Legal limits on the road to food safety:
Establishing sound criteria for compliance decisions
“Regulations and GMO reality”

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Overview

• GMO legislation in the EU
• GMO identification methods
• Harmonization between different levels of labelling requirements
• Recovery correction in analytical results
Legislation on Thresholds for GMOs

Seeds

- Draft Commission Directive…/…/ EC (Setting thresholds)
- Draft Regulation on a Protocol for sampling and testing of seed lots

Traceability/ Environment


Food/Feed

- Regulation (EC) No1829/2003
What does the legislation say?

Regulation (EC) No 49/2000

– ‘material derived from genetically modified organisms…. in a proportion no higher than 1% of food ingredients individually considered or food comprising a single ingredient ….’(Article 1)

– (10) the notion of material derived from genetically modified organisms should be understood as referring to the part of each ingredient that is derived from the genetically modified organisms’
What does the legislation say?

Regulation (EC) No 1829/2003

Labelling is mandatory if,

• food and feed contain or consist of GMO;
• food and feed are produced from or contain ingredients produced from GMO;
• flavourings and food additives contain, consist of, or produced directly from GMOs.

• Introduction of a threshold of 0.9 % (Article 12 - “Scope”)
Food (and feed) containing material which contains, consists of or is produced from GMOs in a proportion no higher than 0.9 % of the food ingredients considered individually or food consisting of a single ingredient, provided that this presence is adventitious or technically unavoidable.

Introduction of a threshold of 0.5 % (Article 47 - transitional measures...)
for the presence of adventitious or technically unavoidable GMO, which are not authorized in the EU, but have an authorization with a positive risk assessment of a country outside EU
What does the legislation say?

Directive 2001/18/EC

amendment by Reg. (EC) No 1830/2003 (Article 7)

„the following paragraph is added to Article 21:

‘3. For products intended for direct processing, paragraph 1 shall not apply to traces of authorised GMOs in a proportion no higher than 0,9 % or lower thresholds established under the provisions of Article 30(2), provided that these traces are adventitious or technically unavoidable.’

The unit used should be related to GMOs, but is not specified
What does the legislation say?

**Seed Legislation – Draft** Regulation on a Protocol for Sampling and Testing of Seed Lots…

- Thresholds defined in the draft protocol relate to % GM seeds in total seeds

*Therefore the unit used should be based on percentage seeds*
Thresholds used in different legislation

Concerns

– Difficulties in implementing legislative thresholds based on % weight GM material: PCR measures copy numbers of target sequence: copy number/DNA correlation? DNA/wt. material correlation?

– % threshold unit?

– Consistency of approach in seed and food/feed legislation?
Detection strategies I

<table>
<thead>
<tr>
<th>Raw material</th>
<th>processed product</th>
<th>target</th>
<th>method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soya</td>
<td></td>
<td>DNA</td>
<td>Polymerase chain reaction (PCR)</td>
</tr>
<tr>
<td>Maize</td>
<td></td>
<td>Protein</td>
<td>Immuno assays</td>
</tr>
<tr>
<td>Rape seed</td>
<td></td>
<td>Fatty acid</td>
<td>(HPLC/GC)</td>
</tr>
</tbody>
</table>
Detection strategies II

Genetic construction

- Promoter “Start”
- Gene “new variety”
- Terminator “Stop”
- marker

Different primer systems for PCR

Different strategies

“Screening”
“construct-specific”
“Event-specific”

Plant DNA → Genetic construction → Plant DNA

5’ → Promoter “Start” → Gene “new variety” → Terminator “Stop” → marker → Plant DNA
Polymerase chain reaction (PCR)

IV. Principle:

1. denaturation (95 °C)

2. annealing (50-65 °C)

3. polymerization (72 °C)

35 - 45 x repetition
Polymerase chain reaction (PCR)

Exponential amplification:

\[ 2^1 = 2 \text{ copies} \quad 2^2 = 4 \text{ copies} \quad 2^3 = 8 \text{ copies} \quad 2^4 = 16 \text{ copies} \quad 2^{35} = 34 \text{ billion copies} \]

Formula: \[ 2^n \]; \( n = \) number of cycles
TaqMan™ system

Target taxon specific

RRS specific

PCR cycle no.

NTC soya
Soya std e
Soya std f
Soya std g
Soya std h
sample

PCR cycle no.

