Critical aspects and specific requirements in GMO analysis:

Measurement of uncertainty and data reporting

Responsibilities for food

Responsibilities according to Regulation (EC) No 178/2002 article 17

At all stages of the food chain Food and Feed Business Operators shall ensure that food and feed satisfies the requirements of the food legislation. They have to verify that such requirements are met.

Verification of the activities of the food and feed business operators are checked by official controls which are performed

- by spot-checks
- unannounced
- in frequency dependent on a risk analyses

Regulation (EC) No 882/2004 of the European Parliament and the Council of 29 April 2004 on ***official controls*** *performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules*

(Official Feed and Food Control legislation)

Article 5, Para 2 (d) ….laboratories operate in accordance with Standards referred to in article 12 (2)

Article 12: …competent authorities may only designate laboratories that operate and are assessed and accredited in accordance with…EN ISO/IEC 17025 on "General requirements for the competence of testing and calibration laboratories"

ISO/IEC 17025

5.4.6.2 Testing laboratories shall have and shall apply procedures for estimating valuating the **uncertainty of measurement**.

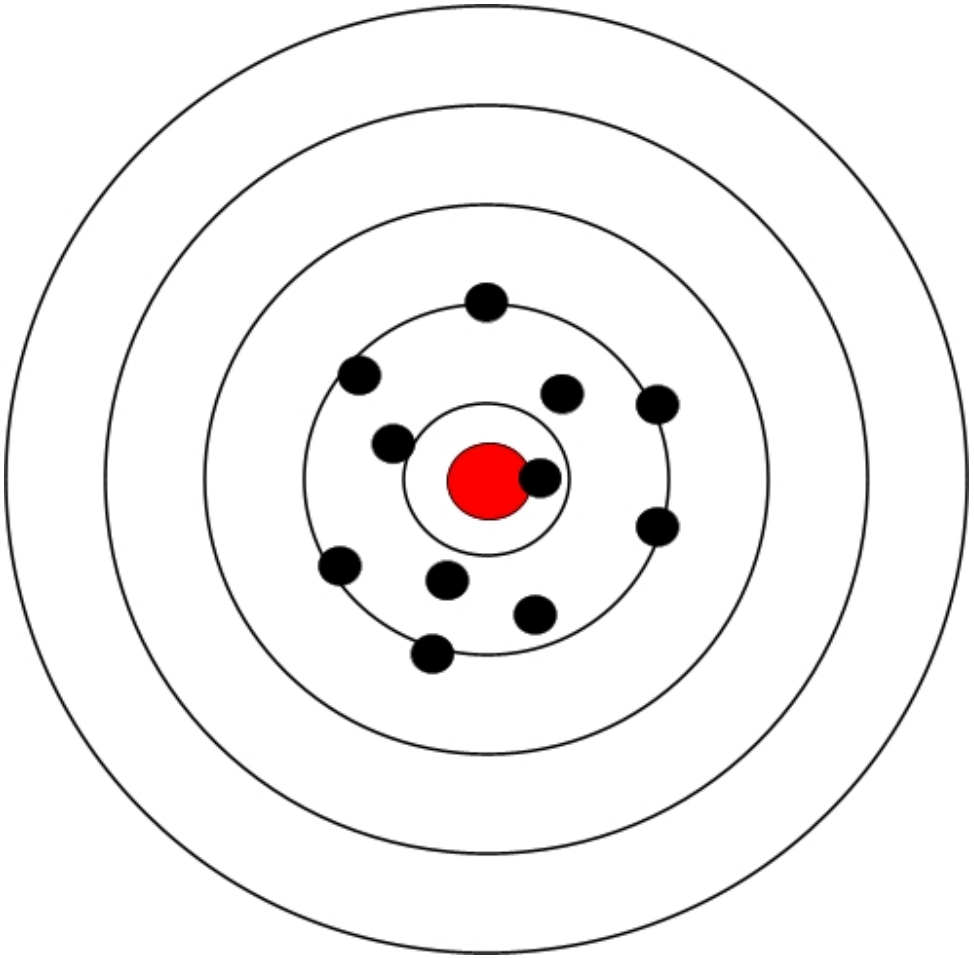
5.4.6.3 When estimating the uncertainty of measurement, all uncertainty components which are of importance in the given situation shall be taken into account using appropriate methods of analysis.

