Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten

GMO analysis of feeding stuffs – current challenges

The following article depicts the current challenges to the analyst for the testing of feed which are consisting, containing or produced from genetically modified organisms (GMOs). The *Working Group PCR Analysis* of the Section Feedingstuff Analysis of the *Verband Deutscher Untersuchungs- und Forschungsanstalten (VDLUFA)* here particularly describes the challenges in testing of feed which arise from the Commission Regulation (EU) No 619/2011 [1] and the rules for the "ohne Gentechnik" ("GMO-free") labelling of food (according to the German Regulation *EG-Gentechnik Durchführungsgesetz*). Also the difficulties in analytical testing of compound feed are examined with regards to botanical impurities of GMO as well as related sampling strategies. Furthermore the analytical detection of EU non-authorised GMO in feed and products produced from such GMO are considered. The fundamental facts of GMO analysis in feed are described in detail in the *Guidance for the Analysis of Genetically Modified Feed* of the Working Group PCR Analysis of the VDLUFA [2].

Keyword:
feed, genetically modified organism (GMO), Commission Regulation (EU) No 619/2011, botanical impurity, compound feed, zero tolerance, methods of sampling, "GMO-free" labelling, not-authorised GMO

Application and special requirements of Commission Regulation (EU) No 619/2011 for GMO testing laboratories

The requirements for the analysis of certain genetically modified material to be used in feed lacking EU authorisation but for which an authorisation procedure is pending or for which the authorisation has expired are defined in Commission Regulation (EU) No 619/2011. For these special cases the legislation lays down the methods of sampling and analysis for offcial control.

The Commission Regulation (EU) No 619/2011 is applying to genetically modified plant material in feed which meet the following criteria:

- → The respective GMO is authorised in a third country for the production of feed.
- → An authorisation procedure is pending in accordance with Regulation (EC) No 1829/2003 [3] for more than three month.
- → The regulation covers GMOs which authorisation has expired in the EU and no application for renewal of the authorisation exists according to Regulation (EC) No 1829/2003 because they are not placed on the market.
- → A favourable risk evaluation of the European Food Safety Authority (EFSA) is available.
- → A validated, event-specific method for quantification has to be published by the European Union Reference Laboratory for GM Food and Feed (EU-RL GMFF)

→ A weight by weight [wt %] certified reference material is available for all laboratories

The GMOs falling under the scope of Regulation (EU) No 619/2011 are listed under the following link: http://ec.europa.eu/food/dyna/gm_register/index_en.cfm

The MRPL (Minimum Required Performance Limit) for GMOs fulfilling the abovementioned criteria corresponds to 0.1 % weight by weight. From the EU-RL GMFF this GMO content is considered as the one which could be determined with satisfactory precision by official control laboratories.

The analysis of such GMO which meet these criteria demands specific requirements to the testing laboratories.

→ All event-specific methods applied corresponding to Regulation EU) No 619/2011 has to be inhoused verified at a level of 0.1 % (wt %).

→ The sample sizes have to be increased so that the aggregate sample weight corresponds to 35,000 grains and the final sample is equivalent to a weight of 10,000 grains. These sample sizes correlate to the following mass-equivalents:

plant	10,000 grains correlate to mass per gram
barley, millet, oat, rice, rye, wheat	400
maize	3,000
soybeans	2,000
rapeseed	40

→ With a GMO content of 0.1 % the laboratories have to ensure that the analysis is performed by a relative repeatability standard derivation at 25 % maximum. For the corresponding validation of these analytical requirements the laboratories need a certified reference material depending on the used matrix with a GMO content of 0.1 %.

→ The analytical result is to be reported with a confidence level of 95 % as x +/- U; x is the analytical result for a transformation event and U is the expanded measurement uncertainty.

→ If the result of a quantitative analysis is determined as relation to the haploid genome copies, the result is to convert into mass fraction corresponding to the respective validation report.

For the analytical implementation of Regulation (EU) No 619/2011 the EU-RL GMFF has published a guidance document [4]

Requirements for the labelling of food "ohne Gentechnik" („GMO-free“)

Within the EU the requirements for the labelling of food and feed consisting, containing or produced from genetically modified plants are laid down in Regulation (EC) No 1829/2003 and Regulation (EC) No 1830/2003 [5]. In Germany the requirements for the voluntary labelling of food produced without genetic engineering are defined by the EG-Gentechnik Durchführungsgesetz (EGGenTDurchfG) [6].

Food according to the EGGenTDurchfG can only be labelled as "ohne Gentechnik" (“GMO-free”) if

- → In food or food ingredient of animal origin the animal from which the food is produced are not allowed to be fed with feed which has to be labelled according to
- → a) article 24 and 25 of Regulation (EC) No 1829/2003, or
- → b) article 4 or 5 of Regulation No 1830/2003 or if it would have to be labelled when placed on the market. For different animal species specific periods prior to the beginning of the food production the feeding of genetically modified feed is not allowed. These animal-specific periods are given in the attachment related to § 3a Abs. 4, Satz 2 EGGenTDurchfG.

