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Marina Miraglia

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The role of sampling in mycotoxin contamination: a holistic view

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The role of sampling in mycotoxin contamination: a holistic view

M. Miraglia*, B. De Santis, V. Minardi, F. Debegnach & C. Brera

The Italian National Institute of Health, National Centre for Food Quality and Risk Assessment, GMO and Mycotoxins Unit, Viale Regina Elena, 299, 00161, Rome, Italy

Abstract

The need of obtaining a representative sample deserves particular consideration since a wrong sampling plan can greatly affect the reliability of the measured levels of mycotoxins. This can even result in legal disputes and barriers to trade.

Reported here is a holistic view for an ideal sampling plan, which is based on two consecutive steps: 1) to establish “*why, where and when*” sampling has to be performed by assessing the purpose, the appropriate time and the site for collecting the samples; 2) to establish “*how*” to draw samples by assessing practical *ad hoc* guidelines, considering that, for bulk goods in particular, mycotoxins are not at all homogeneously distributed in a lot.

So far, step 1 is not yet covered by specific guidelines while for step 2, European regulations establish the procedures for the sampling of bulk and retail products potentially contaminated by mycotoxins.

Key words: sampling, mycotoxins, surveillance, monitoring.

* To whom correspondence should be addressed; e-mail: : miraglia@iss.it

Introduction

The most prominent reason for collecting food samples for the investigation of contaminants such as mycotoxins, is to protect consumer health, mainly verifying the compliance of food and feed with acceptable safety standards.

Sampling is one of the most crucial, but underestimated parts of the multifaceted and complex bulk of activities aimed at addressing and managing food issues. In practice, the overall objective of good sampling is to provide reliable samples to be analysed that can represent the basis for “fit for purpose” investigations.

In most cases, meaningful sampling is a process comprising two very dissimilar steps: i) the first step (hereafter referred to as “primary sampling”) consists in taking the decision on “why, where and when” to collect the samples. In other words, the process of “statistically” locating the sites (populations) from which food samples should be taken; ii) the second step (hereafter referred to as “secondary sampling”) consists of establishing how samples should be collected in order to be representative of the lot under investigation. For both steps the quality and the consequent reliability of the data are strongly dependent on the available resources and on the skill of the people involved.

For this class of contaminants, the need for statistically based planning is particularly relevant for i) the multifaceted implications of mycotoxin contamination (health, trade, ethical issues related to developing countries’ difficulties), ii) the largely inhomogeneous distribution of the toxins within food commodities, with the consequent need for careful secondary sampling.

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Good primary sampling schemes have so far been developed for several classes of contaminants such as dioxins and pesticides (South *et al.* 2004), in contrast to the very few valid ones so far proposed for mycotoxins.

In contrast, a large number of papers have appeared, related to secondary sampling schemes for aflatoxin B₁ (particularly on its distribution in a lot and on related sampling plans) (Whitaker *et al.* 1974, 1976, 1979, 1994), but only a few studies deal with some *Fusarium* toxins (Whitaker *et al.* 1998; Hart and Schabenberger 1998; Whitaker *et al.* 2000). Conversely, specific studies focused on the distribution of OTA-contaminated units are not yet available, apart from the vague assumption that “representative sampling” for aflatoxins is more difficult than sampling for other known mycotoxins in food products. Sampling procedures recommended for aflatoxins should thus be adequate for other mycotoxins (Dickens and Whitaker 1982). Nevertheless, the European legislation dealing with sampling plans for OTA to be used for official control was only recently adopted (Commission Directive 2005/5/EC 2005).

Due to the lack of specific issues on the overall sampling for OTA, this paper is mainly devoted to illustrating a holistic view of sampling for mycotoxins in general, assuming the given considerations could in most cases be adapted to OTA.

Primary sampling schemes: “why, where and when” to collect samples for mycotoxin analysis

The above mentioned health and trade issues represent the overall reasons why samples have to be collected. Where and when to collect samples will depend on the practical purpose for which they are destined. The sampling methodology to be employed should be chosen on the basis of a rationale in accordance with a “fit-for-

