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Fourth meeting
Bonn, 12-16 May 2008
Item 10 of the provisional agenda*

HANDLING, TRANSPORT, PACKAGING AND IDENTIFICATION

Note by the Executive Secretary

Addendum

Compilation, as at 11 April 2008, of additional information submitted by Parties, other Governments and relevant international organizations on experience gained with the use of techniques for the sampling and detection of living modified organisms and on the need for and modalities of developing criteria for the acceptability of, and harmonizing sampling and detection techniques (paragraph 2(a) of Article 18) **

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ADDITIONAL SUBMISSIONS FROM PARTIES AND OTHER GOVERNMENTS

As at 11 April 2008

BELGIUM [11 APRIL 2008]
[SUBMISSION: ENGLISH]

Title: Combinatory SYBR®Green Real-Time PCR screening as a risk management tool for low level presence of materials derived from Genetically Modified Plants

Scope

The presence of non-uniformly distributed Genetically Modified Organisms (GMO) in a product may necessitate the development of a novel GMO detection paradigm. In essence, a uniform ISO-17025 conform platform would be required integrating sensitive detection technology with bioinformatics data-mining tools and a suitable decision support system. Preferentially, such approach should perform adequately at low cost, be easy-to-use but remain highly flexible. Here, the Belgian Authorities introduce the development of a highly sensitive GMO detection tool for the presence at low levels of materials derived from GMO.

Introduction

The recent finding in Europe of several unauthorized GMO on the EU market (e.g. Bt10 and DAS 59132 maize, LL601 and Bt63 rice) is symptomatic of the increasing use of GM-crops in several important commodity-producing countries. It is to be foreseen that such events will become more frequent in the future. Thus, sustaining globally the legal integrity of the different regions with respect to GMO presence in their market products may require adapted decision support systems, based on scientifically sound principles.

At the occurring low levels (e.g. LL601 rice was estimated to be present at below 0.02%), highly sensitive detection methods are required. Although requiring high initial investments in facilities and personnel, real-time PCR methods have to date been chosen as the preferred GMO detection technology. In essence, due to their high sensitivity (virtually a single DNA molecule can be detected), their high flexibility and their (relatively) ease to use, all measures for emergency management within the GMO world have been based on PCR methods. Protein-based methods (e.g. lateral flow strips) could in some cases represent a valuable alternative (see Starlink corn), especially in screening, but require in most cases however a confirmation of the precise nature of the GM-product (e.g. by DNA sequence analysis of a specific PCR-amplified marker).

Combinatory SYBR®Green Real-Time PCR screening: a short description

Each GM-plant can be described as a combination of different genetic/recombinant elements inserted at a unique site in the host genome. While the unique insertion site allows to precisely identifying a particular GMO, any common inserted recombinant elements can be used as screening tags for the presence of GM-material in a product. Most commonly present elements in GMO today, are the 35S promoter element from the Cauliflower Mosaic Virus and the terminator element from the Nopaline Synthetase gene of *Agrobacterium tumefaciens*. Both elements have been often used in screening approaches for GMO presence (for a review see the contribution on this topic from the EC-JRC).

The variety of commercial GM-crops to date invokes however a broader approach. A high number of different GMOs comprise the 35S and/or tNOS element (either as particular GMO or in crosses between 2 GMOs - the so-called "stacked events") in their inserted recombinant DNA; on the other hand, GMO are emerging that do not contain these elements, meaning that they would escape a "simple" 35S/tNOS screening approach.

The Scientific Institute of Public Health (IPH, Brussels, Belgium) depends of the Federal Ministry of Public Health and the Environment of Belgium. In the field of GMOs, the IPH is the co-ordinator of the Consortium of National Reference Laboratories for GMO detection. Within this context, the IPH has developed a new GMO screening platform based on SYBR®Green Real-time PCR methodology. The approach combines the detection of the presence of major commodity crops (such as soy, maize, oilseed rape, rice, cotton) with the detection of common generic recombinant elements (such as the 35S/tNOS elements) and GM-specific elements (such as herbicide resistance genes, insect resistance genes). A limited set of 10-12 targets allows covering the current "GMO universe" for commercial releases, including not only the EU-authorized GMO but also most of the GMO authorized in non-EU countries. The platform has proven to be flexible and has been successfully used in Proficiency Testing and routine analysis for food/feed products and seeds. The GMO screening setup as developed by the IPH has been approved according to the ISO-17025 standard and received a BELAC accreditation (since December 2007).

Applications and opportunities

GM-crops are part of the food chain at a global level and their presence in food and feed products is gradually increasing. The presented GMO detection platform offers a tool allowing investigating the overall presence of GM materials on the market. The platform is highly flexible, proven to be robust in various matrixes and is amendable to cost-friendly kit production. The platform could come together with a decision support system (DSS) that is readily automated via a standard i-Protocol. The DSS could be linked on-line to up-to-date GMO Dbases for real-time interpretation via a web application, offering the possibility to harmonize the determination of GM-material presence over time, space and produces.

EUROPEAN COMMUNITY

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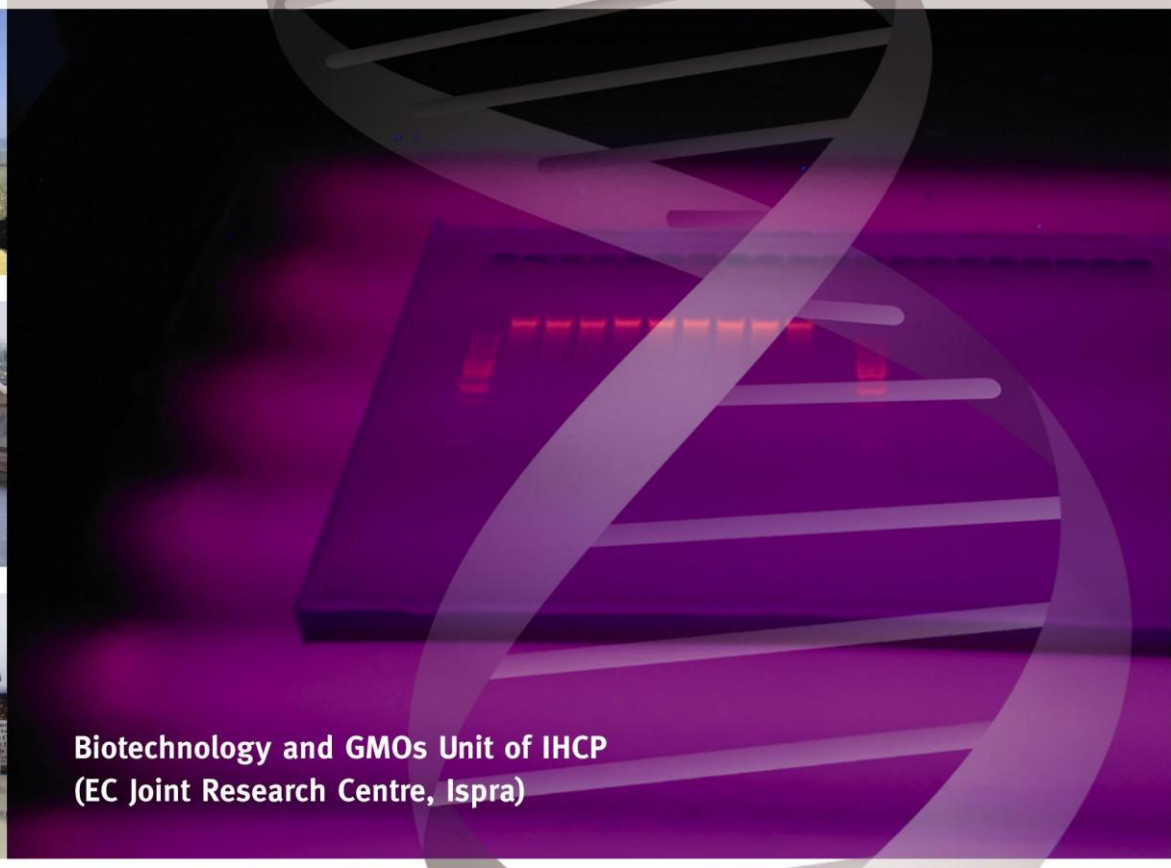
An interactive version of this submission with hyperlinks is also available as record number 43770 in the Biosafety Clearing-House, http://bch.cbd.int/database/record.shtml?id=43770