Definition of **uncertainty (of measurement)**

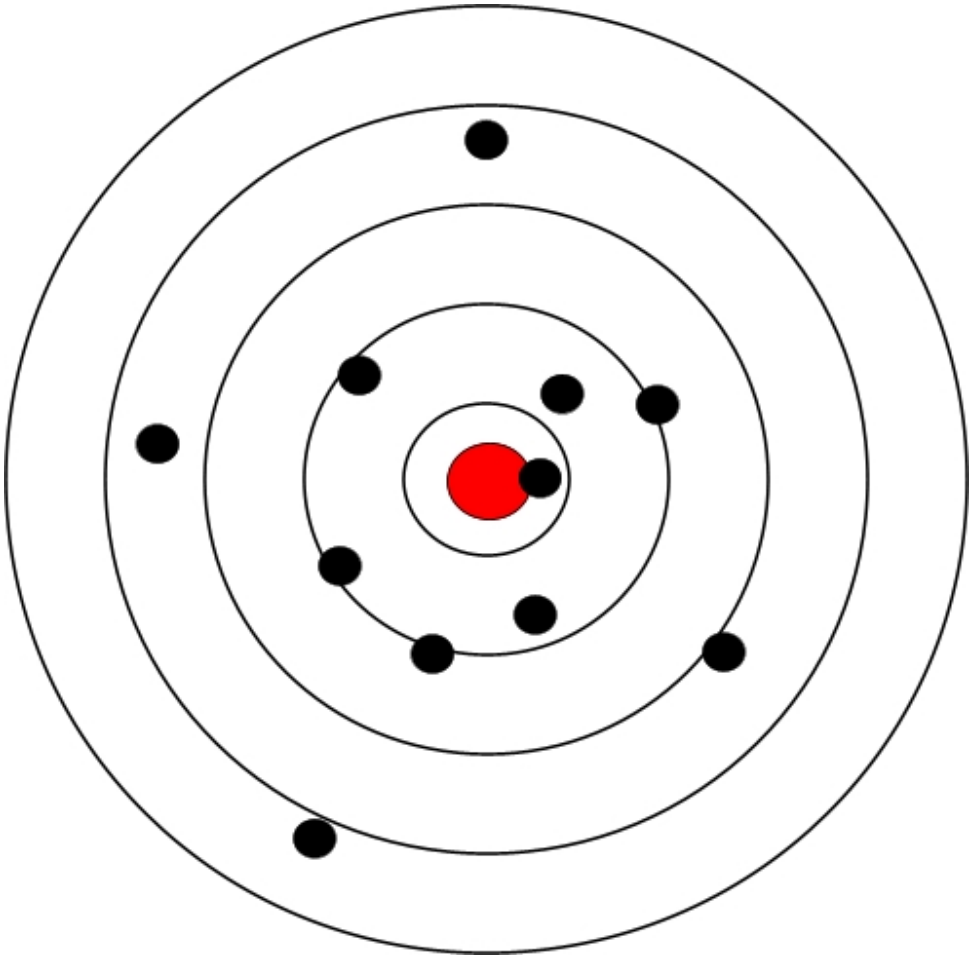
Parameter, associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand

(Evaluation of measurement data — Guide to the expression of uncertainty in measurement
http://www.bipm.org/utils/common/documents/jcgm/
JCGM_100_2008_E.pdf)

uncertainty (of measurement)



uncertainty (of measurement)

