboratory sample size (kg)	10	20

Table 1.4 Minimum number and size of incremental samples as a function of the lot weight for total aflatoxins in treenuts (almonds, hazelnuts, and pistachios) (Codex Standard 193-1995)

Lot weight tons – (T)	Minimum number of incremental samples	Minimum size of incremental sample (kg)	Minimum size of aggregate sample (kg)
$T \geq 15$	100	0.2	20
$10 < T < 15$	75	0.267	20
$5 < T \leq 10$	50	0.4	20
$1 < T \leq 5$	25	0.8	20
$T \leq 1$	10	2	20

The two sampling plans are illustrated in Table 1.3. The number and size of the incremental samples will vary with lot (sublot) size. Table 1.4 is used to determine the number of incremental samples (between 10 and 100) to be taken from lots or sub-lots of various sizes below 25 tonnes. Criteria are provided for the sample preparation and the performance of analytical methods used in the analysis of the aflatoxins in treenuts.

1.3.4 USDA sampling plans for aflatoxins

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 at retail or from bulk

Table 1.5 Incremental sample size and aggregate sample size required for the aflatoxins control programmes in peanuts and tree nuts by the US Food and Drug Administration (FDA, 2009)

Commodity	Control programme	Minimum number of incremental samples	Incremental sample size (kg)	Minimum aggregate sample size (kg)
Peanuts roasted in shell (only for domestic runner variety)	Monitoring	15	0.454	6.8
	Surveillance	75	0.454	34
Tree nuts (except in-shell Brazil nuts and all pistachio nuts in import status) shelled, in-shell slices, pieces, or flour	Monitoring	10	0.454	4.5
	Surveillance	50	0.454	22.7

commodities and the lot size. For each commodity the minimum number of subsamples to be taken and the minimum unit size are specified as shown in Table 1.5 (FDA, 2009).

1.4 Uncertainty estimation and designing sound sampling plans for mycotoxin analysis in food and feed

1.4.1 Uncertainty of the test procedure

Table 1.6 shows the mycotoxin measurement process, starting with sampling of the lot and ending with analytical determination. Each step contributes to the uncertainty of measurement (Ramsey and Ellison, 2007). Even when using accepted sample selection, sample preparation and analytical methods (Campbell *et al*., 1986; Whitaker, 2006), or applying official protocols, there are uncertainties associated with each of the steps in the mycotoxin test procedure. The mycotoxin

Table 1.6 Mycotoxin measurement process

	Sampling		Sample preparation		Analysis		
Lot sampling	Primary sampling	Sub-sampling	Preparation of the laboratory sample	Analytical sample	Test portion	Test aliquot	
Collection of several increments to form the aggregate sample	Comminution and/or splitting	Further comminution and/or splitting	Milling, wet milling, splitting, homogenization	Selection of the sample for chemical analysis	Chemical analysis	Quantification of mycotoxin concentration	Mycotoxin test results

concentration in the lot cannot be measured with absolute certainty and the result should always be reported with an estimate of the uncertainty.

Each of the three steps of the mycotoxin test procedure has an associated variance that contributes to the total variance of the testing scheme. In order to evaluate the sampling strategy, each of these components must be measured and/or modelled and their relationship understood. Amongst the statistical measures of variability only the variance is additive. Therefore, it is assumed that the total variance, V_T associated with a mycotoxin test procedure is the sum of the sampling variance (V_S), sample preparation variance (V_{SP}), and analytical variance (V_A) (equation [1.1]).

$$V_T = V_S + V_{SP} + V_A \quad [1.1]$$

Cheli *et al*. (2009) summarized the variability associated with each of these steps (Table 1.7). The data indicate that for small sample sizes the sampling phase is the largest source of variability of the mycotoxin test procedure for maize and peanuts contaminated with fumonisin and aflatoxin.

1.4.2 Random and systematic aspects of uncertainty

The bias is the difference between the test result (mean) and the true or reference value. Bias accounts for the systematic component of the uncertainty. Accuracy is the closeness of agreement between the result of a measurement and the true/reference value of the quantity being measured. Precision, measured as variability, is defined as the closeness of agreement between independent test results obtained under stipulated conditions. Precision accounts for the random component of the uncertainty.

1.4.3 Sampling variance

The explanation of the uncertainty of sampling is very well described by Ramsey and Thompson (2007): 'We extract a small amount of material (the sample) to determine the composition of a much larger body (the target). This sample should ideally have exactly the same composition as the target, but never does. The discrepancy gives rise to uncertainty from sampling'.