Institute for Health and Consumer Protection

Biotechnology and GMOs

An overview of EU activities for the development and harmonisation of GMO detection methods and sampling procedures



Biotechnology and GMOs Unit of IHCP
(EC Joint Research Centre, Ispra)

JRC
EUROPEAN COMMISSION

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December 2007

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Content

1 Introduction

This report gives a general overview of activities regarding the development, validation and harmonisation of GMO detection methods within the European Union and, in particular, the role of the Joint Research Centre (JRC) of the European Commission (EC). It describes the established structures and research activities that already have led to a well-attuned control system of GMO labelling throughout Europe. These activities also may serve as a model for other countries and regions seeking to achieve harmonisation of methods on an international level.

Complementing the major role of the JRC and its European Community Reference Laboratory on Genetically Modified Food and Feed (CRL-GMFF), individual European nations have been active on the topic of detection and have established discussion and networks of experts within the region and beyond. The driving force behind such activities was the introduction of restrictive food and feed labelling regulations that are aimed at ensuring the freedom of choice for consumers. Since the enactment of new and tighter EU labelling regulations in April 2004, food and feed containing more than 0.9 % GMOs must be labelled, provided that this presence is adventitious or technically unavoidable (see chapter 2 - “GMO labelling regime in the European Union”).

As a natural prerequisite for the proper enforcement of labelling regulations in the EU, extensive activities are underway to develop and to study the performance and harmonisation of GMO detection methods. An overview of GMO detection systems is given in chapter 3 (“Detection methods for GMOs applied in the EU)”.

The EU countries also have established an expert network of regulatory authority laboratories. The European Network of GMO Laboratories (ENGL) is led by the EC’s JRC in Ispra, Italy. The JRC is also responsible for the maintenance of the CRL-GMFF. In close co-operation with the ENGL, the CRL-GMFF is the central pillar for the validation of detection methods for GM products and conducts extensive ring trial work. The ENGL is comprised of more than 120 participating laboratories in the EU, Norway and Switzerland and represents a high-ranking resource for the solution of the technical challenges of GMO detection. In order to support the validation of GMO detection methods, the JRC has developed a unique software tool known as AMPE (see chapter 4 - “Validation and harmonisation of GMO detection methods in the EU”).

A further prerequisite for the successful harmonisation of GMO detection approaches is the dissemination of proper analytical methods. The Biotechnology & GMO Unit of the JRC transfers its knowledge to collaborating laboratories and, in this context, holds a series of practical training courses for the staff of food control laboratories within the European Union and even beyond its borders. In this regard, the JRC is the leading player in the dissemination and harmonisation of GMO detection methods worldwide. Also, an essential task of the B&GMO Unit is the maintenance of a central and comprehensive database containing suitable detection methods for GMOs and general information on each specific GMO. To date, more than 400 PCR- or ELISA-based methods have been entered in this database, which is comprised of more than 50 GMO events (see chapter 5 - “Dissemination and Training Activities”).

JRC and national laboratories in the EU countries have gathered broad experience in the practical application of GMO detection systems for the enforcement of labelling requirements. The implementation of control measures on national level is inspected regularly by the Food and Veterinary Office (FVO) of the EC. It serves as an independent control agency to promote the common and harmonised implementation of European legislation. In most cases, FVO inspectors have concluded that Member States have installed appropriate structures and competent staff to undertake GMO controls. Nonetheless, some authorities have been advised to extend their controls to all EU-approved GMO as well as unapproved GMO that illegally may enter the European market. This point indicates a major challenge to control systems within the EU: the effective exclusion from the regional market of imports of illegal and possibly unknown GMO products. In cases in which illegal GMO products are detected and prompt the need for emergency measures, the B&GMO Unit of the

JRC supports the EU Commission. The established procedures in such cases (such as was caused by the import of commodities containing illegal traces of Bt10 maize or LL Rice601) have demonstrated efficiency in reacting to urgent matters (see chapter 7 - “EU experience on GMO detection techniques”).

Such incidents also illuminate the gaps within the currently available methodology of GMO detection. Research is in progress to make GMO detection more robust and more economic, as well as to address the constantly increasing number of GMO events worldwide. In particular, such research activities are aimed partly at the provision of accurate and certified reference material for enforcement laboratories. Further aims include the development of new high-throughput detection systems and the exploration of more reliable sampling strategies. Another important goal is the improvement of methods for the detection of unknown and illegal GMO products on the European market. The activities of the ENGL and CRL-GMFF are supported by the research and development programmes FP5 and FP6 funded by the European Commission, such as Co-Extra and GMO-Chip (see 3.5 – “Biochips / Micro-arrays”, chapter 6 – “Sampling strategies”, and chapter 8 – “Next generation of detection methods”).

Finally, particular attention shall be focused on the **“1st Global Conference on GMO-Analysis”** to be hosted by the JRC and ENGL on 24-27 June, 2008 in Como, Italy. This conference may present the next major step in the international harmonisation of approaches to GMO detection and will address existing challenges in the fields of sampling for GMO analysis, the appropriateness of analytical tools and the consistency and interpretation of test results. Further and updated information is available at the official website of the Global Conference.