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4 purpose” approach. Where to collect samples refers to the selection of the sites where
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6
7 sampling should be done (ship, dock, container, farm, stable and so on up to the table).
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10 When to collect samples refers to the purpose of the collection, such as mandatory
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12 or targeted investigations.
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16 *“Purposes”: monitoring, surveillance and targeted sampling*
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19 Collecting samples for mycotoxin contamination is mostly performed for
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21 monitoring, surveillance and targeted purposes.
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24 According to the World Health Organization/Communicable Diseases
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26 Department/Communicable Diseases Surveillance & Response (WHO/CDS/CRS),
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28 monitoring is “the performance and analysis of routine measurements, aimed at
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30 detecting changes in the environment or health status of populations”, while
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32 surveillance can be defined as “the ongoing systematic collection, analysis and
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34 interpretation of data, followed by the dissemination of information to all those
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36 involved so that directed actions may be taken” (WHO 2004, Lo Fo Wong *et al.* 2004).
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38 In other words, monitoring activities are somehow both preliminary and routinely
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40 performed activities, while surveillance is undertaken whenever data from monitoring
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42 reveals that standard/legal values have been exceeded, and it aims at providing a basis
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44 for centralized and qualified feed-back (Noordhuizen and Dufour 1997). The amount of
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46 samples to be collected for monitoring should be proportional to the food consumption
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48 rate and take into account the amount of domestic production and the amount of import.
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54 Targeted sampling is instead the action undertaken when there is a concrete
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56 suspicion that contaminants (mycotoxins) are present in excessive amounts as a
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58 consequence of previous findings.
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5 A concise and effective view of the sampling requirements for surveillance of
6 mycotoxins has been given by the Joint FAO/WHO Expert Committee on Food
7 Additives (FAO/WHO 2001a). This points out the relevance of generating meaningful
8 data from surveys through the collection of representative samples. This should reflect
9 the selection of the sites where to collect samples within the food chain and across the
10 countries, taking into consideration also differences in the agro-climatic conditions.
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18 Targeted sampling focuses on sample populations, which are likely to be non-
19 compliant (food and feedstuff) or more sensitive (groups of consumers). As for
20 mycotoxins, suspected samples include goods produced or stored under bad conditions
21 and food derived from animals showing clinical signs of intoxication.
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28 As an example of sampling due to a suspicion of contamination, decisions were
29 taken at the European level imposing special conditions on the import of certain
30 products consigned from countries suspected of production of contaminated goods
31 (Commission Decisions 2002/80/EC; 2002/233/EC; 2002/679/EC; 2003/552/EC;
32 2004/429/EC; 2002/79/EC; 2002/678/EC; 2003/550/EC; 2000/49/EC; 2003/580/EC;
33 2003/423/EC; 2004/428/EC and 2005/85/EC). Actually in this case the border line
34 between surveillance and targeted sampling is rather ambiguous, depending on the level
35 of suspicion.
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47 It should be considered that targeted sampling should not be used for the exposure
48 assessment since it may lead to an overestimation of the exposure (WHO 1997).
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54 **“Secondary” sampling: how to collect samples**

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56 Mycotoxin-contaminated units are not homogeneously distributed throughout the
57 lot and a few units (approximately 0.1% for a wide variety of agricultural products) are
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4 likely to be highly contaminated (mycotoxin clusters), while most of the grains are
5 mycotoxin-free. However, after the lot is milled the contaminants are usually more
6
7 homogeneously distributed throughout the bulk; the mean level in the end product could
8
9 still be unacceptable from the health perspective (Whitaker *et al.* 1969; Johansson *et al.*
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11 2000).
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16 Collecting samples for analysis only from the highly contaminated grains or from
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18 the mycotoxin-free ones will provide incorrect final results. Therefore, it is extremely
19
20 relevant to collect and gather randomly many incremental samples from the grain in
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22 bulk in order for the analysis to be representative of the whole lot.
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26 Without implementing a good secondary sampling plan, an associated error in the
27
28 evaluation of the mycotoxin level of the lot could easily occur, generally leading to an
29
30 underestimation; whenever the sampling is performed for monitoring/surveillance
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32 purposes, a poorly developed secondary sampling plan could produce false information
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34 for risk assessors/managers. For inspection purposes, incorrect secondary sampling can
35
36 result in litigation problems.
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40 In order to define an appropriate sampling plan, knowledge of the distribution of
41
42 contaminated units within the bulk is essential. The matter has been investigated
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44 especially for aflatoxins and, to a lesser extent for deoxynivalenol and fumonisins
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46 (Whitaker *et al.* 1974; 1976; 1979; 1994; 1998; 2000; Hart and Schabenberger 1998).
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51 **Sampling steps**

