# Overview of Sampling for Ochratoxin A in Coffee

**Sampling requirements in force in key markets:**

World exports of green coffee beans totalled 5,233,064 metric tonnes in 2004 (FAO, 2005). It is a well-established practice of the world coffee sector to sample coffee lots marketed globally for various quality factors, which means undertaking sampling from these 5 million tonnes.

Sampling in trade has been performed to guarantee that coffee is traded and priced according to its physical quality (grading and defects), and sensorial (test cup) factors and composition. For this sampling the procedure usually consists of taking small incremental portions (~10 – 30 g) from 100% of the bags in a lot. The increments are combined into a composite sample that is homogenized and, usually, divided into three test samples.

Because of heterogeneity of mycotoxin distribution in a lot, specific sampling plan for ochratoxin A (OTA) needs to be designed and taken into consideration when establishing regulatory sampling criteria. There is a general recognition of the importance of mycotoxin sampling plans and that the generation of meaningful results can only be obtained if representative samples are taken from carefully selected populations of foods (Coker & Whitaker, 2001), and properly homogenized prior to sub-sampling for analysis. Despite this recognition, sampling is still much neglected, and, often, in the drive for rapid methods, because sampling and sample preparation is very time-consuming, proper sampling is frequently overlooked (Gilbert & Vargas, 2003).

According to the Codex Alimentarius, the design of official sampling plans aims to provide international methods to avoid or eliminate difficulties arising from legal and technical disputes related to sampling in trade. Some important criteria should be observed when selecting a official method of sampling.

Preference should be given to sampling methods designed by international organizations dealing with food products and having procedures in place that determine:

- How a representative sample should be taken from a lot;
- The size and number of increments to for a representative sample;
- The administrative measures for taking and handling the sample;
- The statistical criteria for acceptance and rejection of a lot on the basis of the sample;
- The procedures to be adopted in cases of disputes (Codex, 2004).

Regulations for mycotoxins have become more diverse and detailed with newer requirements regarding official procedures for sampling and analytical methods, and the issue of measurement uncertainty has entered the regulations discussion (FAO, 2004). Sampling plans have featured on the agendas of international bodies such as

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Codex Alimentarius and have become an integral part of recent regulations as the one for aflatoxin.

Official sampling plans are important for mutual recognition of certification programs of food products, reducing economic losses for both exporter and importer, and maintaining consumer safety. An example of mutual recognition in certification program is the  memorandum of understanding, which two important peanut markets (the United States of America (USA) and the European Union (EU)) have agreed to recognize US sample testing before export to Europe (FAO, 2004).

Regarding OTA, Table 1 details the worldwide provisions comprising regulation level, sampling plan and analytical method (FAO, 2004).

*Table 1: Status of regulation for ochratoxin A in coffee (FAO, 2004)*

| Country | Roasted Coffee (µg/kg) | Soluble Coffee (µg/kg) | Green coffee (µg/kg) | Status of Sampling plan | Status of Analytical method |
|---|---|---|---|---|---|
| Bulgaria | 4 | Not informed | 8 | Official | Official |
| Cuba | 5* | | | Official | Official |
| European Union | 5 | 10 | Not established – foreseen for 2006 | Official | Method Performance criteria |
| Greece | 20 | 20 | 20 | Non-Official | Non-official |
| Hungary | 10 | 10 | 15 | Not informed | Not informed |
| Indonesia | Not detectable* | Not detectable* | Not detectable* | Not informed | Not informed |
| Italy | 4 | Not informed | 8 | Official | Not informed |
| Lithuania | See European Union | | | Non-Official | Official |
| Poland | See European Union | | | Not informed | Official |
| Serbia and Montenegro | 10 (all foods) | | | Official | Official |
| Singapore | 2.5 | Not informed | 2.5 | Non-Official | Official |
| Slovakia | 10 (foodstuffs) | | | Official | Official |
| Slovenia | See European Union | | | Official | Non-official |
| Switzerland | 5 (all foodstuffs) | | | Official and Non-Official | Official and Non-official |
| Uruguay | 50* | | | Not informed | Official |

* Type of coffee is not specified

The European Union represents the largest number of countries with provision for ochratoxin A comprising regulation level, sampling plan and analytical method (Table 1).  The European Commission set regulatory limits for OTA in roasted and soluble coffee in 2005 (EC, 2005a), together with provisions for sampling roasted and soluble coffee (EC, 2005b).