2 GMO labelling regime in the European Union

The EU recognises the right of the consumer to information and labelling as a decisive tool in making an informed choice. GMO labelling was introduced in the EU to give consumers the freedom of choice between GMOs and conventional products. Since 1997, the labelling of GMOs, either as such or in a food product, has been mandatory.

On 18 April 2004, new regulations for the labelling of genetically modified foods and feed came into effect in the EU. These reinforce labelling requirements and, for the first time, also addressed feed (Regulation (EC) 1829/2003, Regulation (EC) 1830/2003).

EU Regulation 1829/2003 on genetically modified food and feed states which items must be labelled with regard to applications of genetic engineering:

- GMOs for food and feed use (example: genetically modified tinned sweetcorn)
- food and feed produced from, or containing, ingredients or additives produced from GMOs (example: oil from GM soy beans or sugar from GM sugar beet)
- food, ingredients and additives which contain genetically modified organisms (example: wheat beer with GM yeast)

Food and feed which is produced with the aid of genetically modified organisms, or obtained using a genetically modified processing aid, do not have to be labelled. Therefore, for example, labelling is required neither for meat, eggs, milk, and dairy products obtained from animals fed with genetically modified feed, nor is it required for additives, flavours and vitamins produced with the help of GM micro organisms.

The labelling requirements also do not apply to food and feed containing GMOs in a proportion not higher than 0.9 per cent of the food ingredients when considered individually, provided that this presence is adventitious or technically unavoidable.

This threshold applies only to GM content that has been authorised in the EU and which therefore is considered safe. Imported GMOs that have not yet received authorisation in the EU, but nevertheless have been subjected to scientific safety evaluations of the European Food Safety Authority (EFSA), had been transitionally tolerated at a threshold of 0.5 percent until April 2007. Since then, food and feed containing GMOs not approved by the EU generally are not tolerated on the EU market.

This Regulation (EC) 1830/2003 is stricter than the previous legislation and extends mandatory labelling to all food and feed produced from GMOs, without making a distinction between those which contain DNA or protein resulting from genetic modification and those which do not.

Specialised traceability infrastructure had to be developed for the new process-oriented regulatory system. Each stakeholder who produces or trades GM raw materials, ingredients, or foods is obligated to forward relevant information to subsequent stakeholders in the food supply chain.

- Documentation must be retained for five years.
- It must always be possible to trace the route of a GMO from the farm to the final product.
- Upon authorisation, every GMO is assigned an ID number that can be used to identify it at all times.

Local governments are responsible for monitoring the GMO content of products. In the case that analytical tests on a product are unable to confirm that labelling regulations have been upheld, indirect means of enforcement are needed. In such cases, monitoring is conducted through the request of written documentation, such as certificates or results of GMO testing from earlier stages in production.

Analytical tests best can be used for enforcement at early steps in the food supply chain, in which food products retain sufficient intact DNA to enable testing.

Regulation (EC) 1829/2003 establishes that biotechnology companies must develop specific detection methods for GMOs. For validation, applicants must provide these to the CRL-GMFF as part of the complete application dossier.

Labelling requirements for transboundary movements of GMO (EU exports)

The Cartagena Protocol on Biosafety to the Convention on Biological Diversity establishes the importance of organising the supervision and control of transboundary movements of GMOs. This contributes to the conservation and sustainable use of biological diversity and, by taking into account risks to human health, enables citizens to make free and informed choices in regard to GMOs.

Since EU Community legislation does not contain specific requirements for exports of GMOs to third countries, Regulation (EC) 1946/2003 establishes a common legal framework for such exports. Among other measures, it is necessary to ensure the identification of GMOs being exported from the Community. According to the Regulation, exporters shall ensure that the following information is stated in a document accompanying the GMO and is transmitted to the importer receiving the GMO: (a) confirmation that it contains or consists of GMOs and (b) the unique identification code(s) assigned to these GMOs if such codes exist.

3 Detection methods for GMOs applied in the EU - Development and use

3.1 General overview

GMO detection methods are essential not only to detect GMOs in food and feed. They also serve to identify particular GMOs and to quantify their amount in the various ingredients of food and feed.

All GM plants possess at least one new gene that has been inserted into their genomes. In most cases, the new gene or genes lead to the production of new proteins. Therefore, two classical approaches are used today to detect GMO compounds in crops and derived products: detection of the new transgenic DNA or of the new protein or proteins it prompts.

The first approach, the Polymerase Chain Reaction (PCR), is based on the detection of novel DNA sequences present in the genome of a crop. The method indicates the absence or presence of GMO-specific DNA in a given sample. The determination of a specific GMO in a sample allows the segregation of its source and the identification of unapproved GMOs on the market. Traceability thereby becomes possible throughout the supply chain of GM crops.

The second detection approach, called ELISA (Enzyme-Linked Immunosorbent Assay) uses antibodies that specifically bind the new protein compounds of GMOs.

3.2 PCR or protein-based detection?

Both methods also can be used to quantify the amount of GMO compounds in a test sample. To date, DNA detection is the standard method used in the EU to determine the identity and amount of GMOs in a tested product. The reasons for its dominance include the comparatively high sensitivity of PCR-based detection methods and the inability of protein-based approaches to discriminate between varying GMOs that express the same or similar proteins. Additionally, industrial processing easily denatures proteins and impedes the use of ELISA methods for food products.

However, the ELISA-test can be a useful, economic and quick approach to the detection of GMOs, at least in raw products and on the field. A prerequisite of its use is that the GMOs in question produce new proteins in all stages of development and that these proteins also are present in harvested plants and their parts.

Qualitative detection methods can be used for the initial screening of food and feed products. Initially, the goal is to investigate whether GMO-specific compounds such as DNA elements and/or proteins are present in a particular product.

If the presence and identity of GMOs in a sample has been determined, a subsequent quantitative test must be executed in order to determine whether the GMO content in a food or feed sample complies with the EU labelling provisions.

Commission Recommendation of 4 October 2004 establishes that results of qualitative analysis should be expressed in ratio of GMO-specific DNA to taxon-specific plant genome DNA (copy numbers). Consequently, only PCR-based methods are applicable to fulfil this legal requirement. However, if novel protein-based methodologies satisfy legal requirements and are fit for use in GMO analysis, such methods also will become an integral element of official EU control measures. As cost- and time- efficient tools for the screening and traceability of GM events, they may have valuable

practical applications. A number of basic research projects are underway to explore the potentials of protein-based detection methods for advanced and high-throughput detection systems. A selection of such activities conducted by the B&GMOs Unit is described in chapter 8 (“Next generation of detection methods”).

