FOOD ANALYSIS: THE ROLE OF MEASUREMENT UNCERTAINTY INTERNATIONAL CONFERENCE 2ND IMEKOFOODS

THE IMPACT OF MEASUREMENT UNCERTAINTY IN ASSESSING THE COMPLIANCE OF MYCOTOXINS IN FOOD AND FEEDS

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Abstract – Over the last ten years, the role on measurement uncertainty has been embraced in those sectors where, traditionally, metrology has strived to assert the concept and its applications. Its critical role in the compliance assessment and decision making in the context of official control is pivotal and the harmonization of approaches is crucial for the success of the control activities.

Keywords: uncertainty, mycotoxins, layout, references (up to five)

1. INTRODUCTION

Regulation EC/401/2006, laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs, introduced for the first time the mandatory use of measurement uncertainty in the assessment of compliance of a lot.

In fact, what has been set is that the rejection of a lot occurs if the laboratory sample exceeds the maximum limit beyond reasonable doubt, taking into account the correction for recovery and measurement uncertainty.

The meaning of this provision led to a new approach for assessing the compliance, that does not correspond to the simple exceeding of the legal maximum limit but it is rather related to the interval of concentration under which there is a probability that the real concentration level of the lot can fall below the maximum limit.

2. THE COMPLIANCE ASSESSMENT AND DECISION MAKING IN THE CONTEXT OF OFFICIAL CONTROL

The introduction of a target value higher than the maximum level to be considered as decision level for checking the compliance of food and feeds to the cogent legislation was introduced by the Decision EC/657/2002 [1]. In that provision, measurement uncertainty was not introduced yet, but the need to calculate a reference level different from the legal limit was individuated as CCα. This value was defined as a "decision limit" meaning the measurement at and above which it can be concluded with an error probability of α that a sample is non-compliant.

In addition, the article 6 of the same Decision stated that the result of an analysis shall have to be considered non compliant if the "decision limit" of the confirmatory method for the analyte is exceeded.

A further difference was introduced depending on the nature of the legal limit. In fact, in the presence of a permitted limit for a substance, the decision limit referred to the concentration above which it can be decided with a statistical certainty of 1 – α that the permitted limit has been truly exceeded. Viceversa, in the case of no existing permitted limit, the decision limit corresponded to the lowest concentration level at which a method can discriminate with a statistical certainty of 1 – α that the particular analyte is present. For substances listed in Group A of Annex I to Directive 96/23/EC, the α error is 1 % or lower, and for all other substances, the α error is 5 % or lower.

Successively, Regulation EC/401/2006 [2], laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in

foodstuffs, introduced for the first time the mandatory use of measurement uncertainty in the assessment of compliance of a lot.

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The measurement uncertainty 'estimate' describes, in fact, the range around a reported or experimental result within which the true value can be expected to lie within a defined level of probability. This is a different concept to measurement error, which can be defined as the difference between an individual result and the true value.

The official definition of the measurement uncertainty is the following: the parameter, associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand [3]. "The 'parameter' may be, for example, a range, a standard deviation, an interval (like a confidence interval) or half-interval (±u is a statement of a half-interval) or other measure of dispersion such as a relative standard deviation.

The reporting of uncertainty is intended to provide a higher level of confidence in the validity of the reported result.

The range within which the raw analytical figure is likely to fall, i.e. its uncertainty, depends on the intrinsic "trueness" and precision of the analytical method as used in the laboratory.

In general, the uncertainty of measurements encompasses many components, arising from activities

related to the "analytical chain". The uncertainty associated to an analytical result is always influenced by three main steps:

- Before the test portion preparation: sampling (SS), packing, shipping and storage of samples;
- Preparation of test portion: sub-sampling, sample preparation and sample processing (SSp);
- Analysis (SA): extraction, clean-up, dilution, derivatization and instrumental determination.

As far as Mycotoxin analysis and in general all the compounds heterogeneously distributed within the sampling target, either spatial or temporal, the sampling step plays the most crucial role in the variability of the results and reveals as the major contribution to the uncertainty. Unfortunately, the calculation of sampling uncertainty is quite complex even if the EURACHEM/CITAC [4] Guide issued, in 2007, a useful tool to be followed in calculating it.

The first relevant issue to consider is to individuate the different sources of uncertainty within the multiple abovementioned steps related at that peculiar operation. In the EURACHEM guide the following sources of uncertainty as derived from a document provided by et al., in 1995 [5], are reported for sampling and sample preparation (Table 1).

Table 1: Some sources of uncertainty in sampling and sample preparation, adapted from reference [3]

Sampling	Sample preparation
- Heterogeneity (or inhomogeneity) - Effects of specific sampling strategy (e.g. random, stratified random, proportional etc.) - Effects of movement of bulk medium (particularly density selection) - Physical state of bulk (solid, liquid, gas) - Temperature and pressure effects - Effects of sampling process on composition (e.g. differential adsorption in sampling system) - Transportation and preservation of sample	- Homogenisation and/or **sub-sampling** effects - Drying - Milling - Dissolution - Extraction - Contamination - Derivatisation (chemical effects) - Dilution errors - (Pre-)Concentration - Control of speciation effects

Moreover, two basic types of approaches for estimating the sampling uncertainty are the empirical and modelling approach, called also top-down and bottom-up, respectively.

Without entering in detail, the empirical approach, without necessarily knowing any of the sources individually, is based on overall reproducibility estimates from either in-house or inter-organisational measurement trials. In other words, it must be considered random or systematic effects in a general way, estimating the magnitude of each of these effects separately from the properties of the measurement methods, such as sampling precision (for random effects arising from sampling) or analytical bias (for systematic effects arising from chemical analysis). These estimates can be combined to produce an estimate of the uncertainty in the measurement result. In Table 2, the contribution to uncertainty used in the empirical approach are summed up.