The EU sampling plan predicts that the number of incremental samples and the size of aggregate samples depend on the weight of the roasted and ground coffee. In lots ≥ 15 tons (15-30 tons), 100 increments should be taken giving an aggregate sample of 10 kg. For lots < 15 tons 10 to 100 increments should be taken giving aggregate samples of 1 to 10 kg.

The sampling plan specified in EU Directive 2005/5/EC (EC, 2005b) has a major advantage in that it is clear as to how the sample should be taken. Thus, the way in which the regulatory limit is to be applied is unequivocal to both producers and importers. It is not clear, however, what is the operating characteristic curve associated with this plan. Thus, producers/importers do not know the 'producer risk' associated with operating this plan, nor is it clear to those concerned with food safety what are the 'consumer risks'.

For the purpose of risk assessment it is recommended that the minimum acceptable sample sizes (obtained from riffle division of large samples) for ochratoxin A in roasted and ground coffee should be minimum of 1.0 kg (10 x 100 g incremental sample). For green coffee, the minimum recommended sample size is 3.0 kg (30 x 100 g incremental sample) (Coker & Whitaker, 2001).


**Ochratoxin A sampling issues:**

The evaluation of the conformance of a green coffee lot to an acceptance limit is usually based upon the results of an ochratoxin A (OTA) sampling plan which is defined by an OTA test procedure combined with a sample acceptance limit. A test procedure consists of sampling, sample preparation and analytical steps. The sample acceptance limit, $C_a$, is a threshold concentration that may or may not be equal to the regulatory guideline, $C_g$.

The true mycotoxin concentration, C, in a lot is unknown in practice. The true but unknown lot concentration is estimated by quantifying the target mycotoxin in a test sample (called a sample test result) that is collected in a random manner from the lot. Considering that a small percentage of beans are contaminated, the sample has to be selected in such a way that every bean in the lot has an equal chance of being chosen – small incremental portions taken at many different locations throughout the lot (Council for Agricultural Science and Technology, 2003).

The sample test result, $\hat{C}$, is then used either as an estimate of the true lot concentration C or to make a decision as to the acceptability of the lot for food and feed purposes. The values of $\hat{C}$ will differ from C for a specified lot due to the variability associated with the test procedure, e.g. sampling, sample preparation and analytical variances. Because of the variability associated with each step of the OTA test procedure (sampling, sample preparation and analytical variances) (Johansson *et el.*, 2000) the true OTA concentration in a bulk lot cannot be determined with 100% certainty. Discrepancies between the sample and lot concentration values lead to misclassification of lots.

Two types of errors are usually made when using a sampling plan to classify a determined lot based upon its mycotoxin concentration. The first type of misclassification, a false positive, occurs when $\hat{C} > C_a$ (acceptance limit) and the lot is rejected, when in reality $C \leq C_g$ (concentration guideline), and the lot is acceptable. This type of misclassification is also called 'sellers' risk' because a good lot is diverted from the food chain at an unnecessary expense to the seller.

The second type of misclassification, a false negative, occurs when $\hat{C} \leq C_a$ and the lot is accepted, when in reality $C > C_g$ and the lot is not acceptable. When the unacceptable lot is not detected, the lot enters the food chain and becomes a potential risk to the consumer. This type of misclassification is also called 'buyers' risk' because a bad lot is processed into a consumer-ready product and may have to be diverted from the food chain at an expense to the buyer.

The frequency with which these two misclassifications occur depends upon $C_a$, $C_g$, and the design of the sampling plan and can be evaluated with help of an operating characteristic (OC) Curve. A desirable sampling plan design would minimize both the buyers' and sellers' risks (Vargas *et al.*, 2004).