Further reading:

The Analysis of Food Samples for the presence of Genetically Modified Organism. Course introduction (training manual of B&GMOs Unit, JRC)

3.3 Protein-based detection methods

The methods for GM plant detection that currently are commercially available have been developed mainly for insect-resistant Bt crops and for herbicide-tolerant GM plants.

3.3.1 Laboratory based ELISA-methods

The most sensitive protein-based approach is the ELISA method, which is commonly a purely laboratory-based method. It can be used for detection and quantification and can be viewed as a useful tool for screening, for control purposes and for the implementation of traceability.

3.3.2 Lateral flow strip test

The lateral flow strip test or dipstick kits are analyses which do not require a laboratory. The test can be carried out within 10-20 minutes and under field conditions. Typical samples for such a test are seeds or plant fragments. The test is semi-quantitative and therefore cannot be used for accurate quantification.

Further reading and resources:

Quantitative detection of Roundup Ready® Soybean by ELISA (training manual “The Analysis of Food Samples for the Presence of Genetically Modified Organisms” of B&GMOs Unit, JRC)

GMO Method database of B&GMO’s Unit

3.4 PCR-based methods

3.4.1 Qualitative methods (screening and detection)

Such methods are commonly used for GMO detection and identification. Detection typically is the first step in the analysis for GMO content. For screening purposes, PCR methods are applied that can detect common genetic elements found in a range of GM plants. Such elements include the 35S promoter and the nos terminator.

Further reading:

- Characteristics of the qualitative PCR systems (training manual "The Analysis of Food Samples for the Presence of Genetically Modified Organism" of B&GMOs Unit, JRC)
- Qualitative detection of MON810 Maize, Bt-176 Maize and Roundup Ready® Soybean by PCR (training manual "The Analysis of Food Samples for the Presence of Genetically Modified Organisms" of B&GMOs Unit, JRC)

Upon the detection of GMOs in a sample, their identity must be determined. For this purpose, PCR primers are used that are event-specific for the GMOs in question. Specialised PCR approaches also are available for specific applications including:

- nested PCR: 'nested' sets of primers can be used to improve the sensitivity and specificity of a DNA amplification.
- multiplex PCR: multiple pairs of primers are used simultaneously to detect a range of target sequences.

Further reading:

- The Polymerase Chain Reaction (training manual "The Analysis of Food Samples for the Presence of Genetically Modified Organisms" of B&GMOs Unit, JRC)

3.4.2 Quantitative methods

For the quantification of GMO content, two PCR approaches are common: Quantitative Competitive PCR and Real-time PCR. Both methods address the problem of limited correlation between the amount of target DNA and the amount of PCR products generated by amplification. Without a strong correlation, the amount of product DNA is insufficient as a quantitative indicator.

Quantitative competitive PCR:

This method is based on the simultaneous amplification of target DNA of the sample and a defined amount of an internal standard DNA.

Real-time PCR:

This method is a more accurate and more widely used quantitative PCR approach. Real-time PCR-systems monitor the amplification of DNA by a fluorescent signal that is proportional to the amount of PCR product. The first significant increase of the fluorescent signal correlates to the initial amount of target DNA.

Further reading:

- Quantitative PCR for the Detection of GMOs (training manual "The Analysis of Food Samples for the Presence of Genetically Modified Organisms" of B&GMOs Unit, JRC)
- Quantitative Detection of Roundup Ready® Soybean by Real time PCR (training manual "The Analysis of Food Samples for the Presence of Genetically Modified Organisms" of B&GMOs Unit, JRC)
- Real Time PCR based GMO quantification: limits and accuracy. C. Barbati, F. T. Weighardt, S. Kay, C. Paoletti, M. Querci, and G. Van den Eede (2002)
- Review of GMO Detection and Quantification Techniques. L. Bonfini, H. Petra, S. Kay, and G. Van den Eede (2002)
- Food Products Identity: the Need for a High Through-Put Approach in Food Analysis. A. Fantozzi, M. Marini, M. Ermolli, G. Van den Eede (2003)
- Food products identity: the need for a high through-put approach for GMO screening. A. Fantozzi, M. Marini, M. Ermolli, and G. Van den Eede (2003)

3.5 Biochips / Micro-arrays

Micro-arrays based on DNA hybridisation are the most recent tools to be developed and validated in the EU for the detection of GMOs. Since most laboratories test their food and feed products by methods that do not allow a broad sample screening for GM crops, a major problem of the current GMO detection system has become increasingly visible. The number of GM crops worldwide constantly is rising and a corresponding increase of approved and unapproved GMOs in the food and feed chain must be expected. Consequently, there is an obvious need for screening tools that allow the simultaneous detection of different GMOs in a sample in one step. Considerable time and expense may be saved in GMO detection laboratories if an indication exists of which GMO is likely to be present in a sample.

In the course of the EU-funded Co-Extra project, a new method of multiplex screening – the DualChip GMO - has been developed.

A broad range of specific DNA molecules, corresponding to specific DNA elements of GMOs, are immobilized separately on glass slides. The immobilised DNA on the glass slide "captures" specific DNA elements of GMOs – if present in the sample – and bound DNA sequences of GMOs are made visible by a subsequent colorimetric reaction. The result is a pattern of visual spots on the glass slide.

The current version of the DualChip GMO detects six different DNA elements typical for a broad range of GM crops. GMOs in a given sample are identified by a software tool provided with the kit for the analysis of results. The current DualChip GMO micro-array can be used to discover most EU-approved GM crops and the spectrum of detectable GM crops continues to be expanded.

The suitability of the method recently was validated by a collaborative ring trial organised by the EC's Joint Research Centre. The target DNA can be detected to a level of 0.1%.

Further reading:

- Microarray Method for the Screening of EU Approved GMOs by Identification of their Genetic Elements. Report of validation (CRL-GMFF)
- Biochips: A powerful tool for multiple and fast analysis of genes and DNA sequences (BATS)
- Project summary of GMOchips (EU funded research project)
- EU funded research project DNA-Track

4 Validation and harmonisation of GMO detection methods in the EU

4.1 Responsible bodies and supporting networks

The enforcement of the EU legislation on GMO labelling requires GMO detection methods that are sound, precise and robust. It is, therefore, an essential requirement to use validated methods for GMO detection and quantification. Only in this manner can it be assured that independent control laboratories achieve comparable analysis results and are able to fulfil regulatory tasks. EU Regulation (EC) 882/2004 establishes that analytical methods used for food and feed control purposes must be validated by control laboratories before their use. Within the European Union, the validation of analytical methods for GMO analysis is required for the authorisation of a certain GM food or feed product.

Consequently, a centralised validation procedure has been established to validate and harmonise GMO detections methods within European member states and beyond.

For this work, four institutions and networks mainly are responsible:

- The **Community Reference Laboratory on Genetically Modified Food and Feed** (CRL-GMFF) in ISPRA, Italy,
- the **European Network of GMO Laboratories** (ENGL)
- the **Institute for Reference Materials and Measurements** (IRMM) in Brussels, Belgium and
- the **European Committee for Standardization** (CEN).