NTC
RRS std a
RRS std b
RRS std c
RRS std d
sample

Standard Curve - 03-06-2008 Quantify-run

Threshold cycle (Ct)

Starting Quantity

Threshold cycle (Ct)

Starting Quantity

Unrevised

Slope: -3.945

Y-intercept: 32.822

Correlation Coeff: 0.999

Unrevised

Slope: -3.022

Y-intercept: 38.108

Correlation Coeff: 0.996

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Genotypes of seed tissues

a) Embryo

2 n 1 set maternal, 1 set paternal

b) Endosperm

3 n 2 sets maternal, 1 set paternal
(maternal set is identical)

c) Seed coat

2 n both sets maternal
(the 2 maternal sets can be identical)
Genotypes of seed tissues

a) Embryo

2 n  1 set maternal,
     1 set paternal (transgenic)

b) Endosperm

3 n  2 sets maternal,
     1 set paternal (transgenic)
     maternal set is identical

(c) Seed coat

2 n  both sets maternal

(the 2 maternal sets can be identical)

The ratio of "transgenic" chromosome sets to total number of chromosome sets is:
a) Embryo  1:2
b) Endosperm  1:3
c) Seed coat  0:2

The real GMO-content (% GM seeds in total seeds) could be underestimated.
Genotypes of seed tissues

a) Embryo

2 n  1 set maternal (transgenic),
     1 set paternal

b) Endosperm

3 n  2 sets maternal,
     1 set paternal (transgenic)
     (maternal set is identical)

c) Seed coat

2 n  both sets maternal (transgenic)
     (the 2 maternal sets can be identical)

The ratio of "transgenic" chromosome sets to total number of chromosome sets is:

a) Embryo  1:2
b) Endosperm  2:3
c) Seed coat  2:2

The real GMO-content (% GM seeds in total seeds) could be overestimated.
Traceability and Comparability of Measurements

Definition of Traceability:

– Property of the result of a measurement or the value of a standard whereby it can be related with a stated uncertainty, to stated references, usually national or international standards, through an unbroken chain of comparisons (ISO Guide 30 and VIM)

Traceability and ISO/IEC 17025 -Section 5.6
What happens in GM analysis?

*E.g.* Determination of % GM material in a food ingredient

**IRMM CRMs %wt GM material/total wt**

 Extraction of stds, samples; PCR Calibration  
(Ct vs log Copy number)

Output: Copy number ratio

% material containing, etc.
What happens in GM analysis?

E.g. Determination of % GM material in a food ingredient

If the material for the standard is identical to the sample analysed L1 is the same as L2 Consequently there is no influence!
What happens in GM analysis?

**E.g.** Determination of % GM material in a food ingredient

**Example: IRMM CRMs %wt GM material/total wt (homozygous)**

- Extraction of stds, samples; PCR Calibration (Ct vs log Copy number)
- Output: Copy number ratio

**Sample zygosity?**

% seeds containing, etc.

L1 ?

L2 ?

MU
Traceability in GM analysis

Do we have traceability through an unbroken chain to stated references?

Factors to be taken into account in the links L1 and L2?

- Factors influencing Wt material/DNA
  - IRMM say that ‘the ratio of GMO DNA/non-GMO DNA in the reference materials may significantly deviate from the certified powder mass ratio values’
  - Impact of new batch of calibration reference material on L1?
Traceability in GM analysis

- Factors influencing DNA ratio /copy number ratio
  - Zygosity
  - Male or female source of GM in heterozygous seed material
  - Ploidy
  - Number of GM DNA copies inserted
Commission Recommendation

Commission Recommendation of 4 October 2004 on technical guidance for sampling and detection of GMOs and material produced from GMOs as or in products in the context of Regulation (EC) 1830/2003

It is stated in section V.6 of this document that ‘the results of quantitative analysis should be expressed as the number of target DNA sequences per target taxon specific sequences calculated in terms of haploid genomes’.

This applies to food, feed and seed.
RM available for GMO detection
(3rd generation)

**GM Roundup Ready® soybeans**
IRMM-410S; nominal 0, 0.1, 0.5, 1, 2, and 5% GMO

**GM Event176 maize**
IRMM-411R; nominal 0, 0.1, 0.5, 1, 2, and 5% GMO

**GM Bt-11 maize**
IRMM-412R; nominal 0, 0.1, 0.5, 1, 2, and 5% GMO

**GM MON810 maize**
IRMM-413; nominal 0, 0.1, 0.5, 1, 2, and 5% GMO

**WARNING:** IRMM-410S, IRMM-411R, IRMM-412R and IRMM-413 are intended to be used to apply PCR screening for the detection of genetic modification in maize. IRMM-410S, IRMM-411R, IRMM-412R and IRMM-413 were produced using a dry-mixing technique minimising DNA and protein degradation during production. It should be noted that degradation of DNA and/or protein still might have occurred during the production of the test material, which may affect DNA and/or protein based quantification. Additionally one has to be careful to draw quantitative conclusions from measurements of unknown samples, as DNA and/or protein based GMO quantification may depend on varieties.
Recovery Correction of Analytical Results

Should it be mandatory in the EU to use recovery correction for all analytical results?

Methods for the determination of the recovery:

- "Addition to blank"
- "Standard addition"
- Internal (isotope labelled) standards
- Matrix-calibration
- Certified reference material

Cases in which no recovery rate can be determined:

- Qualitative methods with a "yes" or "no" answer
- Empirical methods, where a value can be arrived only in terms of the method *per se*

Recovery rates for varying substances and matrices are very difficult to determine in Routine analysis!
Thank you for your attention

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