If the probability of accepting a lot with and OTA concentration C by a given sampling plan is P(C), then the plot of the acceptance probability, P(C), versus the lot concentration, C, is called an operating characteristic (OC) curve. The shape of an OC curve is shown in Figure 1 and can be used to estimate the buyers' and sellers' risks associated with a given OTA sampling plan. The shape of an OC curve is uniquely defined by a given OTA test procedure where sample size, degree of comminution, subsample size, analytical method, and acceptance limit are designated, as well as the probability distribution of the OTA in the lot (Vargas *et al.*, 2004).

Other aspects such as particle size, and type of mill used also influence the uncertainty associated with measuring the true level of OTA contamination in a green coffee sample (Vargas, Santos & Castro, 2001; Vaegas *et al.*, 2001). Sampling plan designs are usually a compromise of these aspects (Whitaker *et al.*, 1995).
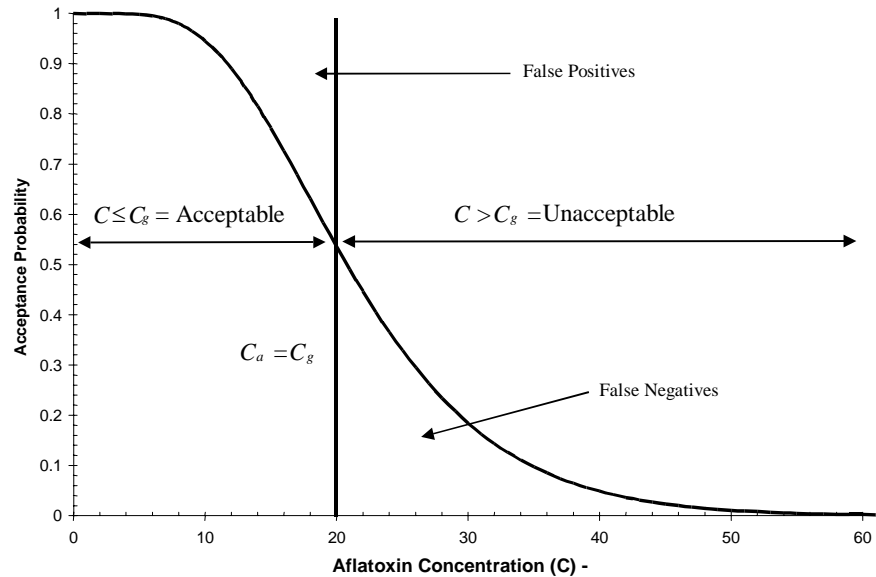
**Figure 1:** *Typical shape of an operating characteristic curve used to evaluate the buyers' and sellers' risks associated with a sampling plan.*

Total variance associated with testing green coffee has been estimated from the analysis of 25 lots contaminated with OTA. Testing a lot with 5 µg/kg ochratoxin A using a 1 kg sample, Romer RAS mill, 25 g subsamples, and high performance liquid chromatography analysis, the total, sampling, sample preparation, and analytical variances have been evaluated as 10.75 (CV=65.6%), 7.80 (CV=55.8%), 2.84 (CV=33.7%), and 0.11 (CV=6.6%), respectively. The percentages of the total variance for sampling, sample preparation, and analytical were 73, 26, and 1%, respectively, and increased with ochratoxin A concentration (Vargas *et al.*, 2004).

The distribution of ochratoxin A in lots of green coffee has been investigated and the three distributions (lognormal, negative binomial and gamma) have provided acceptable fits. Because of its simplicity, the 2-lognormal distribution has been selected to model ochratoxin A test results for green coffee. The percent-contaminated beans were a function of the lot concentration and increased with lot concentration. At a lot concentration of 5 µg/kg, approximately 6 beans per 10,000 beans are contaminated (Vargas *et al.*, 2005a).