The trader has to document the compliance with these requirements.

Feed additives (e.g. vitamins) produced with genetically modified bacteria (not present in the final product) are not to be labelled in accordance with Regulation (EC) No 1829/2003 and No 1830/2003. Consequently they are not taken into account in the EGGenTDurchfG.

Further information about the legal basis and the requirements for the "ohne Gentechnik" (“GMO-free”) labelling are available on the website of the *Verband Lebensmittel ohne Gentechnik (VLOG)*.

„Guidance – botanical impurity“

According to Regulation (EC) No 1829/2003 generally all feed containing, consisting or are produced from EU authorised GMO has to be labelled. Feed containing GMO contents of 0.9 % or lower are exempted from labelling provided that this presence is adventitious or technically unavoidable. The operator has to prove this in an appropriate way towards the competent authorities. According to Regulation (EC) No 1829/2003 article 24 section 2 and in context with Regulation (EC) No 641/2004 article 19 section 2 [7] the legally specified threshold applies to feed and of each feed of which it is composed.

This means that each of the separately labelled components of a compound feed has been compliant with the requirements of the abovementioned regulation concerning the threshold, even if several components of a plant species (for example maize gluten, maize ground and maize flour) are contained in the feed.

The amount of global cultivated GMO steadily increases. As a result the likelihood that such GMOs get into feed as supplemented component or by carryover of feed material also increases. Feed can contain botanical impurity not labelled as feed component or ingredient but in many cases in relation to the impurity they show a high GMO content (> 0.9 %). Attachment I No 2 of Regulation (EC) No 767/2009 [8] on the placing on the market and use of feed lays down the requirements for botanical purity of single feed material. According to this regulation the following requirements have to be met:

The botanical purity of feed materials shall not be less than 95 %, unless a different level has been laid down in the Catalogue referred to in Article 24. Botanical impurities comprise impurities of plant materials which do not have adverse effects on the animals e.g. straw and seeds of other cultivated species or weeds. Botanical impurities such as residues of other oil seeds or oil fruits derived from a previous manufacturing process shall not exceed 0.5 % for each type of oil seed or fruit.

For harmonisation of the control of production, treatment, use and placing on the market of GMO in feed a Task-Force of the “Länderarbeitsgemeinschaft Verbraucherschutz” LAV, working group feeding stuff in collaboration with the federal government and the VDLUFA has prepared a guidance document for the application of the relevant legislation reffered to as “Leitfaden zur Kontrolle von GVO in Futtermitteln“[9]. The document amongst others provides guidance for the handling of GMO material in feed derived from botanical impurities. The following practice related examples are given therein:

For single feed containing botanical impurities of other (not declared) plant species which in turn contain GMO material, compliance with the threshold of 0.9 % for the single feed material (= 100 %) has to be determined.

Example:

GMO soy in maize, amount < 5 % = botanical impurity:

→ The soy material needs not to be indicated as component,

Compound feed can contain feed material with botanical impurities of other plant species which itself contain GMO derived material. Testing for compliance with the threshold (0.9 %) can only be performed with the respective single feed material in which the GMO derived material is present. Therefore the single feed material used for the production of the compound feed has to be available. If the threshold in one of the feed material used as component in the compound feed is exceeded, this single feed material has to be labelled as “genetically modified [name of the organism]” on the declaration of the compound feed.

The approach outlined in the guidance document for the control of GMO in feed for the evaluation of GMO containing botanical impurities with view of labelling requirements the mass fraction of the feed material related to the total mass of the feed has to be determined. Therefore, a method for the valid quantification of the plant species material content in the feed sample in the range of < 1 % is required.

Testing for EU non-authorised GMO and products produced thereof - application of the "zero tolerance"

With the exception of certain EU non-authorised GMO which fall in the scope of Regulation (EU) No 619/2011 the so called "zero tolerance" applies.

Analogous to Regulation (EU) No 619/2011 the Working Group PCR-Analysis of the VDLUFA elaborated a sampling method for the detection of EU authorised GMO [10]. For samples with an inhomogeneous distribution of gm material a size of 10,000 grains is recommended for the **final sample (laboratory sample)** for pre-packaged and non-pre-packaged products. Nevertheless, the quantities given in that method are not sufficient for the aggregate sample to test for EU non-authorised GMO according to Regulation (EU) No 619/2011. The regulation foresees an **aggregate sample** size of 35,000 grains.

The presence of traces of EU non-authorised GMO in rice (LL601 and Bt63), linseed (FP967CDC Triffid) and wheat (MON71800) necessitated the developing of further sampling and analysis strategies.

The Commission Regulation (EU) No 691/2013 [12] lays down the sample size for aggregated and final sample for GMO which are covered by Regulation (EU) No 619/2011,

since Regulation (EC) No 152/2009 [11] does not cover the analysis of GMO and products produced from GMO.