4.1.1 The Community Reference Laboratory on Genetically Modified Food and Feed (CRL-GMFF)

The Joint Research Centre (JRC) has been appointed as the Community Reference Laboratory for Genetically Modified Food and Feed (CRL-GMFF). JRC's CRL-GMFF was established in 2004.

Under Regulation (EC) 1829/2003, the CRL-GMFF has the mandate to validate analytical methods for the detection of GMOs in food and feed. The operations of the CRL-GMFF are performed in line with

- Regulation 641/2004 (EC) (establishes the implementation of Regulation (EC) 1829/2003) and
- Regulation 1981/2006 (EC) (outlines the implementation of Article 32 (Community reference laboratory) of Regulation (EC) 1829/2003).

Within the current framework, detection methods are tested by CRL-GMFF for their 'fitness of purpose' and subsequently are validated by collaborative trials at the expense of the biotechnology company (Overview of CRL-GMFF operations).

Finally, the methods are published on the CRL-GMFF website, facilitating their use by private detection laboratories and official control laboratories. Moreover, the methods are proposed for standardisation in CEN/ISO (see 4.1.4).

Regulation (EC) 1829/2003 establishes that biotechnology companies must develop specific detection methods for GMOs (see box below). For validation, applicants must provide these to the CRL-GMFF as part of the complete application dossier.

Improved conditions for the validation process by Regulation (EC) 1829/2003

Due to the new GM Food and Feed Regulation (EC) 1829/2003 for the validation procedure within the EU, general and major progress has been achieved in this field. In the early years (1997-2002) of GMO authorisation, the applicant biotechnology company was not required to provide a method for the detection, identification and quantification of the GMO in question. Because of this, the heavy burden of the cost, development and validation of GMO tests fell upon the research laboratories involved in GMO traceability projects and upon the national enforcement laboratories.
The European Commission has been convinced by the executing organisations and networks responsible for validation of GMO detection method that new regulations were needed. Consequently, Regulation (EC) 1829/2003 obliges biotechnology companies to develop their own methods of detection and to provide these as part of the complete application dossier to the CRL-GMFF for validation.

In order to be accepted, the method submitted by the applicant must satisfy specific performance criteria. Failure to meet these criteria leads to rejection of the method and, consequently, to a delay in the authorisation of the GMO.

The criteria are recorded in a document provided by CRL-GMFF called "Definition of minimum performance requirements for analytical methods of GMO testing".

This document was compiled by the European Network of GMO Laboratories (ENGL, see 4.1.2).

It describes:

- the "Method Acceptance Criteria" for specific GMO detection approaches, which are to be fulfilled by applicants introducing new GMOs to the authorisation process in the EU (see general principle conditions in Annex I of Regulation (EC) 641/2004) and
- "Method Performance Requirements", which must be met successively in a collaborative inter-laboratory study.

Details on "Method Acceptance Criteria"

Besides criteria such as applicability, practicability and specificity of the method submitted by the applicant, the following conditions also must be met (and are based on the EU's labelling threshold of 0.9% for the adventitious or technically unavoidable presence of GMO):

- The range of the standard test curve should allow reliable testing of GMO concentrations from 0.09 % to 4.5 % (w/w) (the required "dynamic range")
- the accuracy of a given test should be within +/- 25 % of the reference value over the whole dynamic range
- the limit of quantitation must be below 0.09 % (w/w)
- the limit of detection must be below 0.045 % (w/w)

- the results of an given test system should not deviate more than +/- 30 % and should be independent of the variety of instruments and operators as well as of the brand and concentration of reagents and the temperature of the reaction

The final assessment of sufficiency and suitability of the performance of a given GMO detection method is conducted in two independent steps:

- in-house evaluation of method performance characteristics described above (by CRL-GMFF)
- evaluation of method performance characteristics, executed through the analysis of inter-laboratory collaborative trial results (concerning dynamic range, reproducibility, standard deviation and trueness). 'Trueness' is defined as the closeness between the value obtained from collaborative inter-laboratory tests and the accepted reference value. It should be within +/- 20 % of the reference value over the whole dynamic range.

Notes:

(1) Presently, the "minimum performance requirements" for GMO detection methods only refer to DNA-based analytical methodologies. Commission Recommendation of 4 October 2004 establishes that results of qualitative analysis should be expressed in ratio of GMO specific DNA to taxon specific plant genome DNA (copy numbers). Consequently, only PCR (DNA) -based methods are applicable. However, if novel methodologies fulfil legal requirements and are applicable to GMO analysis, the present document will be amended accordingly.

(2) CRL-GMFF provides additional documents for applicants to be used in the frame of the authorisation process of GMOs in the EU, including the contribution of suitable GMO detection methods:

- Guideline for the submission of DNA sequences to the CRL-GMFF
- Format to provide information on GM detection methods and related samples
- Template protocol format for submission of a GMO specific real-time PCR system
- Explanatory notes to applicants (Reg. EC No. 1981/2006)
- Practical instructions for applicants which concern the implementation of procedures on financial contributions to the Community Reference Laboratory for Genetically Modified Food and Feed (CRL-GMFF) as described by Regulation (EC) No 1981/2006.
- Practical instructions concerning the method validation task of the CRL as described Regulation (EC) 1829/2003 and in the Regulation (EC) 641/2004 (so called implementing guidelines). Scientific and technical aspects are described by the CRL in collaboration with the European Network of GMO Laboratories (ENGL) according to the scientific expertise prevalent in these bodies.

The documents mentioned above are regularly under revision taken into account the latest technologies and experiences gained with the requirements set in the documents (see CRL-GMFF website).

In 2004 the JRC also was appointed to be the Community Reference Laboratory (CRL) for GMOs in regard to official controls performed to ensure the verification of compliance with feed and food law.

The duties are related to assistance provided to the National Reference Laboratories (NRL) in fulfilling their official control activities and are read as follows:

(a) providing national reference laboratories with details of analytical methods, including reference methods;

(b) coordinating application by the national reference laboratories of the methods referred to in (a), in particular by organising comparative testing and by ensuring an appropriate follow-up of such comparative testing in accordance with internationally accepted protocols, when available;

(c) coordinating, within their area of competence, practical arrangements needed to apply new analytical methods and informing national reference laboratories of advances in this field;

(d) conducting initial and further training courses for the benefit of staff from national reference laboratories and of experts from developing countries;

(e) providing scientific and technical assistance to the Commission, especially in cases where Member States contest the results of analyses;

(f) collaborating with laboratories responsible for analysing feed and food in third countries.