Process	Effect class	
	Random (precision)	*Systematic (bias)*
Analysis	e.g. *duplicate analyses*	e.g. *certified reference materials*
Sampling	*Duplicate samples*	*Reference sampling target, inter-organisational sampling trial*

Four classes of effects that contribute to the uncertainty of measurements, and methods for their estimation.

Table 2. Contributions in the empirical approach

The modelling approach, or bottom-up, identifies all of the sources of uncertainty, quantifies the contributions from each source, and then combines all of the contributions, as a budget, to give an estimate of the combined standard uncertainty.

The same elements as treated for sampling and sample preparation uncertainty can be applied by the already cited bottom-up or top-down approach and will be object of full explanation by other Authors in this contributing text.

About the relationship between analytical results, measurement uncertainty, recovery factors and the provisions of EU food and feed legislation, DG SANTE of European Commission refers to a paper [6] stating that for chemical analyses, using the results from collaborative trials, it would not be unreasonable to expect that the expanded uncertainty reported by laboratories would be of the following orders:

Concentration	Expanded uncertainty	Range of acceptable concentrations
100mg/kg	16%	84 to 116 mg/kg
10mg/kg	22%	7.8 to 12.2 mg/kg
1mg/kg	32%	0.68 to 1.32 mg/kg
< 100 µg/kg	44%	56 to 144 µg/kg

However, at levels regarding contaminants and undesirable substances, it could be argued that especially for concentrations less than 100 µg/kg, typically related to genotoxic carcinogenic or non-genotoxic carcinogenic compounds, the value associated to the measurement uncertainty seems to be too large to be used, considering the direct impact on the consumer health.

Unlike pesticides, where, as stated in 2006 by CODEX [7] and provided the analytical proficiency of a laboratory has to be established through participation in EU or similar PT studies and/or demonstrated by acceptable long-term precision and bias associated with its test results, an agreement value (50%) has been adopted, in the field of mycotoxins no harmonised approach for calculating the measurement uncertainty is in place, apart an approach reported in regulation EC/401/2006 [2], described further into the text.

The first effect is that, on the same sample, two different laboratories can give a different judgment on compliance based on different approaches for determining the measurement uncertainty, creating contentious and disputes after a Court. Secondly, the lack of a reference approach can create a sort of non-controlled conditions where any single laboratory is left free to choose whatever approach.

Among the different approaches to be followed for calculating the measurement uncertainty, four are noteworthy that are the holistic, the metrologic, the simplified metrologic method and the one based on Horwitz approach.

All these different methods will be treated in full detail by other Authors in this compendium and all the related technical aspects are forwarded to them.

Commonly, the Accreditation body is much in favour of the use of the metrological approach rather than the one based upon the Horwitz equation or other alternative methods and this can be quite reasonable especially in consideration of the assessment of the compliance for genotoxic carcinogenic compounds such as aflatoxin B1.

In general, measurement uncertainty should be evaluated and quoted in a way that is widely recognized, internally consistent and easy to be interpreted.

As previously cited, an approach is given by the Regulation EC/401/2006 [2] in the case where there is a limited number of fully validated methods of analysis. In this case, a 'fitness-for purpose' approach, defined as an uncertainty function (**Uf**) that specifies maximum levels of uncertainty regarded as fit for purpose, is provided by the Regulation.

In these cases, the laboratory may use a method that produces results within the maximum standard uncertainty, as calculated by the following formula:

$$Uf = \sqrt{(LOD/2)^2 + (\alpha * C)} \quad (1)$$

where: — Uf is the maximum standard uncertainty (µg/kg) — LOD is the limit of detection of the method (µg/kg) — α is a constant, numeric factor to be used depending on the value of C — C is the concentration of interest (µg/kg).

Another aspect that should be worthy to be mentioned is the role of the measurement

uncertainty not only in the judgment of the compliance to the legal limits but also towards the so called «safe level».

A level can be considered safe if it does not cause, beyond any reasonable doubt, a quantifiable risk to humans exposed for a lifetime to that dose level.

It is known that the exceeding of the legal limit does not mean that a substance is risky for humans or animals but that it cannot be placed into the market and in case that product will be recalled or withdrawn.

With the introduction of the role of measurement uncertainty as previously described, the value of the legal limit itself slightly looses its importance shifting the target level ahead in correspondence with that value that diminished of the measurement uncertainty falls below or above the legal limit.

Nonetheless, this situation masks two different and converse aspects, both related to the real safety of the consumed product.

One scenario depicts a situation where the value falls between the legal limit and the safe limit. If considering the measurement uncertainty the product is not compliant, it can be risky or not risky for the consumer health, this in direct correspondence with the nature of the toxic propertied of the contaminant. A second scenario reflects a value beyond both the safe limit and the legal limit and in this case the uncertainty doesn't play any critical role since in this case the product is unsafe for the consumer. A third scenario is when the raw value falls below the legal limit but above the safe level as in the case of genotoxic carcinogens substances. In this case, if the raw value falls below the legal limit considering the measurement uncertainty, *de facto* it is in compliance with the law even being unsafe for the consumer.

This last case plays a very relevant burden for consumer health, especially for sensitive groups such as children, since the commercialisation of these products is allowed even being unsafe.

3. CONCLUSIONS

In conclusion, the entry of the concept related to the measurement uncertainty for assessing the compliance of contaminants in food and feeds dramatically changed the way to interpret the analytical results, despite the fact that, at least for mycotoxins, a full harmonisation of the approach to be used for its calculation is still missing; for this reason, a practical effort should be exerted by all the interested parties in finding a solution as soon as possible.

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