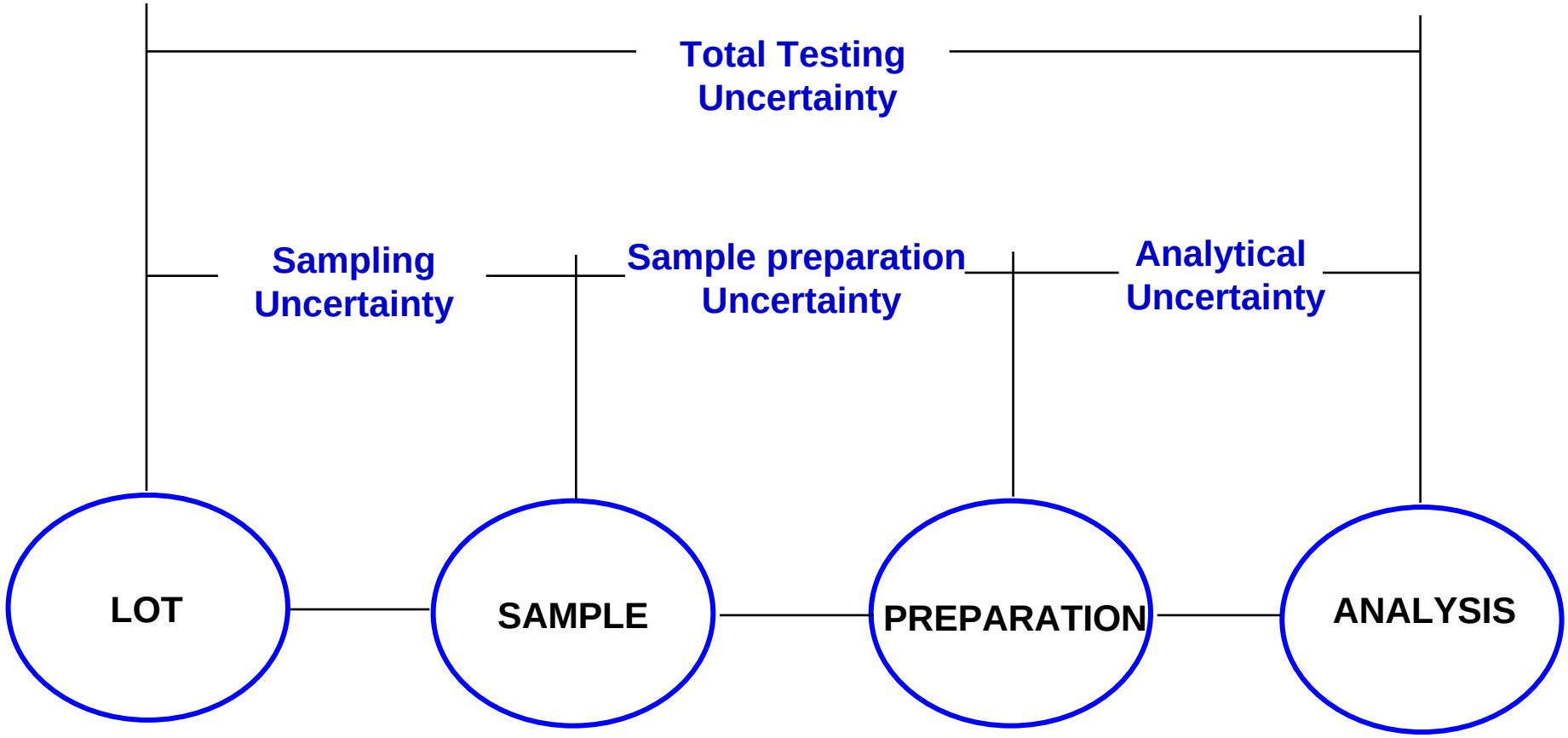


ISO/IEC 17025

5.4.6.3 Note 1
Sources contributing to the uncertainty include,
but are not necessarily limited to,

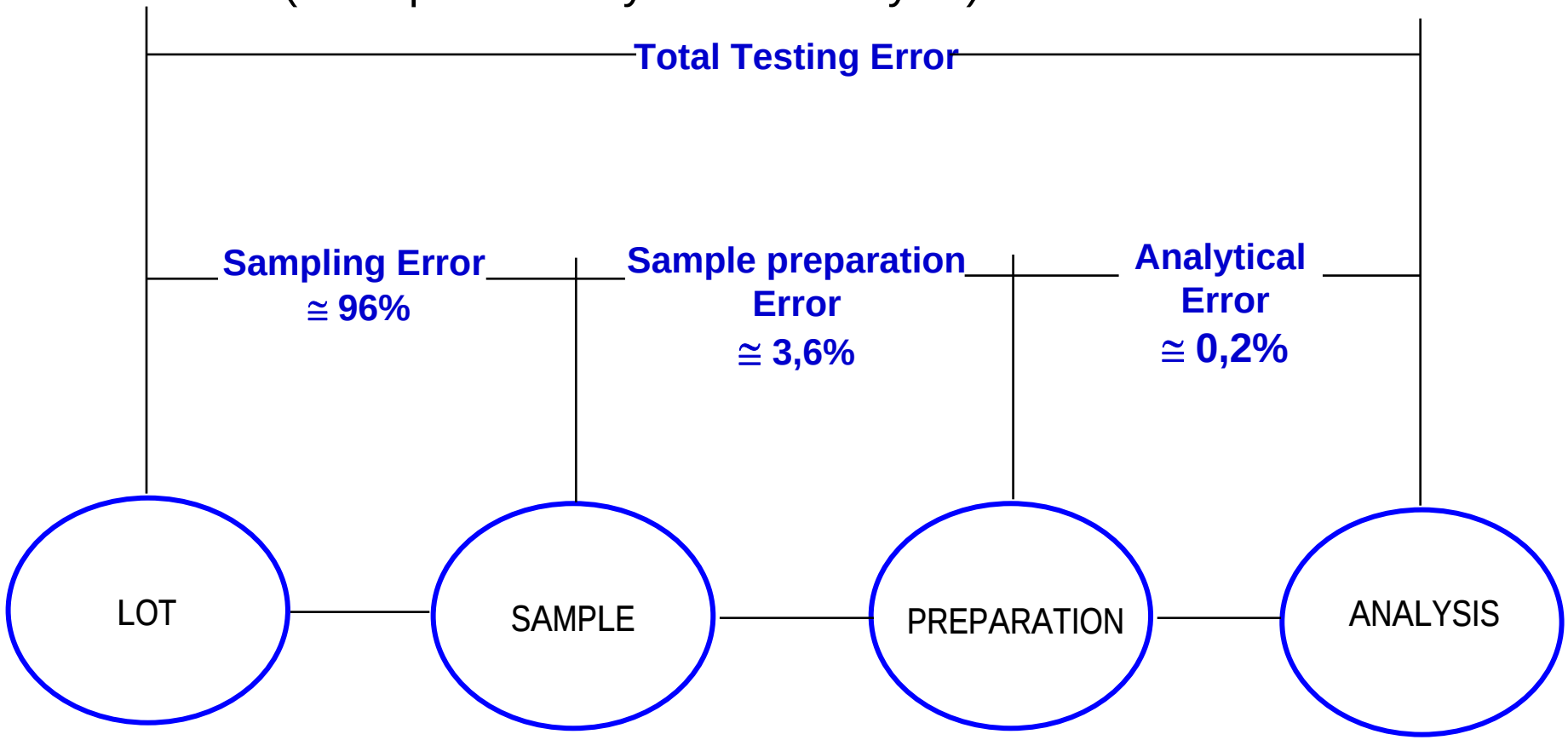
- the reference standards and
- reference materials used,
- methods and equipment used,
- environmental conditions,
- properties and condition of the item being tested or calibrated,
- and the operator.

Uncertainty associated with the analytical chain



TOTAL VARIANCE

• Whitaker et al., J. of AOAC Int., Vol 89, No 4, 2006
(example from mycotoxin analysis)



Interpretation of results including MU (1)

- The MU report of DG SANCO states that **Enforcement Authorities** shall use the measurement uncertainty associated with an analytical result when deciding whether an analytical result falls within the specification for food and feed control purposes…
- The value obtained by subtracting the uncertainty from the reported concentration, is used to assess compliance. Only if that value is greater than the maximum concentration stipulated in legislation, it is sure **'beyond reasonable doubt**' that the sample concentration of the analyte is greater than that prescribed by legislation.