The contribution of the sampling variance to the total variance has been evaluated and quantified in several products. Researchers have developed equations to describe the sampling variance for several commodities and mycotoxins (Whitaker *et al*., 1972, 1974, 1976, 1979b, 1993, 1998, 2000; Schatzky *et al*., 1995a, 1995b; Hart and Schabenberger, 1998; Johansson *et al*., 2000c). These equations show that, especially for small laboratory sample sizes, the sampling step is usually the largest source of variability associated with the mycotoxin test procedure. The sampling variance decreases with increasing lot concentration and laboratory sample size.

The sampling error is inevitably large because of the uneven distribution of mycotoxins amongst contaminated particles within a lot. Studies on aflatoxins in

Table 1.7 Variability associated with each step of the test procedure, sampling (V_S), sample preparation (V_{SP}) and analysis (V_A) expressed as a percentage of the total variance (V_T). Adapted from Cheli *et al*. (2009), with permission

Matrix	Mycotoxin	Limit (μ kg^{-1})	Aggregate sample size (kg)	Sample homogenizer	Subsample (g)	Aliquots for analysis	V_S/V_T (%)	V_{SP}/V_T (%)	V_A/V_T (%)	Reference
Shelled corn	Aflatoxin	20	0.91	Romer mill	50	1	75.6	15.9	8.5	Whitaker (2006)
Shelled corn	Aflatoxin	20	4.54	Romer mill	100	2	55.2	29.1	15.7	Whitaker (2006)
Shelled corn	Aflatoxin	20	1.13	Romer mill	50	1	77.8	20.5	1.7	Johansson *et al*. (2000c)
Cottonseed	Aflatoxin	20	4.54	Romer mill	100		96.8	2.6	0.7	Whitaker *et al*. (1976)
Wheat	Deoxynivalenol	5000	0.454	Romer mill	25	1	22	56	22	Whitaker (2000)
Shelled corn	Aflatoxin	20	5	Romer mill	100	1	59.8	34.5	5.7	Johansson *et al*. (2000a)
Peanut	Aflatoxin	100	2.27		100		92.7	7.2	0.1	Whitaker *et al*. (1994)
Shelled corn	Fumonisin	2000	1.1		25	1	61	18.2	20.8	Whitaker *et al*. (1998)
Shelled corn	Fumonisin	2000	2	Romer mill	25	1	69			Whitaker *et al*. (2007b)
Almond	Aflatoxin	15	10	Romer mill	100	1	96			Whitaker *et al*. (2006)
Green coffee	Ochratoxin A	5	1	Romer RAS mill	25	1	72.6	26.4	1.0	Vargas *et al*. (2004)
Powdered ginger	Aflatoxin		0.14	NA	5	1	87		13	Trucksess *et al*. (2009)
Powdered ginger	Ochratoxin A		0.14	NA	5	1	97.1		2.9	Trucksess *et al*. (2009)

corn and peanuts suggest that about 0.1% of the kernels in a lot are contaminated and the concentration in a single kernel may be extremely high. Cucullu *et al*. (1986) reported that the contamination of aflatoxins could reach 1×10^6 μg kg^{-1} and 5×10^6 μg kg^{-1} for one single peanut kernel and cottonseed, respectively. Shotwell *et al*. (1974) reported findings over 4×10^5 μg kg^{-1} for aflatoxins in a maize kernel.

In order to perform reliable sampling, in theory each individual unit within a lot must have the same probability of being selected. In most cases this is impossible or impractical; consider, for example, sampling a ship load consisting of 500 tonnes of wheat. Two main aspects of the sampling step are critical for the reduction of the uncertainty, the selection techniques and the number and the size of the incremental samples selected from the lot. It is important to note that sampling variance also depends on the mycotoxin/matrix combination. In corn, for example, the curves describing the relationship between the sampling coefficient of variation (CV) and the mycotoxin concentration show the same trend, but the values of CV are higher for aflatoxin than for fumonisin and DON (Johansson *et al*., 2000b; Whitaker *et al*., 1998, 2000).

Systematic effects in sampling are caused by the heterogeneity of the lot, combined with the inability of the sampling method to reflect this heterogeneity properly (Ramsey and Thompson, 2007; Whitaker, 2006). Static lot sampling bias can be caused by, for example, a sampling probe that does not allow larger particles into the probe, a probe that does not reach every location in the shipment and use of a single probing point in a poorly mixed lot. Systematic effects may be difficult to quantify, but they can be reduced, for example by selecting proper sampling devices or by increasing the size of the sample. If the sample for analysis comprises the entire lot, the systematic effects or bias arising from sampling will be negated (although not the bias arising from the analytical stage). In almost all cases this is impossible and/or impractical, but increasing the sample size will give a better representation of the whole lot. Other measures such as grinding solid materials to reduce the particle size, either of the whole lot or of a relatively large sample, and efficient mixing can also reduce bias.

