Application Note V1.0

Use of the harmonised conversion factors to transform PCR results from the DNA copy number ratio domain into the mass fraction domain

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This application note explains how the conversion factor is correctly used to transform a PCR result obtained as DNA copy number ratio into a result expressed as a GM mass fraction.

INTRODUCTION

The current EU legislation regarding the expression of the results of the measurement of genetically modified organism (GMO) content in food and feed prescribes mass fraction (% m/m) as measurement unit.
There is a need to convert analytical data obtained as DNA copy number ratio into a GM mass fraction.

THE CONVERSION - BACKGROUND

In principle three main pathways exist to measure the GM content in a sample. The first two are using conventional quantitative (qPCR) to analyse the samples. qPCR methods have to be calibrated and the measurement unit of the results will depend on the Certified Reference Material (CRM) used for the calibration. Consequently, a result expressed either as GM mass fraction or as DNA copy number ratio is obtained. Most CRMs in this field are certified for their GM mass fraction or their purity, only a few are certified for both their GM mass fraction and DNA copy number ratio and some are certified only for the DNA copy number ratio.
The third pathway employs digital (dPCR), which does not need a calibrant and delivers results expressed as DNA copy number ratio.
In view of the above mentioned legal requirements a conversion from one to the other measurement unit is necessary for being able to exploit all three pathways for compliance control of products.
Unfortunately a simple conversion fulfilling metrological principles per se does not exist.
The exact amount of genetically modified targets and reference targets, which determine the DNA copy number ratio in a CRM, does not only depend upon the zygosity of the material (e.g. seeds) used to produce the CRM but is also influenced by other factors. For instance, this ratio is impacted by the varietal differences in the relative mass fraction of the seed tissues present in a CRM composed of a mixture of GM and non-GM seed powders. In addition, the DNA extractability and DNA content of the GM and non-GM seed as well as the parental origin of the GM are influencing that ratio. In pure GM maize kernels, the contribution of haploid genomes from parental gametes has been experimentally estimated and varies between male and female, which means that a conversion factor could already fluctuate from 0.34 to 0.66 in hemizygous seeds[1].
Because of these various influences on the DNA copy number ratio in a CRM, the EURL GMFF decided to determine the conversion factor in all CRMs associated to the GM events currently authorised in the EU and to publish the harmonised conversion factors[2,3].

THE CONVERSION - THEORY

The relationship between the GM mass fraction, DNA copy number ratio and conversion factor in a CRM is given in Equation 1.

$$CF_{CRM} = \frac{cp_T \times (m_{GM} + m_{nonGM})}{cp_E \times m_{GM}} \qquad [1]$$

with:

CF_{CRM} .... conversion factor linked to the CRM

cp_T .... copy number concentration of the transgenic target (expressed as cp_T/µl)

cp_E .... copy number concentration of the endogenic target (expressed as cp_E/µl)

m_{GM} .... mass of the GM powder

m_{nonGM} ...... mass of the non-GM powder

The conversion factors are determined experimentally and are therefore accompanied by a measurement uncertainty. This uncertainty has to be included in the total uncertainty budget of subsequent GMO measurements.

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[1] Zhang D., et al. (2008) Transgenic Res. 17:393-402.
[2] https://gmo-crl.jrc.ec.europa.eu/doc/CF-CRM-values.pdf
[3] Corbisier P. et al. (2021) Food Control https://doi.org/10.1016/j.foodcont.2021.108626

THE CONVERSION - PRACTICE

The conversion of results from the DNA copy number ratio domain to the GM mass fraction domain is easy. The result obtained as copy number ratio is divided by the corresponding conversion factor. Attention has to be paid as the conversion factors are method-dependent and are only valid for results obtained using the same targets. In a second step, the measurement uncertainty has to be calculated. Therefore the relative standard uncertainties of the measurement (u_m) and of the conversion factor (u_{CF}) are combined as provided in Eq. 2.

$$u_{comb} = \sqrt{u_m^2 + u_{CF}^2} \qquad [2]$$

EXAMPLE

A practical example on how to use the conversion factor on a real sample is illustrated here considering a fictitious sample consisting of a mixture of GM maize and GM soya.