Interpretation of results including MU (2)

- DG Health and Consumer Protection: Report to the Standing Committee on the Food Chain and Animal Health on the relationship between analytical results, the measurement uncertainty, recovery factors and the provisions in EU food and feed legislation with particular focus on the community legislation,
- http://ec.europa.eu/food/food/chemicalsafety/contaminants/report-sampling_analysis_2004_en.pdf

Guidance Document on Measurement Uncertainty for GMO Testing Laboratories

S. Trapman, M. Burns, H. Broll, R. Macarthur,
R. Wood, J. Zel

EUR 22756 EN/2 - 2009

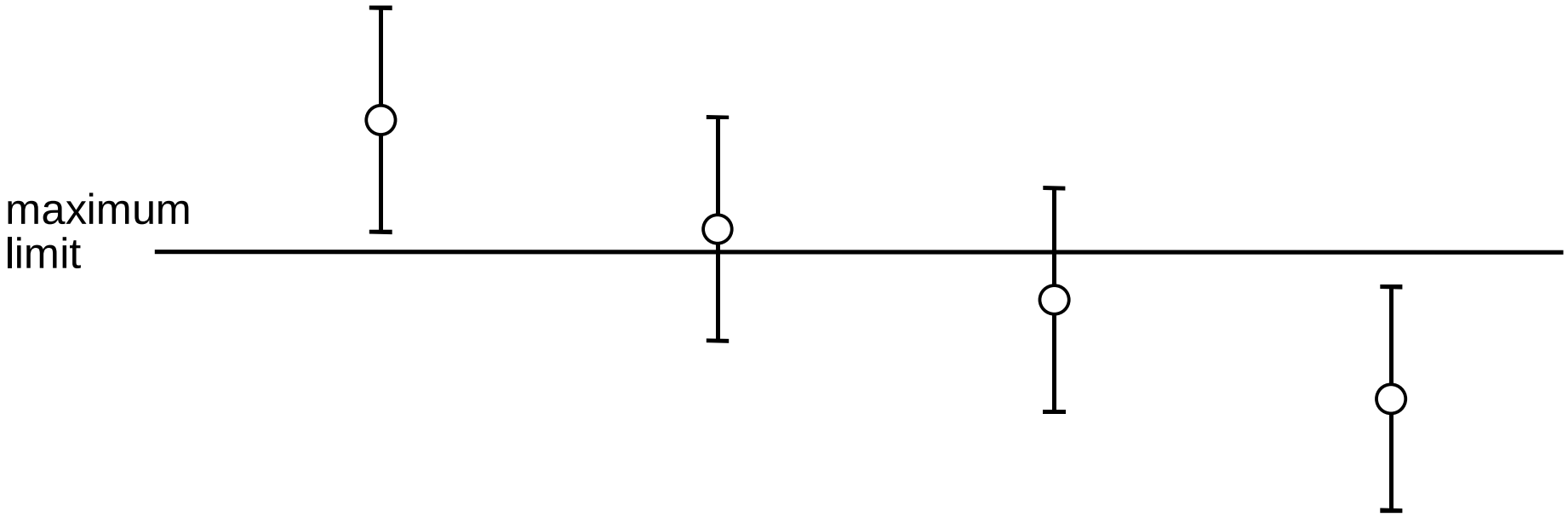
JRC EUROPEAN COMMISSION

irmm

Prepared by:
Task Force Group of Members of the European network of GMO laboratories

http://www.irmm.jrc.be/reference_materials_catalogue/user_support/Documents/eur22756en.pdf