Random effects in sampling are mainly caused by variations in the composition of the sample in space or in time, by the use of different sampling methods, by the sampling procedure or the handling of the sample (e.g. by different samplers) and by variability in the performance of the sampling equipment. The most obvious approach to reducing the random effects is to increase the number of samples taken, which will result in a smaller standard deviation of the mean result. An equivalent approach is to increase the number of sub-samples or increments taken to produce one aggregate sample for analysis. A careful investigation of the variations in time and space, carried out as part of the validation of the sampling procedure, might be needed to select the proper sampling frequency or spatial distribution for the given quality requirement. Collecting too many samples will be more expensive, but will not necessarily give more or better information and thus should be avoided. The methodologies and equipment employed in collecting incremental samples are crucial in reducing the errors associated with the mycotoxin test procedure.

Table 1.8 Methodology for estimation of precision and bias of sampling and analysis in the empirical approach according to the Eurachem/Citac guide (Ramsey and Thompson, 2007)

	Sampling	Analysis
Precision	Perform duplicate sampling	Perform replicated analysis
Bias	Use a reference sampling lot (target), participate in inter-organizational sampling trial	Use certified reference materials (CRM)

Measurement uncertainty arising from sampling: the Eurachem/Citac guide

Eurolab, Nordtest and the UK RSC Analytical Methods Committee have jointly produced a Eurachem/Citac guide on measurement uncertainty arising from sampling (Ramsey and Ellison, 2007). Two main approaches to quantify uncertainty are described in the guide, the empirical approach and the modelling approach.

The empirical (also defined as 'experimental', 'retrospective' or 'top-down') approach uses repeated sampling and analysis, under various conditions, to quantify the effects caused by factors such as heterogeneity of the analyte in the sampling target and variations in the application of one or more sampling protocols. This approach relies on overall reproducibility estimates from either in-house or inter-organizational measurement trials, without necessarily trying to quantify any of the sources of uncertainty individually. The Eurachem/Citac document gives guidance on how to quantify the systematic and random errors in sampling and analysis to provide an estimation of the overall uncertainty (Table 1.8).

The guide describes four types of methods to estimate the combined uncertainty empirically. The 'duplicate method' often gives a reasonably reliable estimate of uncertainty. This method is the simplest and probably the most cost-effective of the four methods described by the guide for the empirical approach. It is based upon a single sampler carrying out the same sampling protocol and taking duplicate samples from at least eight sampling lots, selected at random. If only one lot exists, then all eight duplicates can be taken from it, but the uncertainty estimate will only be applicable to that one lot. Both of the duplicate samples are sub-sampled, resulting in two separate test samples. Duplicate test portions are drawn from both test samples and each is then analysed in duplicate (i.e. duplicate chemical analysis). This system of duplicated sampling and chemical analysis is known as a 'balanced design' (Fig. 1.4). To calculate uncertainty, the random component of the uncertainty can be estimated by applying analysis of variance (ANOVA) to the measurements of concentration on the duplicated samples. A 'samplers guide' is available on the internet which offers excel spreadsheets for ANOVA calculation (Groen, C).

The modelling (also defined as 'theoretical', 'predictive' or 'bottom-up') approach uses a predefined model that identifies separately each of the components of the uncertainty and sums them in order to make an overall estimate. Models from Gy's sampling theory (see next section) can sometimes be used in this approach to estimate some of the components. Further examples are given in the Eurachem/Citac guide.

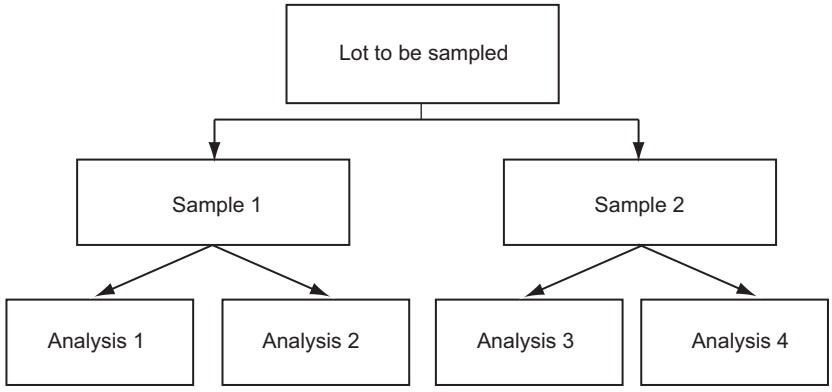

Fig. 1.4 A balanced design.

Theory of sampling (TOS)

The theory of sampling (TOS) was introduced by Pierre Gy in 1950. The TOS provides a description of all errors involved in the sampling of heterogeneous materials as well as the tools necessary for their evaluation, elimination and/or minimization. A comprehensive summary of the TOS has been prepared by Petersen *et al*. (2005), and provides many practical examples. The total measurement error, which Gy called the global estimation error (GEE), is the sum of the total sampling error (TSE) and total analytical error (TAE).