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53 The steps usually employed in the evaluation of the mycotoxin level in a lot
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55 include: sampling (random collection of incremental samples throughout the bulk),
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57 sample preparation (gathering and grinding the incremental samples) and analysis
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(generally on the slurry aggregate samples). It is widely recognized that the sampling step is by far the largest contributor to the total error and the associated variability is largely dependent on the level of the toxin. It has been recognized since 1993 that the sampling plan is a function both of the employed testing procedures and of the acceptance/rejection limit (FAO 1993). An exhaustive and updated review on the overall issue of secondary sampling has been provided recently by Whitaker (2004), who in the last decade successfully studied the contribution of sampling error to the total variance during mycotoxin determination. Unfortunately, no similar study has so far been performed for OTA. Nevertheless pragmatic sampling plans are available (Commission Directive 2005/5/EC 2005), based on the assumption that OTA distribution is somehow less heterogeneous than for aflatoxins. The following considerations, mainly derived from the Whitaker paper referring to the issue of sampling for mycotoxins in general, are likely to be applicable to OTA.

Variance (V) in the evaluation of mycotoxins

The total error (TV), associated with the evaluation of mycotoxins is obtained by summing up the error associated with the sampling of incremental samples from the lot (SV = sampling variance), the error of the sample preparation (SPV = sampling preparation variance) and the error of the analytical determination (AV = analytical variance) (Figure 1):

$$TV = SV + SPV + AV$$

It has been shown that the SV is the biggest contributor to the total variance due to the large variability among the contaminated units. Using a 0.91 kg sample of shelled corn with a 20 $\mu\text{g}/\text{kg}$ concentration of aflatoxin, grinding the test sample in a Romer

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4 mill and quantifying by immunoassay, the contribution of SV , SPV and AV was 75.6,
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6 15.9 and 8.5 % respectively (Whitaker 2004).
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9 The sampling variability has been quantitatively studied for many agricultural
10 products (peanuts, corn, soybean, cottonseed, pistachio, wheat, figs) mainly for
11 aflatoxins, but also for deoxynivalenol in wheat and fumonisin in maize (Whitaker *et al.*
12 1998; 2000; Hart and Schabenberger 1998). Equations linking the sampling error to the
13 concentration of the toxin and to the size of the aggregate (gathered) sample have been
14 derived, for aflatoxins, fumonisins and DON. In all cases SV increases with the
15 lowering of the toxin concentration. (Figure 2a, b, c). The sampling variance pattern is
16 similar for the three toxins, but the AFB_1 values are higher than for the other two.
17 Therefore, AFB_1 is probably less homogeneously distributed in the bulk.
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30 The variability associated with the sample preparation has so far been described
31 for aflatoxin in several crops, for fumonisins in corn and for deoxynivalenol in wheat.
32 In all cases, for a given size of sub sample, a decrease of the variance with a decrease of
33 the particle size has been demonstrated.
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40 The analytical variability will be discussed in a separate paper in this issue.
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45 **Uncertainty in the mycotoxin evaluation and OC curves**

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47 Due to the variance associated with the mycotoxin evaluation, a 100% level of
48 certainty is far from being achievable. An overestimation is a risk for the
49 seller/producer, that a good lot could be wrongly rejected, while an underestimation
50 could result in a risk for the buyer/consumer that a bad lot could be wrongly accepted.
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56 For a given sampling plan the Operating Characteristic (OC) curve (Figure 3)
57 describes the probability of acceptance of a lot as a function of its actual quality (Codex
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4 Alimentarius 2004). For mycotoxins, an OC curve links the concentration of the toxin
5 in the lot (x-axis) to the probability of accepting that lot (y-axis). For a prefixed limit of
6 acceptance the OC curve evaluates the risks of the seller/producer and the
7 buyer/consumer (marked areas in Figure 3). For every sampling plan those risks are,
8 defined by sample size, preparation and size of the sub sample, number of analyses and
9 methodology.
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18 The aim of any good sampling plan is to reduce the above mentioned areas. The
19 risks are reduced when both the aggregate sample and the sub sample size are increased
20 or when the ground particle size is decreased.
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25 Lowering the limit of acceptance increases the seller's risk, while an increase of
26 that limit reduces the buyer/consumer risk.
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30 OC curves have been drawn for several sampling plans for aflatoxins (Dickens
31 and Whitaker 1982), but there is a lack of information on the OC curves for OTA.
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37 **Sampling protocols**