A study by the UK Food Standard Agency evaluated the application of the US, UK, Dutch and EU sampling plans for the determination of aflatoxins in foods for the determination of ochratoxin A in coffee. It found that the distribution of ochratoxin A in coffee was found to be relatively uniform (Coker & Whitaker, 2001).

Equation 1 predicts the variance associated with an OTA test procedure that uses any size sample, ns, any size subsample, nss, and any number of aliquots, na, to measure OTA in a green coffee lot (Vargas *et al.* 2005b):

$$S^2_{(t)} = (1/ns)1.350C^{1.090} + (25/nss)\,0.272C^{1.457} + (1/na)0.008C^{1.605}$$

, where C is OTA concentration ($\mu$g/kg) in the lot (FAO, 2005).

For a sample accept/reject limit of 5 $\mu$g/kg ($C_a$), increasing sample size and increase number of samples increase the percentage of lots accepted at concentrations below the regulatory limit (good lots) while increasing the percentage of lots rejected at concentrations above the regulatory limit (bad lots). Increasing sample size reduces the uncertainty associated with the OTA test procedure, which reduces both the buyers' and sellers' risks (Figures 2 and 3).

Increasing sample size is often the first approach taken to reduce uncertainty and risks because sampling accounts for most of the total variability (73% at 5 µg/kg for a 1 kg sample) associated with the OTA test procedure (Vargas *et al.*, 2004).

The same sample size effect has been also observed for other mycotoxin and commodities such as aflatoxins in peanuts and corn (FAO, 1993; Defize & Marres, 1995). An example of a sampling plan that uses a single sample is that designed by FAO/WHO to detect aflatoxin in raw shelled groundnuts destined for further processing (EC, 1998). The FAO/WHO aflatoxin-sampling plan uses a single 20 kg sample (1x20 kg) with an accept/reject limit of 15 $\mu$g/kg total aflatoxin (Vargas *et al.*, 2005b)

Increasing the number of samples of a given size that are taken from a contaminated lot also has an effect on both the buyers' and sellers' risks associated with classifying lots. If all sample test results are averaged, the effect is the same as increasing the size of a single sample (Figure 2). However, if all sample test results from multiple samples are required to test less than the accept/reject limit, the effect on the OC curve and thus the buyers' and sellers' risks are very different from averaging multiple sample test results.

Three sampling plans showing the effect of requiring either one, two, or three 1 kg samples (1x1kg, 2x1kg, and 3x1kg) to all test less than or equal to the accept/reject limit of 5 $\mu$g/kg is shown in Figure 3. The remaining sampling plan design parameters nss and na are equal to 25 g  and 1 aliquot, respectively. As the number of samples that are required to test less than or equal to the accept/reject limit increases, the OC curve shifts to the left reducing the buyers' risk, but increasing the sellers' risk. This type of sampling plan is often used late in the marketing system on consumer-ready products destined for human consumption because the product has little chance of containing a mycotoxin concentration above the regulatory limit. The buyer is placing most of the risk on the seller with this type of sampling plan design (EC, 1998).  An example of a sampling plan that uses multiple samples is that designed by the EU to detect aflatoxin in consumer-ready groundnuts. The EU uses three 10 kg (3x10kg) samples where all three samples must test less than the accept/reject limits of 2 $\mu$g/kg $B_1$ and 4 $\mu$g/kg total aflatoxin (EC, 1998).

For 1 kg samples, decreasing the sample accept/reject limit relative to the regulatory limit decreases the percentage of lots accepted while increasing the percentage of lots rejected at all ochratoxin A concentrations (Figure 4). The effect

of changing the accept/reject limit relative to the regulatory limit on the buyers' and sellers' risks when testing green coffee lots for OTA is shown in Figure 4.