The components of TSE can be divided into two major groups:

1. Errors of incorrect sampling. Examples are gross errors and as such they are excluded from estimates of uncertainty. Incorrect sampling errors are unpredictable and arise from sampling equipment and procedures that do not follow the rules of sampling correctness defined in the sampling theory. These errors can be minimized or eliminated by carefully checking the performance of the equipment and procedures, by replacing inappropriate devices and procedures with those that comply with sampling rules and by sufficient training of sampling personnel.
2. Errors of correct sampling. These are a consequence of the material structure and are therefore inevitable, leading Gy to describe sampling as 'a science that falls in the province of the calculus of probability'. The material heterogeneity can be divided into two classes, constitution heterogeneity (CH) and distribution heterogeneity (DH).

CH is inherently dependent on the physical properties (composition, shape, size, density, etc.) of the particles making up the lot. Mixing and blending does not change the CH. The only way to alter the CH is by crushing/comminuting, for example by milling grain to reduce particle size. DH is dependent on the spatial distribution of the particles in the lot and shows the local stratification/segregation of particles in the lot. For example, particles with large differences in size and/or density tend to segregate or stratify heavily, with the smallest and/or densest

particles at the bottom of the lot. Thus, all particles do not have the same probability of being selected for sampling.

Gy's fundamental sampling error (FSE) is the minimum error of an ideal sampling protocol. It depends on the number of critical particles in the samples. The Eurachem/Citac Guide provides Gy's formula and accompanying explanation and examples on the estimation of the relative variance of the FSE.

Gy's formula is:

$$S^2_{(\mathrm{FSE})} = Cd^3\left(\frac{1}{M_S} - \frac{1}{M_L}\right) \approx \frac{Cd^3}{M_S} \qquad [1.2]$$

where M_S is the mass of the sample, M_L is the mass of the lot, C is the sampling constant and d is a measure of the coarsest fragment size (top 95% retaining sieve mesh size).

According to the Eurachem/Citac Guide, the empirical approach to the quantification of uncertainty tends to be more generally applicable and does not depend on prior knowledge of all of the sources of uncertainty. This approach is less time consuming and therefore less costly. The modelling approach gives more information about the individual sources of uncertainty and is perhaps more appropriate when elaborating a sampling plan that should be long term and with a very specific purpose.

1.4.4 Sample preparation variance

Once a representative primary aggregate sample has been selected from the lot, a laboratory sample must be taken from the aggregate sample. To save money and time, the mass of the primary sample should be reduced before transportation, storage or analysis. This is a critical phase; it is important that sub-sampling retains the representativeness of the lot.

The laboratory sample must be processed/comminuted for mycotoxin quantification. It is highly recommended that the entire laboratory sample is comminuted before a test portion is taken for analysis. The particle size must be sufficiently small to allow effective mixing, giving a distribution of the mycotoxins that is as homogeneous as possible, thereby reducing variability in the sample preparation step (Spanjer *et al*., 2006). This also applies to the preparation of the aggregate sample to obtain the laboratory sample. The most efficient grinders are those that can reduce the particle size of the laboratory sample to the smallest size possible. Several studies have been conducted on sample reduction techniques. The water slurry technique is the preparation of a homogeneous paste by blending an already milled sample with an appropriate amount of water at high speed in a slurry mixer. Velasco and Morris (1976) and Schatzki and Toyofuku (2003) demonstrated that the application of the slurry technique reduces the clogging of samples that have a high oil content and, in addition, produces a smaller particle size and more homogeneous samples. Spanjer *et al*. (2006) also showed that for pistachios, coffee beans and spices the slurry technique gives a lower variability than dry milling.

Petersen *et al*. (2004) presented a comprehensive survey of 17 types of field/laboratory mass reduction equipment and compared them with respect to accuracy (bias), reproducibility (precision), representativeness, material loss, operation time as well as ease of cleaning. It was shown that many devices have inherent design faults. All of the tested sample splitters and related dividers were orders of magnitude superior to various spoon and shovel methods. It was concluded that grab sampling and shovelling methods should be totally avoided. Although grab sampling is fast, easy and cheap, it also leads to heavily biased samples.

To find a robust, quick and efficient method for soil splitting in the field, Gerlach *et al*. (2002) evaluated five sampling techniques: riffle splitting, paper cone splitting, fractional shovelling, coning and quartering and grab sampling. Riffle splitting performed best, while grab sampling performed by far the worst, with 15–20% bias.

Despite the use of correct sample reduction procedures, a certain degree of variation among different laboratory samples is unavoidable and represents the main source of sample preparation variability. Small particle size, large sample size and low contamination levels are associated with a reduction of the sample preparation variability.

