doi: 10.1255/tosf.9

Sampling errors undermine valid genetically modified organism (GMO) analysis

Kim H. Esbensen, Francis Pitard and Claudia Paoletti

Esbensen, Paoletti and Pitard. While the first is trying to get a sampling issue across, the latter two take a much more relaxed boat ride on Lake Como (2008).

This letter was written to the Joint Research Center, European Commision, some time ago, but the authors were asked not to publish it because "appropriate measures" were about to be undertaken. However, five years later, nothing has happened and the letter is therefore very much still relevant today. The authors are pleased that *TOS forum* has offered it an airing; the issue is serious.

The First Global Conference on GMO Analysis, 24–27 June, 2008, held in Cernobbio, Italy, was a scientific success, and very well organised. Its many purposes were all achieved: a broad and comprehensive scientific overview of all relevant issues related to genetically modified organism (GMO) detection and quantification was offered to an audience which represented just about every country, academic institution, industrial company and regulatory body involved, on a truly global scale (more than 70 countries were represented). In the matter of GMO policy enforcement, the entire world looks to Europe, with good reason. The European Commission has charged the Joint Research Centre, Ispra, with the responsibility of developing and supervising application of appropriate methods for GMO detection and quantification. We congratulate the organising and scientific committees for the substantial breakthrough of providing all stakeholders with an opportunity to see the entire width of the GMO playing field: detection, analysis, documentation, accreditation and harmonisation.

However, we want to point out and to express our grave concern about one salient matter that in our view was decidedly under-achieved at this conference, indeed in the GMO field ever since.

This timely conference also highlighted a dramatic weak point which threatens to undermine the legitimacy of GMO detection and quantititation in particular, viz. the issue concerning *sample representativity*. Primary samples, which form the input to all GMO laboratories and their subsequent quantitation constitute the singular critical factor concerning whether an analytical result will be reliable for decision making; or not. Although there is an alarming need for a unifying standard, it has not been possible to reach agreement between the relevant CEN and ISO parties on even the basics of this issue; amongst other reasons this is a matter of a marked transatlantic disparity regarding perceived GMO risk with derived different policies in Europe and the US. As a result, primary sampling issues today have no unifying common basis but standardisation is predominantly carried out on a case-to-case basis with a plethora of sub-optimal attempts to formulate principles and rules—alas with very disappointing efficiency, indeed none realising representative sampling. The issue can be stated with clarity: if a sample arrives at any GMO laboratory without proper provenance documentation (without documentation of being representative), the entire detection/analysis/validation/documentation chain is without merit, reliability or value. All non-representative samples are in reality not worth analysing, since the analytical result will only relate to the minute amount of material analysed (typically of the order of 50 mg). Failure to provide scientific and legal *proof* of a fully representative sampling and sub-sampling process disqualifies such "samples", because the analytical result cannot be reliably attributed to the original lot, which is the whole objective of analytical characterisation. This goes both for detection and quantitation.

However, a complete framework for representative sampling does exist, called the Theory of Sampling (TOS), which has been in existence for more than 50 years.

Sampling for trace concentrations (the legislative EU GMO threshold for adventitious occurrence of GMO is 0.9%) suffers from highly significant Total Sampling Errors (TSE) typically of a magnitude of 20–100× the analytical error. It therefore makes little, or no, sense to continue to focus overwhelmingly on analytical precision, if primary sample representativity cannot be reliably documented, i.e. if the accuracy of the analytical result is left unknown: accuracy concerns trueness (representativeness) with respect to the original lot from where the primary sample was taken (shipload, truckload, field etc.).

The Theory of Sampling constitutes the world's only complete scientific framework for all aspects of representative sampling, it covers all types of lots and materials, at all scales, including "from farm to fork". It especially also holds all principles for representative mass reduction in the analytical laboratory, where one typically finds appreciable representativity violations, GMO laboratories not excluded, as was indeed also demonstrated at the conference. Instead of continuing the tradition of one standard for each analyte, TOS forms an overarching framework, in fact constituting a much needed unifying standard for representative sampling. Full documentation has been available in the literature for more than 25 years.