Further reading:

Description of CRL-GMFF Validation Process (CRL-GMFF)

In case of disputes

Under REGULATION 1981/2006 (EC), the CRL-GMFF also has the mandate to provide scientific and technical advice in the case of disputes between EU Member states concerning the results of GMO analysis. In such cases, the CRL may reanalyse submitted samples and integrate appropriate procedures into the overall Quality System concerning GMO detection throughout Europe.

Validated methods by CRL-GMFF

A compilation of validated GMO detection and DNA extraction methods (full method reports) within the frame of the Regulation EC 1829/2003 is given in Annex III. The table list indicates “fit for use” methods for about 30 GMOs. Additionally, GMO-specific methods are listed which, in November 2007, currently are in the process of validation. The validation reports and full method reports are available in pdf-format.

4.1.2 The European Network of GMO Laboratories (ENGL)

In 2002, the European Network of GMO Laboratories for GM food and feed (ENGL) was established as a consortium of national enforcement laboratories. The network supports CRL-GMFF in evaluating new methods and is coordinated by JRC’s Biotechnology & GMO Unit (Institute for Health and Consumer Protection).

A reason for the establishment of the network was dissatisfaction with the enforcement of labelling requirements in the EU. Prior to the ENGL, no systematic coordination existed between enforcement

laboratories. In developing reliable GMO detection methods, these laboratories consequently were hampered by the lack of sufficient reference material and sequence information.

In regard to the sampling, detection, identification and quantification of GMOs, the ENGL represents a unique platform for experts from throughout Europe. The twin goals of such networking are the international harmonisation of analytical approaches and the solution of the many technical and analytical problems faced by enforcement laboratories in addressing GMOs in food and the environment. For example, ENGL members discuss the technical and analytical challenges of the implementation of Regulation (EC) No 258/97 and of the labelling of food products containing more than 0.9% GMO.

Since 2004, ENGL provides assistance to the CRL-GMFF, particularly with respect to the validation of analytical methods for the event-specific quantification of GMOs.

The ENGL supports the CRL-GMFF in the following activities:

- development of methods for qualitative and quantitative analysis
- dissemination of proven detection technologies through training and capacity building
- harmonisation of control and exchange of information
- validation of screening and quantification methods for GMO detection
- development of sampling strategies for different GM commodities such as seeds, grains, raw material or processed food products
- development of supporting tools for reliable GMO detection, such as GMO sequence databases with GMO-specific molecular data and bioinformatics
- initiation of research on new detection methods, e.g. within the EU 6th Framework Programme

Today, the network is comprised of members from more than 120 laboratories (representing all 27 EU Member States as well as Norway and Switzerland). In addition, laboratories from other countries (e.g. China, Turkey) participate as observers in the network.

4.1.3 Institute for Reference Materials and Measurements (IRMM) - Provision of Certified Reference Material

Certified reference material (CRMs) are needed for reliable calibration and quality control of the quantification methods applied. The Institute for Reference Materials and Measurements (IRMM) is supporting the ENGL with the production of certified reference materials and by delivering advice on the correct use of GMO CRMs. The delivery of GMO CRMs for further method validations provides additional direct support to the ENGL.

The IRMM provides powder material derived from seeds containing mixtures of certified quantities of GMO and non-GMO material. Certified reference material (CRM) is available for every approved GMO in the European food and feed chains. CRM is incorporated into every worldwide GMO detection test. Currently, efforts are being made to certify the powder materials not only for their mass fraction of a specific GMO event but additionally for the copy number ratio as recommended in 2004/787/EC. To support GMO laboratories, a calibrant also has been made available. The first set of calibrants and quality control material was released at the beginning of December 2007.

Further reading and resources:

- GMO reference materials (IRMM)
- Certified Reference Materials – Catalogue 2007 (IRMM)
- IRMM catalogue: Plasmid DNA Fragments of MON 810 maize
- Use of Certified Reference Material for the quantification of GMO in DNA copy number ratio (IRMM)
- Past and Future of Reference Materials (IRMM, 2002)

4.1.4 The European Committee for Standardization (CEN)

Once validated by CRL-GMFF in cooperation with ENGL, a GMO detection method may be accepted as an international standard by the European (CEN) or international (ISO) standardisation body.

Standardisation of reliable detection methods is an important tool for fair trade under the umbrella of the World Trade Organization (WTO). The European and international standardisation organisations (CEN and ISO) have established common standards for GMO detection, including a general document on performance criteria and laboratory organisation requirements. These standards currently serve as models in several other detection areas, beginning with the modular approach and including the general requirements of PCR-based detection methods. In this way they save time and contribute to the global harmonisation of molecular biology based detection methods.

In order further to contribute to the harmonisation of sampling, the B&GMOs Unit continues to provide technical advice to CEN towards the definition of new CEN sampling protocols, (CEN/TS 21568 : 2005), which is in line with the sampling protocol for grains proposed within the frame of EC Recommendation 787/2004.

CEN generally has approved a set of six general standards on methods of analysis for the detection of genetically modified organisms and derived products (CEN/TC 275 - Food analysis - Horizontal methods). The standards comprise methods of sampling, DNA extraction, and methods of protein and DNA analysis. All standards mentioned in the table below also have been accepted by the International Organization for Standardization (ISO), are now ISO Standards and have been adopted worldwide:

CEN/ISO methods of analysis for the detection of genetically modified organisms and derived products	
General requirements and definitions	EN ISO 24276:2006
Sampling strategies	CEN/TS 15568:2006
DNA extraction	EN ISO 21571:2005
DNA analysis (qualitative)	EN ISO 21569:2005
DNA analysis (quantitative)	EN ISO 21570:2005
Protein analysis	EN ISO 21572:2004

Publications of the standard methods are commercially available.

Further reading:

CEN/TC 275 WG11 on Genetically Modified Foodstuffs (IRMM)

4.1.5 Contributing to an internationally harmonised validation process: European activities at Codex Alimentarius

Delegates of European Member States largely have contributed to the development of international standards for the validation process of GMO detection methods. Codex Alimentarius, the joint FAO/WHO Food Standards Programme, is an international harmonising network. At the twenty-fourth Session of the Codex Alimentarius Committee on Methods of Analysis and Sampling (CCMAS), papers were discussed that provided the methods collated by the ad hoc Intergovernmental Task Force on Food Derived from Biotechnology (see CX/MAS 02/8). These papers also outlined general considerations in regard to analytical methods for the detection and identification of foods derived from biotechnology (see CX/MAS 02/9).

CCMAS addressed the novelty of such methods by establishing a working group led by the UK and Germany. This group will consider whether methods for the detection or identification of food ingredients from biotechnology fit existing criteria for analytical methodologies. The suitability of proposed methods to become Codex standards also will be assessed.