If the accept/reject limit and the regulatory limit are both 5 μg/kg, then the areas in Figure 4 representing the buyers' risk and sellers' risk are similar. Changing accept/reject limit to a value less than the regulatory limit of 5 μg/kg (e.g. 2 versus 5 μg/kg) shifts the OC curve to the left. Compared to the sampling plan where the accept/reject limit is 5 μg/kg, the buyers' risk decreases, but the sellers' risk increases. Often, importers will prefer to contract for products where the sampling plan uses an accept/reject limit below the regulatory limit because it reduces the importers' or buyers' risk and forces the exporter (seller) to take the largest share of the risk. If the accept/reject limit becomes larger than the regulatory limit of 5 μg/kg (e.g. 10 versus 5 μg/kg), the OC curve shifts to the right. As a result, the sellers' risk decreases but the buyers' risk increases. Changing the accept/reject limit relative to the regulatory limit can reduce only one of the two risks, because reducing one risk will automatically increase the other risk. Using an accept/reject limit greater than the regulatory limit, while rarely used, is sometimes used early in the market system when a processor can clean up or reduce contamination by processing the product (Vargas et al. , 2005b).
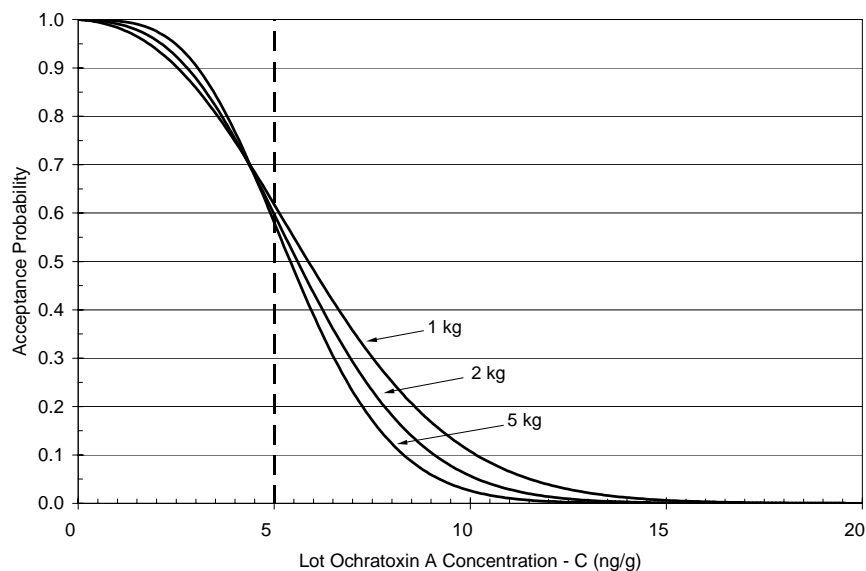


**Figure 2:** *OC curves for testing ochratoxin A in coffee using 1, 2 and 5 kg samples. Romer Ras type Mill (Marconi CF/920, 80% of sample with particle size < 28 mesh), 25 g subsample, 1 aliquot, HPLC, and an accept/reject limit of 5 μg/kg.*