… BEYOND REASONABLE DOUBT



(i)
Result less uncertainty above limit

(ii)
Result above limit but limit within uncertainty

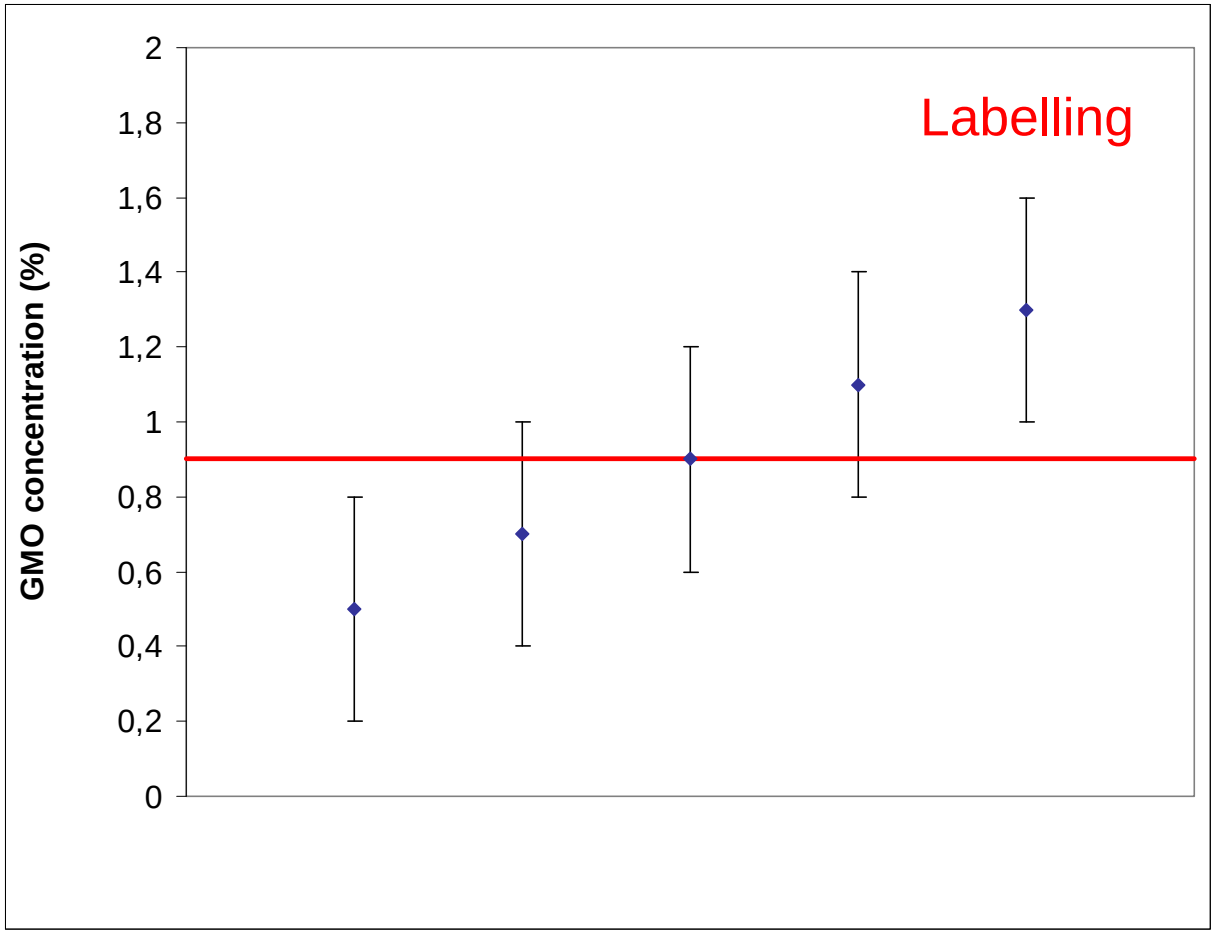
(iii)
Result below limit but limit within uncertainty

(iv)
Result plus uncertainty below limit

RESULT - MEASURMENT UNCERTAINTY = RESULT FOR OFFICIAL CONTROL

in the case of labelling of food and feed containing approved gmo or material derived from approved gmo

Example


GMO concentration (%)
2
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
Labelling
0,9%
Legal treshold

Bottom up approach (1)

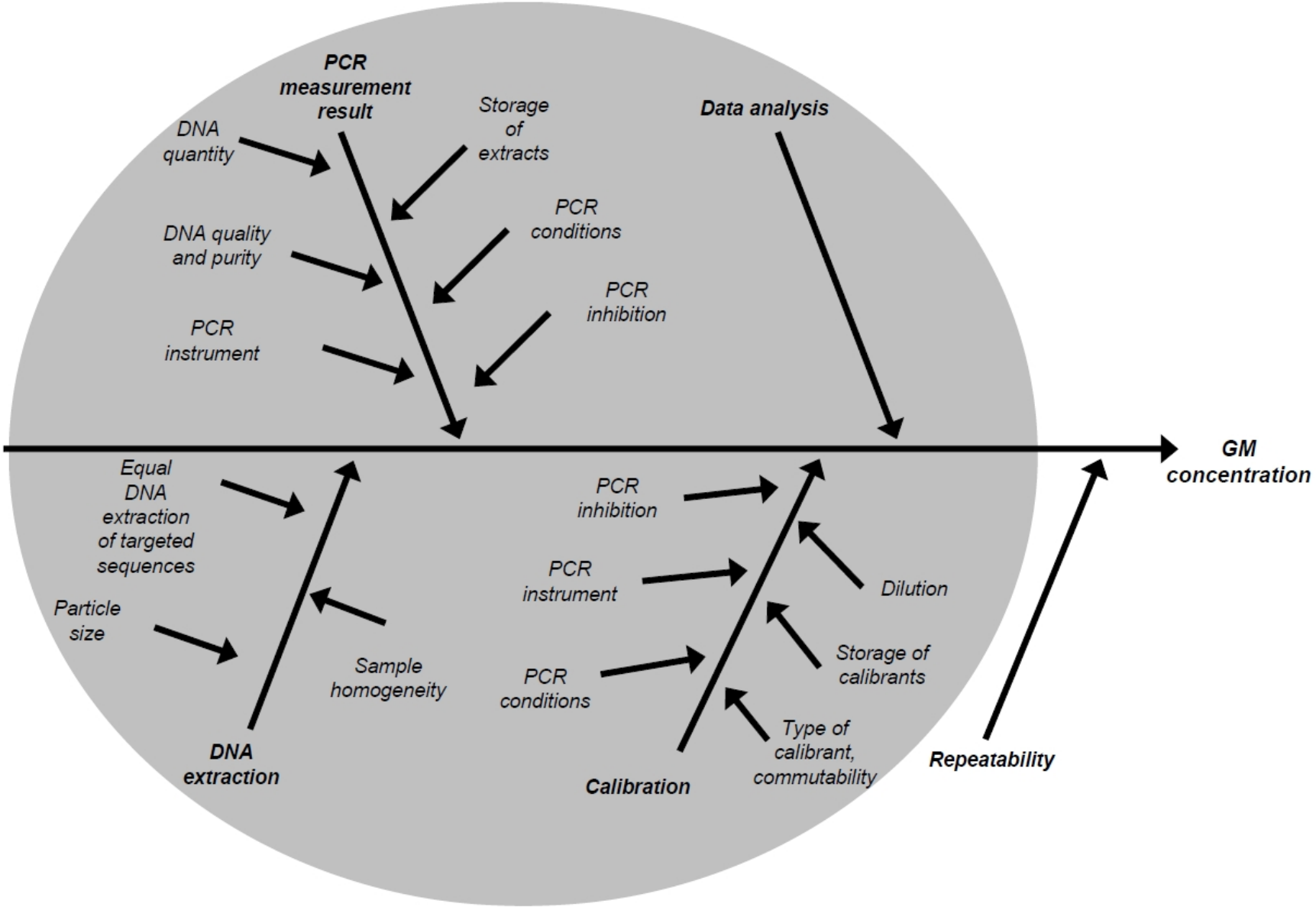
Influence factors:

Sampling
Storage Conditions
Instrument effects
Reagent purity
Measurement conditions
Blank Correction
Operator effects
Sample effects
Random effects

Combined measurement uncertainty

Calculations for individual components of uncertainty

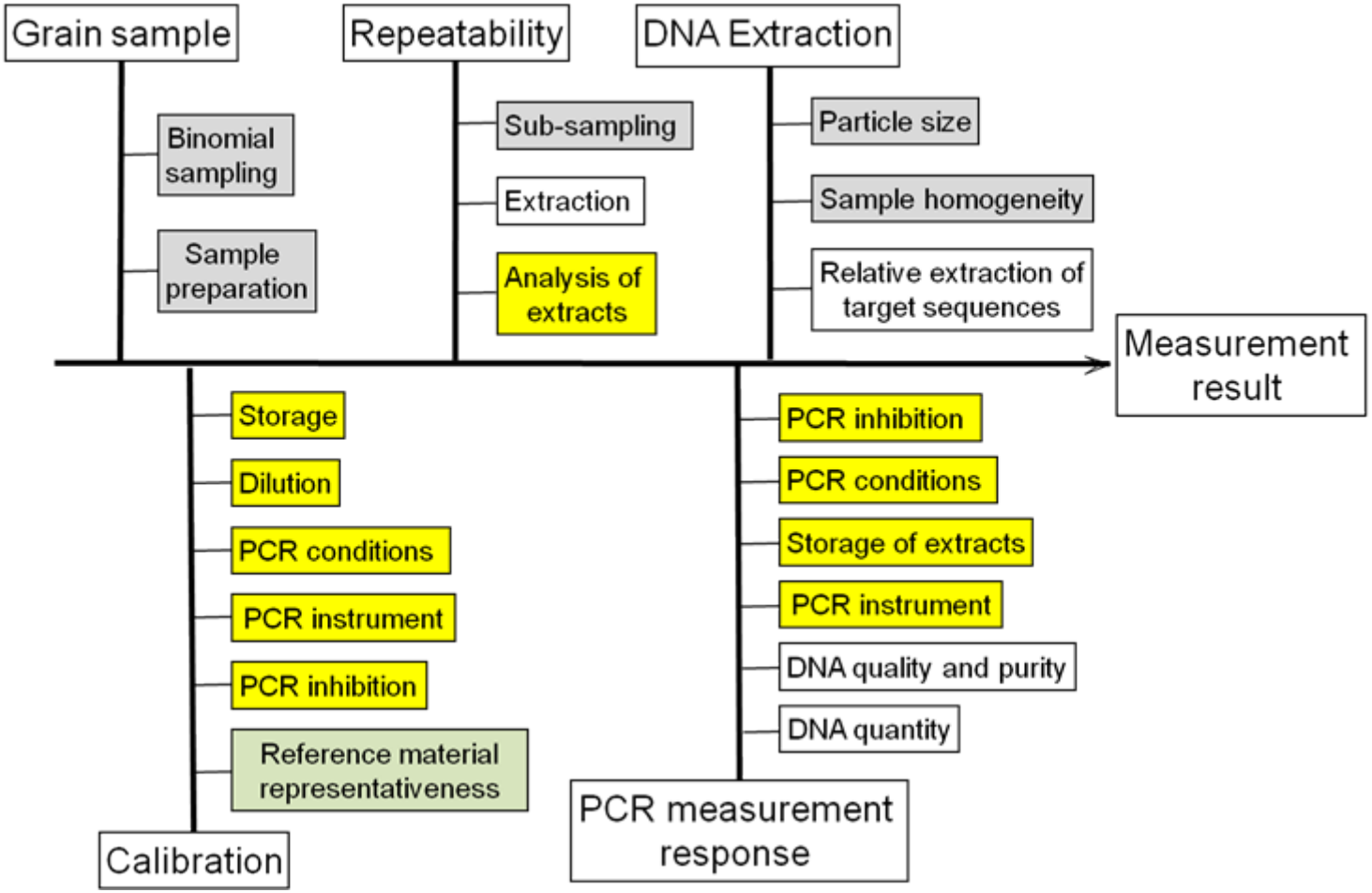
Bottom up approach (2)