1.4.5 Analytical variance

Recent advances in analytical methodology have been applied to improve significantly the capabilities for the efficient detection and quantification of mycotoxins in agricultural commodities. As discussed above, the variance associated with the analytical step is usually lower than for the sampling or sample preparation steps. The analytical variance is a function of the mycotoxin concentration and the number of aliquots analysed.

Whitaker and co-workers (1996a, 2003) showed that an increase in the mycotoxin concentration generated a higher analytical variance, whilst an increase in the number of aliquots analysed reduced the variance. Whitaker also discussed the influence of the analytical method on the variance. He demonstrated that an HPLC method for the analysis of aflatoxins in corn produced less variability than TLC and enzyme-linked immunosorbant assay (ELISA) methods. European Commission Regulation EC 401/2006 (2006) specifies minimum performance criteria at different levels of contamination for methods of analysis for mycotoxins and this is an integral part of the sampling protocol.

1.4.6 Reducing variability of a mycotoxin test procedure

As discussed above, biases have the potential to occur in the sample selection process and sample preparation process, and in the quantification steps of the mycotoxin test procedure. Biases should be the easiest component of uncertainty to control and reduce to acceptable levels, but methods to reduce bias are difficult to evaluate because of the difficulty in knowing the 'true' mycotoxin concentration of the lot (Whitaker *et al*., 2009). However, biases can be minimized by ensuring

that sample selection and sample preparation equipment are continuously checked for performance. Analytical methods must undergo a validation process to show that they are 'fit for purpose' and biases can be minimized and corrected by the use of certified reference materials if available, or by the use of recovery tests.

In summary, the only way to obtain a precise estimate of the 'true' lot concentration is to reduce the total variability of the mycotoxin test procedure by reducing the variability associated with each step, sampling, sample preparation and analysis.

- Sampling variability can be reduced by increasing the size of the aggregate sample.
- Sample preparation variability can be reduced by increasing the analytical portion size and/or increasing the degree of comminution (number of particles per unit mass).
- Analytical variance can be reduced by increasing the number of aliquots analysed and/or by using a better performing analytical method (less uncertainty).

1.4.7 Uncertainty estimation and 'fit for purpose' concept

The Eurachem/Citac Guide emphasizes that uncertainty of sampling must be embedded in the concept of fitness for purpose. The uncertainty level tolerated by the user of the results should be carefully considered before designing a testing plan. If the performance level is set too high (too stringent), the investigation will be unnecessarily expensive. Ramsey and Thompson (2007) analysed the best division of resources between sampling and analysis. In general, measurements should be performed in such a way that the uncertainty is the lowest that can be achieved. However, reducing the uncertainty of a measurement result involves rapidly escalating costs. The true cost of a decision is the sum of the measurement costs and the costs deriving from incorrect decisions. This sum has a minimum value at some particular level of uncertainty and this uncertainty level defines fitness for purpose (Ramsey *et al.*, 2001). As a rule of thumb, an inverse-square relationship can be applied between cost and variability (measured as standard deviation): if the total standard deviation is cut to half, the cost will increase four times (Minkkinen, 2004).

To summarize, the contribution of the sampling to the overall uncertainty is occasionally small, but is often dominant (larger than 90% of the total measurement variance). This suggests the need for an increased proportion of the total expenditure involved to be invested in sampling, rather than chemical analysis, in order to reduce total uncertainty and achieve fitness for purpose.

1.4.8 Operating characteristics curves

Whitaker *et al*. (1970) developed operating characteristic (OC) curves for several commodities. An OC curve is a plot that has a unique shape for a particular

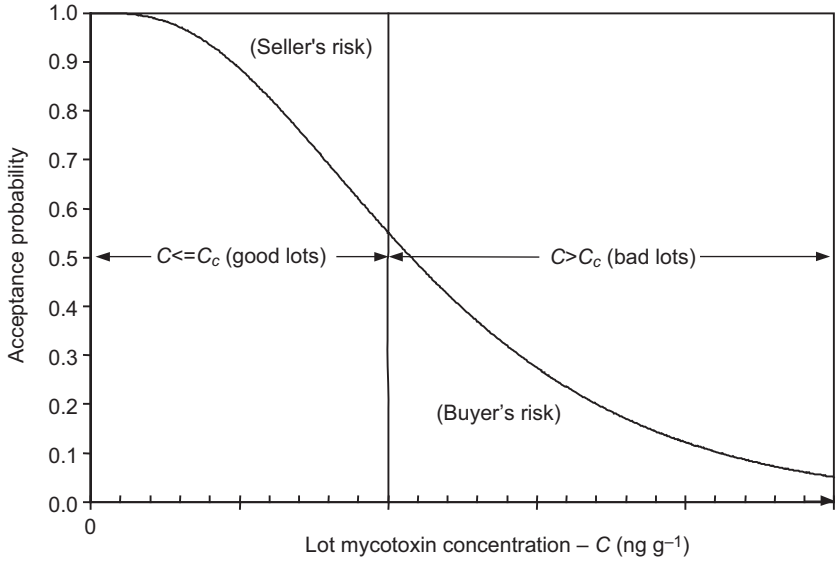

Fig. 1.5 Operating characteristics curves.

sampling plan, showing the relationship between the probability of accepting a lot with a particular mycotoxin concentration and the lot mycotoxin concentration (Fig. 1.5). The OC curve can be considered a footprint of a specific sampling plan and is defined for designated values of sample size, degree of comminution, sub-sample size, analytical method, number of analyses and an accept/reject limit.