The proposal currently under discussion provides comprehensive information required for the validation of quantitative and qualitative methods. Such information includes characteristics that could be used to consider existing validated methods as well as to assist laboratories in the determination of measurement uncertainty. The draft report also contains a list of validated methods for GMO detection and, in addition to PCR-based methods and the modular approach to GMO testing and validation, includes protein-based methods.

Participants from the EU stated that international standards for GMO detection are needed to ensure traceability. Tracing requires adequate methods of analysis and, in light of several problems of methodology in the identification of foods derived from biotechnology, the EU participants further stressed the importance of such standards.

It was concluded that the criteria approach should be applied in the selection of methods by the Codex for the analysis for foods. The next meeting of the Codex Committee on Methods of Analysis and Sampling will be held in Budapest, Hungary in March 2008.

The actual document is not yet part of the official work programme. However, the inclusion of the above-mentioned document in the stepwise procedure according to Codex rules currently is under consideration.

Further reading:

Report of the twenty-sixth session of the Codex Committee on methods of analysis and sampling - Budapest, Hungary, 4-8 April 2005

Report of the twenty-seventh session of the Codex Committee on methods of analysis and sampling - Budapest, Hungary, 15 - 19 May 2006

4.2 AMPE Software: A tool for a standardised validation of GMO detection methods

AMPE ("Analytical Method Performance Evaluation") developed by the B&GMOs Unit is a software tool designed to evaluate/validate the performance of analytical methods under standardised conditions. The performance of a given detection method is qualified by characteristics that include the limits of detection and quantitation as well as the accuracy, specificity, and linearity of responses.

In the context of control purposes, method validation is requested by EU legislation. In many cases, it is a pre-requisite for the acceptance of a specific method. It is important that method performance be evaluated among different Member States and Competent Authorities in a harmonised and standardised manner. Based on the principles of ISO 5725 (1994), AMPE supports standard validation procedures. Alternative procedures also are provided. The software enables the comparison of different detection methods and the evaluation of their adequacy with respect to user-specific analytical needs.

The software was developed using MS Visual Basic. It runs over Microsoft Windows operating systems and is available free of cost.

Recently a report was prepared by IRMM to give guidance on measurement uncertainty for GMO testing laboratories. The report describes two alternative ways to determine the uncertainty related to a specific result, either by collaborative studies or by considering results already obtained in previous control measures.

Further reading:

- Analytical Method Performance Evaluation (AMPE) - a software tool for analytical method validation. Acutis M, Trevisiol P, Confalonieri R, Bellocchi G, Grazioli E, Van den Eede G, Paoletti C. (2007)

5 Dissemination and Training activities

5.1 General dissemination activities

Over years, the Biotechnology and GMOs Unit (B&GMOs Unit, JRC) has developed a profound knowledge on the different aspects related to GMO detection and quantification. The Unit also has designed, adapted or validated advanced methods for their detection and quantification.

A central task of the B&GMO Unit is the harmonisation and dissemination of proper analytical approaches for GMO detection. Knowledge on these techniques is transferred to collaborating laboratories through publications, collaborative projects, individual training or specific courses. Technical details are provided to trainees as oral presentations or brief written outlines.

In this context, the Unit holds a series of training courses for food control laboratory staff within the European Union but also beyond the borders of European member states. The aim is to provide analytical biotechnology skills and to promote the use of validated and harmonised methods for the detection, identification and quantification of GMOs in food and feed.

The courses specifically address laboratory personnel who possess a good level of analytical knowledge but have little or no expertise in GMO detection.

Besides such regular training courses, the B&GMOs Unit offers individual training according to specific needs. Training in this important area frequently has been requested, due to the increasing need to comply with current European legislative framework.

Since 2000, the B&GMOs Unit (JRC) and the World Health Organisation (WHO Food Safety Programme in Europe) have collaborated in the organisation of such training courses to promote issues related to food safety . The training courses are offered particularly to laboratories from EU Accession Countries, as well as Central and Eastern Countries with economies in transition. As a response to the increasing collaboration with countries beyond the European borders, these training activities have been enlarged to address Africa. Eleven regular training courses have taken place.

The training activities are supported by a written training manual, which describes a selection of basic techniques currently used in EU enforcement laboratories and which reflects the most updated and harmonised approaches. The subject matter covers a wide variety of techniques for the detection, identification, characterisation and quantification of GMOs, and includes important theoretical background information.

The following are the topics covered by the training courses:

- DNA extraction from raw and processed materials
- Screening of foodstuffs for the presence of GMOs by simple Polymerase Chain Reaction and by nested Polymerase Chain Reaction
- Quantification of GMOs in ingredients by Real-time Polymerase Chain Reaction
- Quantification of GMOs in ingredients by the Enzyme-Linked ImmunoSorbent Assay

Further reading and resources:

- User Manual "The Analysis of Food Samples for the Presence of Genetically Modified Organisms" (last update:2006)
- Foreword
- Overview, general introduction on Genetically Modified Organisms (GMOs), EU legislation
- Manual presentation, working methods and course introduction
- Samples used during the course
- Extraction and purification of DNA
- Agarose Gel Electrophoresis
- The Polymerase Chain Reaction (PCR)
- Characteristics of Roundup Ready® Soybean, MON810 Maize and Bt-176 Maize
- Characteristics of the qualitative PCR systems described in the Manual
- Qualitative detection of MON810 Maize, Bt-176 Maize and Roundup Ready® Soybean by PCR
- Quantitative PCR for the detection of GMOs
- Quantitative detection of Roundup Ready® Soybean by Real-time PCR
- Quantitative detection of Roundup Ready® Soybean by ELISA

Additionally, the B&GMO Unit offers an interactive course on DVD (“Detecting GMOs”, The JRC Advanced Training Series, KJ-53-03-491-EN-Z) that integrates the information provided on the one-site training courses and includes an overview of EU legislation, experimental set-up, sample preparation, agarose gel-electrophoresis, qualitative PCR, quantitative real-time PCR and a protein-based approach for GMO detection.

Further reading:

- Information on DVD course
- Fact sheet

5.2 GMO Methods Database

The adoption of appropriate and cost-effective screening strategies for the analysis of GMOs in the food and feed chain requires access to reliable, comprehensive, up-to-date genetic and regulatory information on the GMOs approved worldwide, as well as to information on standards for their corresponding detection, identification and quantification.

Therefore, one of the central tasks of the B&GMO Unit of IHCP is the provision of a central database containing suitable detection methods for GMOs and general information on each specific GMO. To date, more than 400 PCR- or ELISA-based methods have been entered in the comprehensive database comprised of more than 50 GMO events.

The data originate from publications in peer-reviewed journals and from reports on collaborative studies, such as ring trials of the ENGL. Additionally, the database is comprised of information from the CODEX database that also may have been reported by non-EU member countries. The database provides general information on the GMO and detailed technical information on the method and method performance of the corresponding validation trial, as well as comments thereto.

