It must be noted that, regarding the minimum requirements for the scope of analysis in Chapter 1.1 to 2.2, not all GMOs were taken into account that are authorised in the EU or tolerated for feed within the meaning of EU Regulation No. 619/2011. Furthermore, GMOs not authorised in the EU are not part of the minimum requirements. In the event of an examination of the marketability and proper labelling of a feed, other GMOs would be taken into account (this includes other GMOs authorised in the EU, GMOs tolerated in feeds pursuant to EU Regulation No. 619/2011, and GMOs not authorised in the EU).

In consultation with laboratories, VLOG regularly checks and updates the following minimum requirements in Chapter 1.1 to 2.2 concerning the scope of analysis of raw materials and feeds. In the event of developments that other GMOs become relevant (e.g. RASFF reports), VLOG will provide its members and VLOG-certified companies with corresponding analysis requirements/guidelines in a timely manner.

This does not mean, however, that the companies participating in the VLOG system are dispensed from their own due diligence obligations to regularly check and, if necessary, update the scope of analysis.

1. Minimum requirements for raw materials / single component feed

1.1. Minimum requirements for raw soy materials / soy-based single-component feed

Determination and assessment of the summation value of the most relevant soy GMOs:

- Quantification of GTS 40-3-2 (RRS- 1)
- Quantification of MON89788 (RRS-2)
- Qualitative detection of A2704-12
  In the event of positive result for A2704, the quantity of this GMO can, for example, be estimated using the ΔΔct method or similar method ensuring that sufficient quantities of species DNA are present. For values over 0.1%, a quantification must be carried out.

Alternately, the laboratory may work with screening parameters that detect at least the GMOs mentioned. In subsequent identification / quantification of positive findings, at least all GMOs (if corresponding elements are positive) mentioned here must be quantified.

1.2. Minimum requirements for raw corn materials / corn-based single-component feed

1. **Screening for 35S Promoter (p35S) and NOS Terminator (tNOS).**
   Other screening elements can be implemented to narrow the corresponding GMO down.
2. **If positive:** Analysis at least for NK603, TC1507, MON810, MON89034 + RRS-1

If using the positive screening parameters, one or more of these GM corn types can be ruled out, then the same number of commercialised GM corn types that come into question must be searched for instead.

Positive screening results must be clarified; if none of the 4 GM corn types are positive, other GM types must be analysed.

3. **Determining the summation value of the corn GMO**
   Identified varieties must be quantified if the estimation of the concentration, when using, for example, the ΔΔct method or another similar method ensuring that sufficient quantities of species DNA are present, leads to values over 0.1%.

RRS-1 positive:
Estimating the soy mass and assessing the amount of soy: Is it a relevant amount or minimal traces? If a botanical contamination containing GMO is determined, an assessment according to the official guidleine[2] must take place.

1.3. Minimum requirements for raw canola materials / canola-based single component feeds

1. **Triple screening** that detects all relevant GM canola varieties (e.g. tNOS, pat gene (or LibertyLink construct), CTP2-CP4epsps (or pFMV))

2. **ID depending on positive screening results**
   - tNOS positive: at least RRS + bar gene for MS8 / RF3 or both directly
   - pat gene / LibertyLink positive: at least canola T45
   - CTP2-CP4epsps / pFMV positive: at least GT73

3. **Determining the summation value of GM canola**
   Identified GM canola varieties must be quantified if the estimation of the quantity, when using, for example, the ΔΔct method or another method ensuring that sufficient quantities of species DNA are present, leads to values over 0.1%.

Positive screening results must be clarified.

If no canola GMO is detected, the presence of a botanical contaminant containing GMO with soya or corn GMO must be clarified (estimation and assessment of masses). Is it a relevant quantity or minimal traces? If a botanical contamination containing GMO is determined, an assessment according to the official guidline[2] must take place.

1.4. Minimum requirements for rice and rice products

1. **Preparation of laboratory samples:**

   Two subsamples of at least 250 g each are to be created from the laboratory sample sent, and each is to be analysed separately (1 extraction, 2 PCRs per subsample:).

2. **Element-specific screening:**

   p35S + tNOS + cry1Ab/cry1Ac sequence

3. **Design-specific proof:**

   Identification, by agreement between the company and the laboratory, of GMO events that cause a positive screening result (see 1).