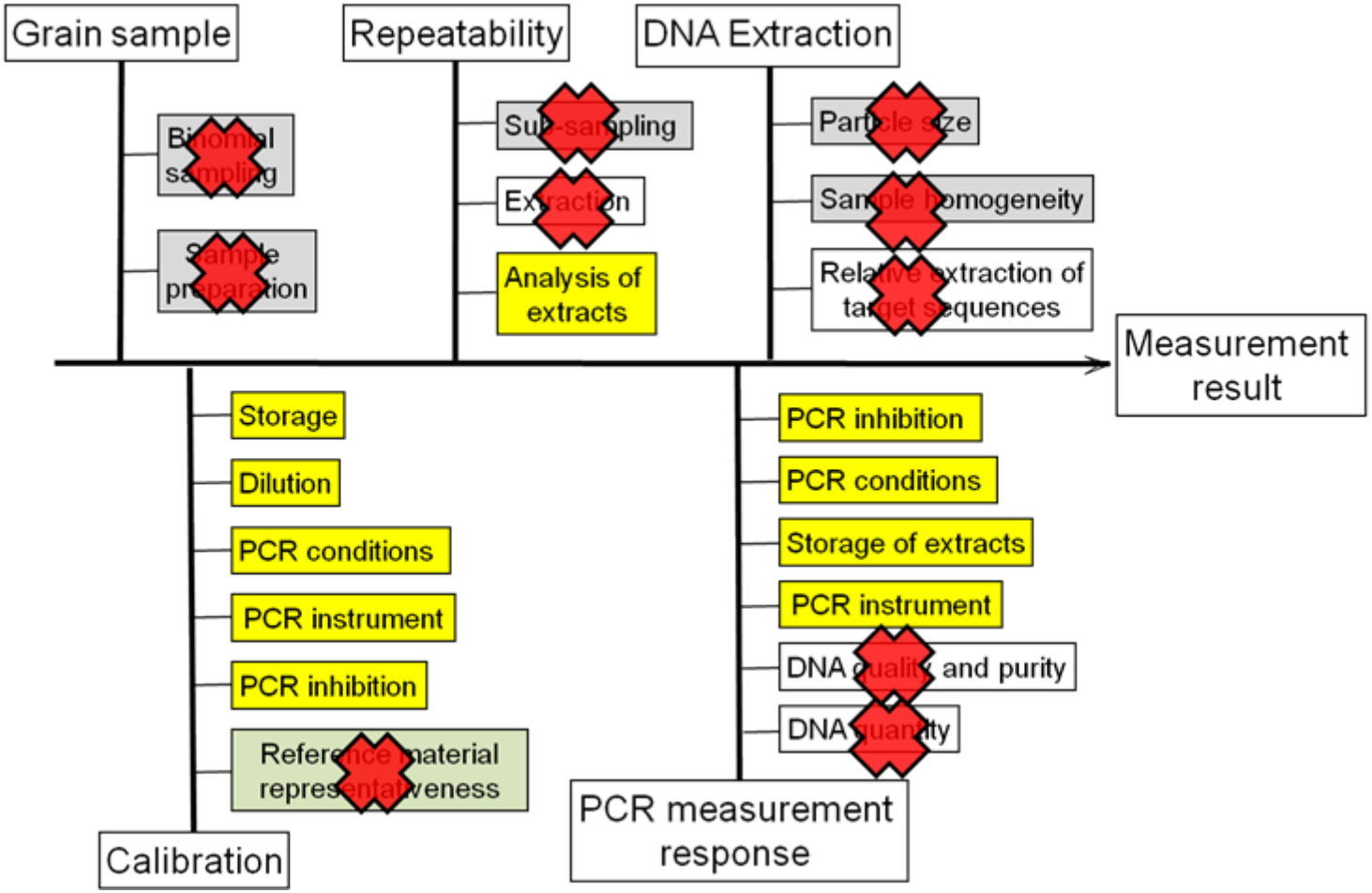
Top down (empirical) approaches

- Data from Interlaboratory method validation study
- Within laboratory data (method validation, quality control)

Sources of uncertainty



Uncertainty reduced with validation of PCR module



General guidance on uncertainty

For EURL validated PCR modules:

- **Uncertainty is a factor of app. 2** (RSD_R < 35%)
- **Result > 1.8% demonstrates need for labelling**
- **Result < 0.45% proves no need for labelling**