For a given sampling plan design, lots with a mycotoxin concentration M will be accepted with a certain probability $P(M)$ (the acceptance probability) which is the probability that a sample test result, M, arising from the sampling plan is less than or equal to the sample accept/reject level, M_c.

$$P(M) = \text{prob}(M < M_c) \qquad [1.3]$$

For a given sampling plan, it is possible to calculate the probability of rejecting a good lot (type I error, false positive sample) or accepting a bad lot (type II error, false negative sample) as a function of the mycotoxin concentration and the risk associated with a specific sampling plan. The accept/reject level (the 'sample acceptance limit') may be a legal limit or a product quality limit. The areas delimited by the curve and the accept/reject level (see Fig. 1.5) describe an economic risk (area above the curve) or a consumer risk (area below the curve).

1.4.9 Design of sound sampling plans

Since the slope of the OC curve has high economic and health relevance, it is crucial, when designing a sampling plan, to maximize the slope of the OC in order to reduce consumer and producer risks and minimize the risk of lot misclassification (Johansson *et al.*, 2000b; Whitaker, 2006). To evaluate the performance of a

specific mycotoxin sampling plan, information must be available on the variability associated with the mycotoxin test procedure and the distribution of the mycotoxin test results.

Johansson *et al*. (2000b) showed that, in the case of sampling plans for aflatoxins in shelled corn, both false positive and false negative risks can be reduced by increasing the size of the aggregate sample, the degree of sample comminution, the subsample size, or the number of aliquots that are analysed. MacArthur *et al*. (2006) examined some retail-sampling strategies for the measurement of mycotoxins in dried vine fruit and nuts. A simulation was used to examine how the uncertainty associated with measurement results could be expected to change with the number of increments used to form the aggregate sample. The simulation accounted for one, 10 and 30 1-kg increments. For simulated measurements based on 10 increments, the probability of a false negative result was less than 5% for lots containing 24 μg kg^{-1} of OTA. If less than 10 increments were taken, the uncertainty was very high. On the other hand, increasing the number of increments beyond 50, given the analytical uncertainty, resulted in little improvement in measurement uncertainty (Spanjer, 2007).

The important conclusion of this study is that a sampling design that has not been adequately researched cannot be said to offer either effective protection to consumers or value for money. For example, a design that is effective for the measurement of OTA in dried fruit is likely to leave consumers largely unprotected if it is applied to the measurement of aflatoxins in pistachio nuts; whereas a design that is effective for the measurement of aflatoxins in pistachio nuts would represent a waste of resources if applied to the measurement of OTA in dried fruits (Spanjer, 2007). The extensive data available on the variability associated with sampling, sample preparation and analysis, has enabled the establishment of a series of sampling plans for the control of aflatoxins, DON, fumonisins and OTA in several commodities, as summarized in Table 1.9.

1.5 Quality assurance and quality control procedures in sampling and arrival of the samples at the analytical laboratory

1.5.1 Quality assurance and quality control procedures in sampling

The Eurachem/Citac guide (Ramsey and Thompson, 2007) mentions the importance of implementing validation and regular quality control procedures for the sampling step of a sampling plan. Quality assurance/quality control procedures applicable to the sampling step include the following:

- Ensure the presence of trained samplers.
- Ensure the availability of written instructions regarding sampling and transport of the samples to the laboratory.
- Ensure the availability of a sheltered unloading area, proper unloading equipment and appropriate sampling devices.

Table 1.9 Sampling plans for mycotoxins in different commodities

	Aflatoxin	Fumonisin	Deoxynivalenol	Ochratoxin A
Peanuts	Whitaker *et al*. (1974, 1995, 1999), Knutti and Schlatter (1982)			
Peanut butter	Waltking (1980)			
Maize	Johansson *et al*. (2000a)	Whitaker (2000); Whitaker *et al*. (1998, 2007b)		
Cottonseed	Velasco *et al*. (1975), Whitaker *et al*. (1976), Park *et al.* (2000)			
Wheat			Whitaker *et al*. (2002)	
Coffee				Vargas *et al.* (2006)
Ginger roots	Trucksess *et al*. (2009)			Trucksess *et al*. (2009)
Pistachios	Schatzki (1995a,b, 2004)			
Almonds	Whitaker *et al*. (2007a)			
Hazelnuts	Ozay *et al*. (2007)			
Figs	Sharman *et al*. (1994)			
Feedstuff	Coker *et al*. (2000)			
Grapes				Battilani *et al*. (2006)