38 Due to the inhomogeneous distribution of contaminated kernels in a lot and the
39 consequent relevance of gathering small incremental samples to form an aggregate
40 sample, methodologies and equipment employed in collecting such incremental samples
41 are crucial in reducing errors.
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51 *CODEX approach.* According to FAO (FAO 2001b), the most accurate and precise
52 procedure for taking random incremental samples for aflatoxins is to draw small
53 samples while the lot is transferred, that is, during the loading or unloading of the
54 product (dynamic sampling). For big lots this methodology is unfortunately rather time-
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4 consuming, since the procedure implies drawing samples at regular intervals of time
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6 which may be all night and day, sometimes interrupting the procedure due to the forced
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8 closure of a hold, as in the case of rain. The deployment of automated sampling
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10 equipment, such as crosscut samplers, could greatly assist the process. (Codex
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12 Alimentarius 2004).
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16 Whenever dynamic sampling is not applicable, as in the case of static lots,
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18 sampling probes have to be used. This could be the case when large volumes of a
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20 product are stored in a single bin (buck, railcar) or in many small containers (bags or
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22 sacks). According to Codex the probes should be carefully selected on the basis of the
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24 type of container, since all the units should have the same chance of being selected.
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30 *European approach.* Since 2001, the European Union put in force a package of
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32 Directives concerning sampling procedures for the most prominent mycotoxins, namely
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34 aflatoxins, patulin, *Fusarium* toxins and ochratoxin A.
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37 For OTA, Directive 2005/5/CE on sampling methods, amending the previous Directive
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39 2002/26/CE, has been very recently endorsed. This Directive deals with provisions both
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41 for bulk and packaged commodities such as cereals, dried vine fruits, roasted and
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43 soluble coffee, grape juice and wine.
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46 For lots in bulk, the aggregate sample sizes depend on the commodity and lot size,
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48 ranging from 1 kg to 10 kg. For wine and grape juice, the minimum number of
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50 incremental samples to be taken ranges from 3 to 10 for juice and from 1 to 3 for wine.
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53 As for lots traded in individual packages, the sampling frequency, both for
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55 aflatoxins (Commission Directive 98/53/EC 1998) and for OTA (Commission Directive
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57 2002/26/EC 2002) is the following:
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$$SF = \frac{\text{lot_weight} \times \text{incremental_sample_weight}}{\text{aggregate_sample_weight} \times \text{individual_packaging_weight}}$$

where SF is the sampling frequency (every n^{th} sack or bag from which an incremental sample must be taken)

All weights have to be expressed in kilograms.

Monitoring and surveillance activities

The status of monitoring and surveillance activities for mycotoxins is widely different from country to country. Developed countries have developed for a number of years monitoring programmes for selected mycotoxins such as aflatoxins, ochratoxin A and more recently *Fusarium* toxins (MAFF 1994; CFIA 2002). The results have been usefully employed for advanced surveillance programmes on selected goods.

In the past decades, coordinated programmes have been launched by the EU aimed at the surveillance of the status of contamination by aflatoxins in spices, pistachios and baby food and by ochratoxin A in cocoa and coffee. Those activities were carried out before the recent European enlargement and efforts should be devoted to compare the contamination of those mycotoxins in the new European countries, in order to verify discrepancies in frequency and level of contamination for health and trade purposes. For relatively “new” mycotoxins such as *Fusarium* toxins, for which legislation has been recently enforced, although monitoring activities were somehow carried out, not many surveillance activities have so far been undertaken. As for OTA residues in food of animal origin, a particularly careful surveillance sampling is directed to pork tissues in Denmark: in 1978, Denmark established a guideline to control ochratoxin A levels in pork products in their slaughterhouses. In the case of macroscopic changes, pig kidneys are analysed for their ochratoxin A content. If the ochratoxin A level of pig kidney is higher than 25 $\mu\text{g}/\text{kg}$, the entire pork carcass is

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4 rejected as the meat is also suspected to be highly contaminated as a consequence. If the
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6 level is between 10 and 25 µg/kg, edible offals are eliminated and if lower than 10
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8 µg/kg, only pig kidneys are discarded (Buchman and Hald 1985).
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11 Work aimed at collecting and elaborating mycotoxin occurrence has been
12 performed by the SCOOP tasks so far developed for aflatoxins, ochratoxin A (SCOOP
13 Task 2002) patulin and *Fusarium* toxins (SCOOP Task 2003). Actually, the ultimate
14 scope of the above tasks was to evaluate the exposure of those mycotoxins in the
15 European population, but the work included the evaluation of information derived from
16 the random or targeted sampling employed for each set of data.
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28 Conclusion