MU in GMO detection

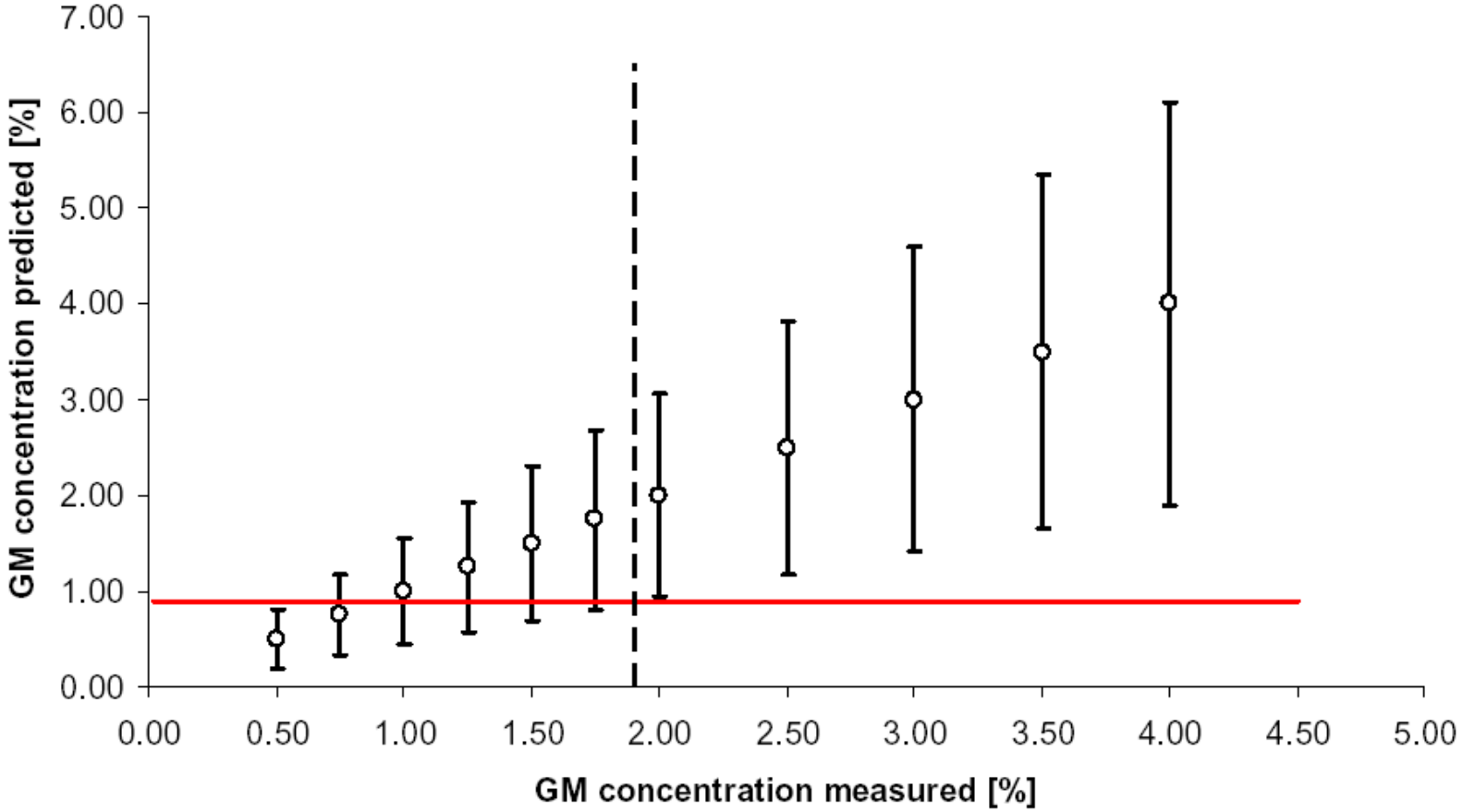


Figure 6: Enforcement level calculated for RRS collaborative trial given in Annex II, expressed in mass/mass %

Using the data obtained during the method validation of RRS by collaborative trial (Annex II), it can be concluded that samples for which a GM concentration above 1.8 % (dashed line) is measured (using the RRS method) contain more that than the legal threshold of 0.9 % GM (red line).

MU in GMO detection

MU from collaborative trials			
GM event	**matrix**	**expanded uncertainty (%) at labelling threshold (0,9%)**	**Estimated enforcement level (%)**
RRsoya	flour	0.53	1,92
NK603 maize	flour	0.66	3.33
GA 21 maize	flour	0.58	2.46
MON 863 maize	flour	0.34	1.46
Bt 11 maize	DNA	0.31	1.38
TC 1507 maize	DNA	0.42	1.67
LL62 rice	DNA	0.25	1.24

ISO 21569 Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Qualitative nucleic acid based methods

9 Expression of results and quality assurance

9.1 General

The results shall be expressed unambiguously, i.e. not as „+ -“

A negative result shall never be expressed as „GMO no present“.

ISO 21570 Foodstuffs — Methods of analysis for the detection of genetically modified organisms and derived products — Quantitative nucleic acid based methods

9 Expression of results

The results shall clearly state the quantity of the GM target sequence relative to the target taxon-specific sequence.

The results should also provide values for the measurement uncertainty, such as the standard deviation or relative standard deviation. Furthermore, the LOD and LOQ of the method and the practical LOD and LOQ should be reported.

The target sequences may or may not be detected, or the quantity of at least one of them may be below the limit of quantitation. Table 1 describes the four alternative cases and the corresponding expression of the result to be included into the test report.

ISO 21570

From Table 1 — Expression of results

if	The target taxon-specific sequence and the GM target sequence are both detected and the quantity is above the LOQ for both target sequences.
then	For each GMO, state:
	“The content of GMO (specify the GMO) derived DNA as determined by detection of (specify target sequence) derived from (specify species) is X ± uncertainty %.” (Specify unit used.)

The GMO-derived DNA content may also be reported as being above or below a specific value, taking into account the measurement uncertainty.

Interpretation of results including MU

5.10.3.1 …… test reports shall, where necessary for the interpretation of the test results, include…:
…. c) where applicable, a statement on the estimated uncertainty of measurement; information on uncertainty is needed in test reports when it is relevant to the validity or application of the test results, when a customer's instruction so requires, or when the uncertainty affects compliance to a specification limit; …