For official control of GMO and products produced from GMO *Recommendation 2004/787/EC on technical guidance for sampling and detection of GMO and material produced from GMO as or in products* [13] and also CEN/TS 15568: *Foodstuff – Methods of analysis for the detection of genetically modified organisms and derived products – Sampling strategies* are available [13]. These sampling strategies are taken into account in sampling method proposed by the Working Group PCR-Analysis of the VDLUFA.

The Working Group Chemistry Experts of the Federal Countries and the Federal Office of Consumer Protection and Food Safety (BVL) recommends for the sampling of non-pre-packaged products especially for soy, maize, rice and rapeseed that the laboratory sample comprises at least of 50,000 grains for example if there is suspected contamination of EU non-authorised GMO or products produced thereof.

From this laboratory sample four sub-samples have to be prepared each containing 10,000 grains [15]. Theoretical a relative limit of detection of 0.03 % with a confidence interval of 99 % is attainable with this procedure for rice grains [16]. This approach corresponds to the Commission Decision 2006/754/EC [17] as it was established for rice 2006. Under the current Commission Implementing Decision for detection of genetically modified rice from China the size of the test sample can be reduced to 125 g for processed food and feed, respectively [18]. The Canadian Grain Commission (CGC) laid down the standards for the sampling and testing of event FP967 in linseed 2009. According to that sampling procedure four subsamples of 60 g each (corresponding to 10,000 linseed kernels) have to be analysed separately. The sample is considered as negative if all subsamples are negative [19]. This approach was implemented in the BVL guidance for sampling and analysing for the detection of genetically modifications in linseed [20].

From the EU-RL GMFF a detection strategy was published for MON71800 [21]. In accordance with EU-RL GMFF for the testing of wheat MON71800 in the case of grain material from a homogenous laboratory sample (2.5 kg) a test sample of 400 g (ca. 10,000 wheat grains) should be taken for the analysis. For processed feed the laboratory sample can be reduced to 500 g and the test sample to 125 g.

Because of that the impurities with EU non-authorised GMO or products produced from GMO are not generally distributed homogenously or occur just in traces in feed, it cannot be ruled out that based on of the low frequency and the inhomogenious distribution in the batch that the results can be different between test laboratories. This applies in particular to samples consisting of whole grains. If a batch is containing only traces of impurities, analysis results are sometimes not reproducible in every (sub) sample. This will be attributed wrongly on the analysis but is in fact an expectable and well known phenomenon when testing for trace amount of an inhomogeneous distributed analyte. For example, if the feed sample is impurified only with one GMO grain, this grain only can be present and detected analytically in one from the final samples (e.g. A-sample) prepared from the aggregate sample. Consequently with the second sample (B-sample) containing no GMO grain the analysis result will be negative. As expected the positive result from the A-sample is not reproducible. This is significant because the legislator concedes a right to perform a second (counter) analysis (Commission Recommendation on technical guidance for sampling and detection of genetically modified organisms according to Regulation (EC) No 1830/2003).

The "Seed Concept" of the Sub-Committee for Method Development of the German National and Federal Countries Joint Committee on Genetic Engineering (2006) [22] indicates that when a laboratory detects a GMO in the range of the detection limit a second analysis of a retained sample (B-sample or second sample) in a second laboratory not necessarily leads to the same result because of statistical reasons.

If no event-specific detection method is available a non-authorised GMO can be detected and characterised by performing a combination of different element-specific and construct-specific detection methods. In an overview article of the European Network of GMO laboratories (ENGL) GMOs are classified into four categories [23] based on the knowledge of their genetical structure. This knowledge has a direct effect on their analytical detectability and identifiability. For example glyphosate resistant wheat MON71800 was classified as category 2 at the time this gm event emerged. This means that GMO screening elements for the detection of MON71800 are known, but an event specific method for the identification is (still) not available. For that reason if only GMO screening elements are detectable like for wheat MON71800, it has to be (emerimentally) excluded that the screening elements detected are related to other plant species (e. g. soy, maize) [21]. Similarly a natural contamination with a donor organism like e.g. *Agrobacterium tumefaciens* or Cauliflower Mosaic Virus have to be excluded by testing for these donor organisms. The number of GMOs which are not detectable by standard screening elements is increasing. Event specific detection methods for these GMO have to be included in feeding stuff analysis.

The European Rapid Alert System for Food and Feed (RASFF) informed about the presence of not-authorised GMO.

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[6] **EGGenTDurchfG:** Gesetz zur Durchführung der Verordnungen der Europäischen Gemeinschaft oder der Europäischen Union auf dem Gebiet der Gentechnik und über die Kennzeichnung ohne Anwendung gentechnischer Verfahren hergestellter Lebensmittel. EG-Gentechnik Durchführungsgesetz vom 22. Juni 2004 (BGBl. I S. 1244, das zuletzt durch Artikel 2 des Gesetztes vom 9. Dezember 2010 (BGBl. I S. 1934) geändert worden ist

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