A screening approach revealed the presence of one soya GM event (MON-89788-1) and two maize GM events (DAS-4Ø278-9 and MON-ØØ863-5) in a sample. For reporting the GM content of this sample in mass fraction the experimental data measured by digital PCR in DNA copy number ratios are converted into mass fractions as follows:

$$\text{GM soya \%}\left(\frac{\text{m}}{\text{m}}\right)\text{for MON} - 89788 - 1 = \frac{\text{cp}_{\text{MON}-89788-1\text{ soya}}}{\text{cp}_{\text{taxon specific sequence}}} * \frac{1}{\text{CF}_{\text{AOCS 0906}-\text{B}}} * 100$$

$$\text{GM maize \%}\left(\frac{\text{m}}{\text{m}}\right)\text{for DAS} - 4\text{Ø}278 - 9 = \frac{\text{cp}_{\text{DAS}-4\text{Ø}278-9\text{ maize}}}{\text{cp}_{\text{taxon specific sequence}}} * \frac{1}{\text{CF}_{\text{ERM®}-\text{BF433d}}} * 100$$

$$\text{GM maize \%}\left(\frac{\text{m}}{\text{m}}\right)\text{for MON} - \text{ØØ}863 - 5 = \frac{\text{cp}_{\text{MON}-\text{ØØ}863-5\text{ maize}}}{\text{cp}_{\text{taxon specific sequence}}} * \frac{1}{\text{CF}_{\text{ERM®}-\text{BF416d}}} * 100$$

The conversion of the GM % from cp_T/cp_E ratio into mass fraction is given in the table below. The CRMs listed in this example are the CRMs reported in the Commission Decisions authorising the placing on the market of products consisting of, or produced from GM events pursuant to Regulation (EC) No 1829/2003.

The percentage of GM soya remains unchanged as the CF_{CRM} is close to 1. The converted percentages of GM maize increase as the CF_{CRM} are smaller than 1. The expanded uncertainty estimated for the final result is only marginally increased because the uncertainty associated to the conversion factors is low.

Example for the use of conversion factors to transform a result expressed in DNA copy number ratio into the legally required GM mass fraction

Event	% GM (cp_T/cp_E) ± 2u	$u_{m,\%}$	CRM	CF_{CRM} ± 2u	$u_{CF,\%}$	$u_{comb,\%}$	% GM (m/m) ± 2u
MON-89788-1 soya	0.6 ± 0.1	8.3 %	AOCS 0906-B2	0.981 ± 0.021	3.6 %	8.4 %	0.61 ± 0.05
DAS-4Ø278-9 maize	10 ± 3	15 %	ERM®-BF433d	0.36 ± 0.05	6.9 %	16.5 %	28 ± 10
MON-ØØ863-5 maize	9 ± 2	11 %	ERM®-BF416d	0.62 ± 0.08	6.5 %	12.8 %	15 ± 4

u standard uncertainty
$u_{m,\%}$ relative standard uncertainty reported for the measurement
$u_{CF,\%}$ relative standard uncertainty for the CF
$u_{comb,\%}$ relative standard uncertainty combined of $u_{m,\%}$ and $u_{CF,\%}$
% GM (m/m) mass fraction of GM event

The calculation of the DAS-4Ø278-9 maize event % GM (m/m) and its associated expanded uncertainty is detailed:
$u_{m,\%} = (3/2)/10 * 100 = 15\ \%$; $u_{CF,\%} = (0.05/2)/0.36 * 100 = 6.94\ \%$; $u_{comb,\%} = \sqrt[2]{15^2 + 6.94^2} = 16.53\ \%$;
% GM (m/m) = 10/0.36 = 27.78, rounded to 28 with an expanded uncertainty 2u = 2 x (27.78 x 16.53/100) = 9.18 rounded to 10.