**Figure 3:** *Operating characteristic (OC) curves for testing ochratoxin A in coffee using 1x1kg, 2x1kg and 3x1kg multiple samples, Romer Ras type Mill (Marconi CF/920, 80% of sample with particle size < 28 mesh), 25 g subsample, 1 aliquot, LC, and accept/reject limit of 5 µg/kg. All samples must test ≤ 5 µg/kg for a lot to be accepted.*
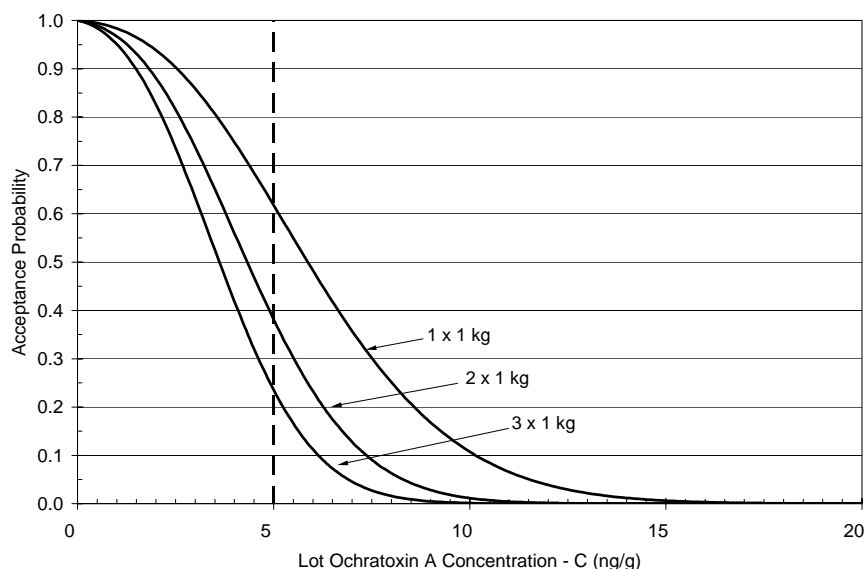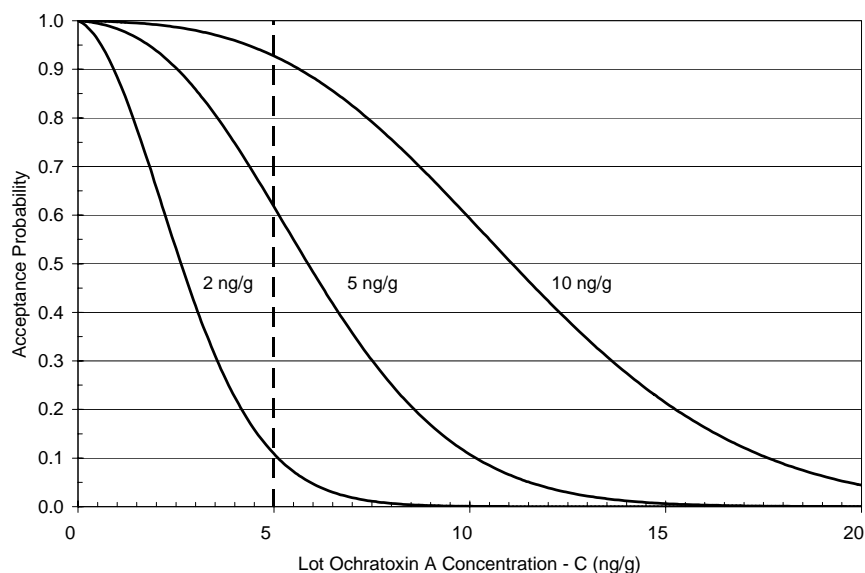


**Figure 4:** *Operating characteristic (OC) curves for testing ochratoxin A in coffee using a 1 kg sample, Romer Ras type Mill (Marconi CF/920, 80% of sample with particle size < 28 mesh), 25 g subsample, 1 aliquot, LC, of 2, 5 and 10 µg/kg.*

This type of sampling plan design reduces the buyers' risk while increasing the sellers' risk and is often used to inspect consumer-ready products. Increasing the number of samples taken from a lot that must all test less than the accept/reject limit increases the number of good lot rejected (sellers' risk) and decreases the

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number of bad lots accepted (buyers' risk) by a sampling plan (Council for Agricultural Science and technology, 2003).

Perhaps some of the most important observations coming out of sampling is the ability to identify a lot associated with the highest risk of contamination. For ochratoxin A lots, the presence of defects impacted significantly and negatively on the incidence and levels of OTA in coffee.

Among the defects the sour, insect damaged-bluish, insect damaged, black, malformed and broken beans were the ones that most contribute to the incidence and levels of OTA contamination in coffee.

The defects black, stick/stalk, bean in parchment and insect damage-bluish were more present in samples with contamination >5 ng/g. This information can be employed (Vargas *et al.*, 2005c) to evaluate the performance of ochratoxin A sampling plans based on preferential analysis of sorted fractions. This approach is attractive in that it offers the possibilities of remedial action though sorting coupled to the sampling plan at an early stage (Vargas *et al.*, 2005c).

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