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30 An effective plan to evaluate statistically the level and impact of mycotoxin
31 contamination in general and of OTA in particular, should consist of a “statistically”
32 based identification of the sites where samples are to be collected and by the reliable
33 evaluation of the status of contamination at that site.
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40 As far as specifying the sites where collected samples are to be taken is
41 concerned, any specific guideline on where and when to take samples depends on the
42 reason why the samples are collected (health, trade, control). In this respect no specific
43 guideline for monitoring and surveillance purposes is so far available for mycotoxins.
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49 The reliable evaluation of the status of contamination at each site should be
50 performed through sampling, sub sampling and analytical steps. Great efforts so far,
51 have been devoted to improve the reliability of the analytical measurements. Sampling
52 has also been extensively studied for several mycotoxins except for OTA.
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5 Generally, it should be noted that data derived from monitoring/surveillance
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7 activities should be updated and ameliorated. In this respect, there is a paucity of data
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9 from the new European countries.
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12 In addition, the acquisition of new databases developed on soundly performed
13
14 primary and secondary sampling should be encouraged.
15

16 17 18 **Acknowledgements** 19

20
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22
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28
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30
31 necessarily represent the views of ILSI Europe.
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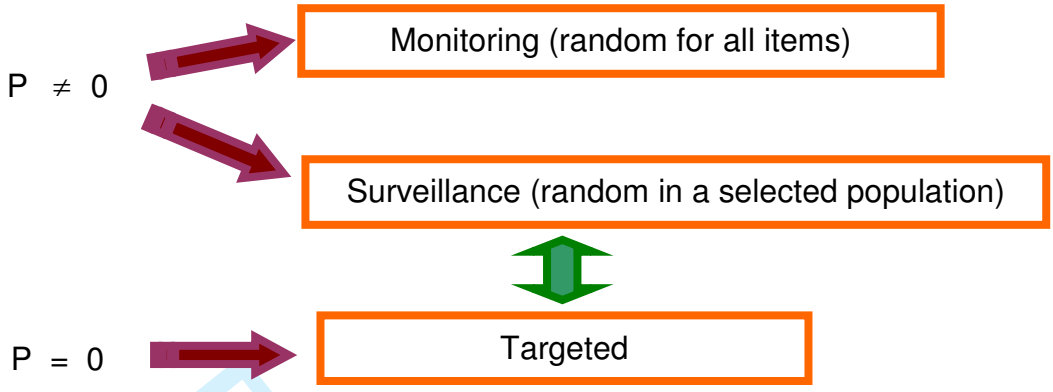
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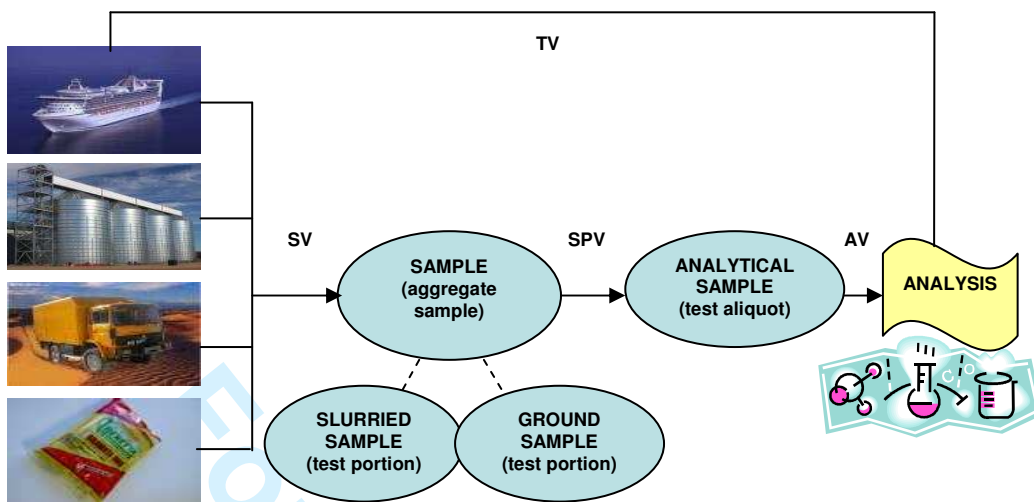
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