- Use clean sampling equipment and sample bags and containers free of contamination to avoid any cross-contamination.
- Take increments from the same lot.
- Avoid any changes which would affect the mycotoxin content, i.e. do not open packaging in adverse weather conditions or expose samples to excessive moisture or sunlight.
- Take the correct number of incremental samples of the appropriate weight at various places distributed throughout the lot.
- Ensure that access lanes in the storage facility are accessible in all directions.
- Place samples collected in a clean, dry, opaque, leak-proof container that can be securely sealed.
- Record as much information as possible about the lots from which samples have been taken to provide as much traceability data as possible.
- Store all samples in a cool dark place and segregate different lots. Apply good storage practices.
- Dispatch samples to the laboratory as soon as possible after sampling.
- Ensure samplers are wearing the correct personal protective equipment and apply good sampling practices.

1.5.2 Arrival at the laboratory and sample preparation

After the actual sampling, the aggregate sample may go through a number of further steps before reaching the laboratory. In this process all possible precautions should be taken to avoid any alteration of the sample by human error, spillage, contamination, packaging, adhesion of critical components to the sides of containers/bags, loss of fine particles during handling or crushing, moisture uptake/loss, biodegradation, and so on.

Upon arrival at the laboratory, the aggregate sample container should be checked to ensure that it is sealed. The accompanying documentation should give details of whether the consignment is intended for direct human consumption or will be subjected to sorting and/or other physical treatment before human consumption. The fine grinding, slurrying and mixing of the laboratory sample should be carried out using a process that has been previously validated as fit for purpose. The sample preparation procedures must be validated at the laboratory and available as standard operating procedures (SOPs).

According to European legislation, if the case applies, formal official aggregate samples are mixed and then split at the laboratory into three sub-samples and only then can each sub-sample be homogenized. In the case of products intended for direct human consumption, one analytical sample, one defence sample and one reference sample are taken from each sub-sample (laboratory sample). For every official aggregate sample taken from a batch of pistachios, for example, nine samples in total are obtained from the homogenized subsamples: three analytical samples, three defence samples and three reference samples.

1.5.3 Analysis

For official control purposes the analysis should be carried out by an official laboratory accredited according to ISO/IEC 17025 (ISO, 2005). It is becoming more common for laboratories to accredit their sampling procedures (Minkkinen, 2004). The basic requirements for accreditation are that the sampling equipment is correct, the uncertainties of the methods have been estimated, procedures are regularly audited and personnel have been adequately trained for their jobs.

1.6 Strengthening national food control systems

1.6.1 Training sampling inspectors

To implement sound sampling plans, it is essential that samplers are well trained and competent in carrying out their jobs. Without training on at least the basics of sampling theory, it is difficult to obtain representative samples, especially when dealing with heterogeneous contaminants. However, very few universities or academic institutions provide courses on sampling. At the national level, the responsibility for the education and training of samplers lies with national inspection bodies, which are the official institutions that share responsibility for food and feed quality and safety.

Capacity building and technical training requires, inter alia, basic infrastructure and investments, information technologies, knowledge of the national food control strategy, food legislation, food inspection services, food control laboratories, collaboration and cooperation among control agencies, sound scientific and technical expertise, and financial resources. In order to be effective, trained food inspection officials should have well planned food inspection programmes, should understand their duties and responsibilities, and should maintain close collaboration with other food control services. This requires adequate management, training and equipment.

1.6.2 Sampling responsibility

The Eurachem/Citac guide (Ramsey and Ellison, 2007) suggests that the responsibility for sampling should be clearly assigned to one organization/individual, while defining responsibilities for the other steps of the measurement process. If possible, the analyst who performs the analysis should also be involved in the planning of the experimental design before analysis. If this is not possible, it is important that the responsibility for sampling, chemical analysis and data analysis is shared between the individuals/organizations involved. Information about the contributions arising from the different steps in the measurement procedure to the overall uncertainty should also be shared among all participants/organizations.

1.6.3 Holistic approach to control of mycotoxins

Producing safe and good quality food is a prerequisite for successful domestic and international trade and key to the sustainable development of national agricultural resources. Despite many years of research and the introduction of good practices in the food production, storage and distribution chain, mycotoxins continue to be a problem, especially in developing countries. In this context it is of utmost importance that a holistic approach is adopted for the control of mycotoxins.