4. **<u>Exclusion of botanical impurities</u>** (GMO carryovers from other plant species) from corn, soy, cotton and (naturally occurring) Cauliflower Mosaic Virus

If the element-specific screening yields a positive result, design-specific proof is to be provided as the next step. In combination with the exclusion of botanical impurities and the Cauliflower Mosaic Virus, an investigation is to be made of whether the sample contains genetically modified rice.

5. **<u>Evaluation of the PCR results:</u>**

   If the targeted sequence of genetically modified rice is proven for at least one of the subsamples analysed, this result is to apply to the entire sample and the batch. The batch cannot be marketed in the EU and cannot be labelled with the “Ohne GenTechnik” seal.

2. Minimum requirements for compound feed

1.1. Minimum requirements for compound feed containing soya

Determination and assessment of the summation value of the most relevant GMOs:

Soy:

- Quantification of GTS 40-3-2 (RRS- 1)
- Quantification of MON89788 (RRS-2)
- Qualitative detection of A2704-12
  In case of positive result for of A2704, the quantity of this GMO can, for example, be estimated using the ΔΔct method or a similar method ensuring that sufficient quantities of species DNA are present. For values over 0.1%, a post-quantification must be carried out.

In case of limited analysability of the soya ingredient, the practical LOD must be indicated.

For corn ingredient:

Additional qualitative detection of the 3 commercialised corn varieties: NK603, TC1507, MON810

In case of positive result, the quantity of this GMO can, for example, be estimated using the ΔΔct method or a similar method ensuring that sufficient quantities of species DNA are present. For values over 0.1%, a post-quantification of the GMOs detected must be carried out.

In the event of limited analysability of the corn ingredient, the practical LOD must be indicated.

For canola ingredient:

Additional qualitative detection of GT73

In case of positive identification, quantification of GT73 must take place if the estimation of the quantity using, for example, the ΔΔct method or another similar method ensuring that sufficient quantities of species DNA are present, leads to values over 0.1%.

In case of limited analysability of the canola ingredient, the practical LOD must be indicated.

Alternately, the laboratory may also work with screening parameters that detect at least the GMOs mentioned (soy, canola, corn). In subsequent identification / quantification of positive results, at least all GMOs (if corresponding elements are positive) mentioned here must be identified and, if necessary, quantified.

1.2. Minimum requirements for soy-free compound feed

Determination and assessment of the summation value of the most relevant GMOs:

Estimating the soy mass:

In a first step, the mass of soy in the feed is estimated. For quantities over 0.9%, the quantity of soy GM must be determined (cf. Minimum requirements for feed containing soy) and an assessment according to the official guideline[1] must take place.

For canola ingredient:

Qualitative evidence of canola GT73 + canola MS8 or canola RF3 (or bar gene)

In the event of positive identification, quantification of GMO or GMOs found must take place if the estimation of the quantity when using, for example, the ΔΔct method or another similar method ensuring that sufficient quantities of species DNA are present, leads to values over 0.1%.

In the event of limited analysability of the corn ingredient, the practical LOD must be indicated.

For corn ingredient:

Qualitative evidence of 3 corn varieties used commercially: NK603, TC1507, MON810

In the event of positive identification, quantification of GMO or GMOS found must take place if the estimation of the quantity when using, for example, the ΔΔct method or another similar method ensuring that sufficient quantities of species DNA are present, leads to values over 0.1%.

In the event of limited analysability of the corn ingredient, the practical LOD must be indicated.

Alternately, the laboratory may work with screening parameters that detect at least the GMOs mentioned (soy, canola, corn). In subsequent identification / quantification of positive results, at least all GMOs (if corresponding elements are positive) mentioned here must be identified and, if necessary, quantified.

3. Other products / raw materials

The strategies for analysing GMOs in other single-component feeds, raw materials, (food) ingredients, intermediate products or foods must continue to be agreed upon with the commissioned laboratory, taking into account the composition and origin of the products.

[1] Leitfaden zur Kontrolle von GVO in Futtermitteln (Stand November 2011). Überwachung des Herstellens, Behandelns, Verwendens und Inverkehrbringens von Futtermitteln im Zusammenhang mit gentechnisch veränderten Organismen (GVO). … Erarbeitet von der PG GVO in Futtermitteln der LAV Arbeitsgruppe Futtermittel unter Beteiligung des Bundes und des VDLUFA, http://www.ohnegentechnik.org/Leitfaden_Futtermittel