At the conference there was a conspicuous lack of appreciation of the value (economic, societal, public safety) of the imperative of documentation that every primary sample can be documented to be representative. Very few presentations (lectures, posters) presented anything akin to compliance with the Theory of Sampling (TOS). In its place there was a widely felt complacency in referencing to the only current CEN technical specification dealing with this issue [CEN TS 15568: 2007: *Foodstuffs—Methods of analysis for the detection of genetically modified organisms and derived products—Sampling strategies*]. Unfortunately this

document comprises only a small first step towards harmonisation with TOS, and most emphatically cannot serve as the needed guarantee. There were scores of important presentations covering every conceivable aspect of laboratory estimation of GMO *measurement uncertainties* (MU) which in the case of GMO is considerable, a survey of the many contributions dealing with TAE alone reached a consensus of some 15–20% (rel). It is highly significant that this metrological term (MU) hardly includes any type of sampling error (only one of out seven sampling errors at best)! There were but a few contributions related to field sampling, but exactly **zero** empirical contributions concerned with uncertainty estimation from the primary sampling stage. Due to the foresight and diligence of the scientific organising committee, there were, however, three invited introductory contributions outlining all essential principles and procedures in the Theory of Sampling, including the pivotal fact that sampling errors are typically 20–50× larger than the total analytical error itself, TAE. This fact was uncontested at the conference, yet there was very little evidence of anything but lip service to the mandate of doing something about this.

The consequences of non-compliance with TOS are several: scientific, economic, authority. Non-representative sampling will perforce give rise to a significant, inconstant sampling bias (always present, but varying in magnitude with every new sampling operation), a bias which is not estimable and therefore not amenable to the classical bias correction we know from conventional statistics. The consequences of not focusing on reducing the Total Sampling Errors (TSE) as much as possible will necessarily also have economic and decision-making consequences—maybe severe—at least there are potential consequences regarding public health concerning non-authorised GMO.

The Joint Research Centre serves the European community and its citizens by providing scientific and technical support to European policy makers as a reference centre. Based on the success of the Global GMO conference, we here call upon the JRC to build on its unequalled success in establishing the ENGL network of harmonised and standardised national GMO laboratories, which covers all aspects of GMO detection and quantitation other than sampling, also to take up the critical success factor of introducing authoritative representative sampling criteria. We have also taken other appropriate scientific actions in the present context,[1–3] the above issues are here offered in the interest of optimal follow-through of the conference.[4] The political aspects of this task are best left with the JRC, but the scientific imperative is very clear:

"Statistical considerations include the accuracy of the analytical estimation with respect to a pre-selected level of tolerable 'risk' or 'uncertainty': It is understood that the lower the tolerable uncertainty, the more laborious the sampling will have to be (the more costly, perhaps somewhat more 'impractical' than today's procedures, which do not originate from in-depth understanding of heterogeneity or representative sampling). It is here essential to be able to distinguish between a sampling bias (which can be reduced/eliminated following TOS, but which is often neglected due to 'practical and economical reasons') and the remaining sampling variance (these two aspects are clearly discriminated in TOS' definition of 'representativity'). Within TOS' framework it is indeed possible to derive complete objective, reliable estimates of the Total Sampling Errors (TSE) accompanying existing or suggested sampling plans and how to decide on the most appropriate sampling procedures."

"Non-statistical considerations include such factors as financial, labor efforts and time constraints. Unfortunately these often dominate or downright rule current sampling protocols design (ISO vs CEN approaches), with the consequence that more approximate sampling protocols with large risks and uncertainties are routinely used—ostensibly 'to save time and money'. While it is not responsibility of science to define the acceptable risk threshold (clearly a political responsibility), science would be remiss if it did not elucidate the very serious consequences of a irresponsible, voluntary, slack acceptance of only these non-statistical issues."[1–3]

We call upon JRC to take the appropriate initiatives without hesitation. We are naturally at your disposition in this endeavour.

References

1. K.H. Esbensen, C. Paoletti and P. Minkkinen, "Representative sampling of large kernel lots I. Theory of Sampling and variographic analysis", *Trends Anal. Chem. (TrAC)* **32,** 154–165 (2012). doi: 10.1016/j.trac.2011.09.008
2. P. Minkkinen, K.H. Esbensen and C. Paoletti, "Representative sampling of large kernel lots II. Application to soybean sampling for GMO control", *Trends Anal. Chem. (TrAC)* **32,** 166–178 (2012). doi: 10.1016/j.trac.2011.12.001
3. K.H. Esbensen, C. Paoletti and P. Minkkinen, "Representative sampling of large kernel lots III. General considerations on sampling heterogeneous foods", *Trends Anal. Chem. (TrAC)* **32,** 179–184 (2012). doi: 10.1016/j.trac.2011.12.002
4. F.F. Pitard, *Pierre Gy's Sampling Theory and Sampling Practise. Heterogeneity, Sampling Correctness and Statistical Process Control*, 2nd Edn. CRC Press (1993). ISBN 0-8493-8917-8