Mycotoxins may originate in the field and therefore mycotoxin control must start during crop production. Unquestionably, prevention is the best method for controlling mycotoxin contamination. FAO has published codes of practice for the prevention and control of mycotoxins; for example, the code of practice for the prevention and reduction of aflatoxin contamination in peanuts (Codex Alimentarius Commission, 2004; CAC/RCP 55-2004), and the code of practice for the prevention and reduction of ochratoxin A contamination in wine (Codex Alimentarius Commission, 2007; CAC/RCP 63-2007).

Pre-harvest mycotoxin formation can be partially controlled through good agricultural practices (GAP) which include insect management, good irrigation and mineral nutrition, crop rotation and the use of mould resistant crop varieties. Rapid field detection methods can be of great help in this respect. Post-harvest prevention procedures include good storage procedures, minimization of moisture exposure, insect infestation prevention and cleaning and disinfection of storage containers and transportation equipment (Boutrif and Canet, 1998). In addition, in storage, the initial grain condition is critical. Good quality, clean, sound grain is easier to maintain in storage than physically damaged grain.

The development of internationally harmonized regulatory measures for mycotoxins is paramount in the global strategy for minimizing mycotoxin contamination while maximizing the availability of food. To this end, national participation in the process of the elaboration of guidelines by bodies such as the Codex Alimentarius Commission is important. This allows discussion of the problems encountered in many countries and the development of consensus guidelines to address such issues. Interaction with the Codex Committees is especially important for developing countries, who can both input into and benefit from the guidelines developed (Boutrif, 1995).

1.6.4 Conclusion

The adoption of the best agricultural practices in the field and throughout the whole farm–fork chain, coupled to the best sampling practices and the use of validated and fit-for-purpose methods, together with accreditation and participation in proficiency testing are the recommended means of ensuring the recognition of mycotoxins test results worldwide. Assistance by international organizations such as FAO may be necessary, particularly in developing countries, to stimulate and implement the necessary food control systems (FAO, 2001).

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1.7 Sources of further information and advice

1.7.1 Books on sampling for mycotoxins

The authors are not aware of any single volume dedicated specifically to sampling for mycotoxins. Individual chapters can be found in the following books:

Adams J. and Whitaker T. B. (2004). 'Peanuts, Aflatoxin and the U.S. Origin Certification Program', in *Meeting the Mycotoxin Menace*, Barug D., van Egmond H., Lopez-Garcia R., van Osenbruggen T. and Visconti A. (eds), Wageningen Academic Publishers, The Netherlands, 183–96.

Anon (2004). 'Sampling', in *Grain Fungal Diseases and Mycotoxin Reference*, USDA, Grain Inspection, Packers and Stockyards Administration (GIPSA), 24–9.

Miraglia M., De Santis B., Pannunzi E., Debegnac F. and Brera C. (2008). 'Mycotoxin concentration data quality: the role of sampling', in *Mycotoxins Detection Methods, Management, Public Health and Agricultural Trade*, Leslie J., Bandyopadhyay R. and Visconti A. (eds), CAB International, Oxfordshire, UK, 185–94.

Whitaker T. B. (2001). 'Sampling techniques', in *Mycotoxin Protocols*, Trucksess M. W. and Pohland A. E. (eds), Series: Methods in Molecular Biology, Vol. 157, Humana Press, Totowa, New Jersay, 11–24.

Whitaker T. B. (2004). 'Sampling for mycotoxins', in *Mycotoxins in Food: Detection and Control*, Magan N. and Olsen M. (eds), National Food Administration, Sweden. Woodhead Food Series No. 103, Woodhead Publishing, Cambridge, UK, 69–87.

Whitaker T. B. (2005). 'Sampling feeds for mycotoxin analysis', in *Mycotoxin Blue Book*, Diaz D. (ed.), Nottingham University Press, Bath, UK, 1–23.

Whitaker T. B., Dickens J. W. and Giesbrecht F. J. (1991). 'Testing animal feedstuffs for mycotoxins: sampling, subsampling, and analysis', in *Mycotoxins and Animal Foods*, Smith D. and Henderson R. (eds), CRC Press, Boca Raton, Florida, 153–64.

Whitaker T. B., Hagler W. M. Jr., Johansson A. A., Giesbrecht F. G. and Trucksess M. W. (2001). 'Sampling shelled corn for fumonisin', in *Mycotoxins and Phycotoxins in Perspective at the Turn of the Millennium,* deKoe W. J., Samson R. A., Van Egmond H. P., Gilbert J. and Sabino M. (eds), Wageningen Academic, The Netherlands, 97–107.

1.7.2 Gy's theory of sampling

Gy's sampling theory is well covered in the following books:

Gy P. M. (1998). *Sampling for Analytical Purposes*, John Wiley and Sons, Chichester, Sussex, UK.

Pitard F. F. (1993). *Pierre Gy's Sampling Theory and Sampling Practice*, 2nd edn, CRC Press, Boca Raton, Florida.