When available, specific information on the performance of the method and its validation status are given. The database also indicates the international standardisation organizations that have received or approved a certain method.

The database supports competent authorities and controlling bodies in EU Member States and beyond in complying with the new EU regulation on traceability and labelling of GMOs and traceability of food and feed produced from GMOs (Regulation (EC) 1830/2003).

In support of the EC Regulation 1830/2003, the database has been included as a general reference resource for methods of GMO analysis in the Commission Recommendation on technical guidance for sampling and detection of GMOs.

If no validated method is available for a specific food or feed sample under analysis, the regulation directs the selection from the database of a method that has been validated in regard to a similar matrix or raw material.

The database currently is being expanded with administrative, genetic and sequence information on worldwide-approved GMO crops and the corresponding plasmid standards.

5.3 Reports on DNA-based and on protein-based GMO detection methods submitted to Ring-Trial

A summary of PCR-based GMO detection methods also is provided. The data has been extracted from the GMOs Methods database published on the Biotechnology and GMOs Unit website (http://biotech.jrc.it/methodsdatabase.htm). It includes general information on the GMO in question and on the corresponding method. It also includes detailed technical information on the assay, on validation data providing the reference of the published article or validation report, on the description of the collaborative study and on its validation and standard status definition. The section indicates whether the assay has been submitted and accepted by standardisation bodies, such as the European Committee for Standardization (CEN, Brussels, Belgium), CODEX and the International Organization for Standardization (ISO, Geneva, Switzerland).

5.4 Harmonising GMO detection internationally: 1st Global Conference on GMO-Analysis 2008

This forthcoming conference, an initiative of the EC DG Joint Research Centre and of the European ENGL, may present a major step in the dissemination and the harmonisation of GMO detection approaches on an international level.

The growing worldwide production of GM crops and derived food and feed has led to increased challenges for producers and traders throughout the various supply chains. In order to secure identity

preservation of GM and non-GM commodities according to specific market demands, further scientific and technical progress must be made to enable the successful functioning of global marketing. The conference will address a broad range of topics related to a functional and internationally harmonised GMO control and analysis system. The conference will bring together international experts to promote scientific dialogue across interdependent areas such as the following:

- Existing challenges of sampling for GMO analysis
- Analytical tools and applied procedures along the commodity production chains
- Consistency of test results, result interpretation and reporting
- Harmonisation standards for the detection of genetically modified traits

This conference is aimed at all stakeholders involved in GMO control and analysis, including industry representatives, regulators and others.

Conference details:

1st Global Conference on GMO-Analysis

Villa Erba, Como, Italy (24-27 June 2008) Website

6 Sampling strategies

The need for proper sampling procedures for reliable analysis

GMO detection aims to gain information on the composition of a large body of target material. Since only a small portion of sample material is subject to the analytical procedure, reliable results are guaranteed solely by appropriate sampling strategies.

A fundamental problem is presented by differing distribution of potential GMO components in the tested material. In seed lots, for example, a homogenous distribution may be assumed and available standard sampling strategies therefore are applicable. However, in cases involving bulk commodities or grain lots, a heterogeneous distribution of GM material must be expected. Appropriate new sampling guidelines must be devised.

Among all currently used sampling guidelines for GMO testing, only one (Recommendation (EC) 787/2004) was specifically developed for GMO surveys. It is free of assumptions regarding distribution and therefore is applicable even in cases of heterogeneity.

Nonetheless, in respect to GMO legislative requirements in the EU, the extent of knowledge on distribution patterns in kernel lots is a pre-requisite to the development and recommendation of suitable sampling plans. The B&GMO Unit/IHCP and ENGL have launched several projects that support the understanding of distribution patterns in kernel lots. Their final goal is the availability of reliable statistical tools that accurately can predict sampling errors. In this manner, such tools support the design of appropriate and harmonised sample strategies for different commodities in all EU member states.

Further reading:

- Sampling - Theoretical Work (B&GMOs Unit / JRC)

6.1 KeLDA (Kernel Lot Distribution Assessment)

KeLDA was an ENGL collaborative research project coordinated by the B&GMOs Unit (JRC). KeLDA represents the first case study to assess the real distribution of GM materials in soybean grain lots. Its results indicated that GM material distribution in soybean lots is heterogeneous, which highlights the need to develop sampling protocols based on statistical models that contain no assumptions on the GMOs that potentially are present.

Further reading:

- Kernel Lot Distribution Assessment (KeLDA): a study on the distribution of GMO in large soybean shipments. Claudia Paoletti et al.
- European Union Perspective – Sampling for Testing of Genetically Modified Impurities. C. Paoletti, M. Donatelli, A. Heissenberger, E. Grazioli, S. Larcher, and G. Van den Eede (2005)
- Sampling strategies for GMO detection and/or quantification. S. Kay and C. Paoletti (2002)

6.2 Supporting software tools

6.2.1 KeSTE (Kernel Sampling Technique Evaluation) - Evaluating sampling strategies as function of lot properties

In order to support industry, GMO control laboratories, and other parties dealing with sampling and GMO detection, software tools for the design of robust sampling strategies already have been developed or are under development.

A new approach has been designed towards investigating of the effects of heterogeneity on the accuracy of different sampling plans for the detection of GM contamination within kernel lots. The proposed model allows the simulation of large kernel lots without imposing constraints on the distribution of GM material.

Using this model, the software tool KeSTE supports the evaluation of the reliability of different sampling plans as a function of certain properties of raw materials. On a case-by-case basis and according to the defined needs of its user, the tool provides reliable estimates of the sampling error associated with sampling plans.

The program was developed using MS Excel, which is needed to run the program.

Further reading and resources:

- KeSTE (Kernel Sampling Technique Evaluation): software download
- KeSTE Manual

6.2.2 CoDE (Contaminant Distribution Estimate)

Quantifying the sampling error associated to different sampling protocols

CoDE is a new software tool (under development) for the determination of sampling errors of sampling protocols. CoDE will use a new statistical model* to estimate the sampling error as a function of both the number and the size of samples taken from any kind of consignment. The novelty of the model is its complete freedom from distribution constraints.

CoDE is aimed at serving as a general supporting tool for the harmonisation of sampling approaches. CoDE will be developed for a Windows 2k-XP operation system.

Further reading:

- *GMO analysis in large kernel lots: modelling sampling of non-randomly distributed contaminants. C. Paoletti, M. Donatelli, E. Grazioli and G. van den Eede
- Comparison of sampling approaches for grain lots. S. Kay (2001)
- Sampling strategies for GMO Detection and/or Quantification. S. Kay and C. Paoletti (2001)

6.2.3 SISSI (Shortcut In Sample Size Identification).

SISSI (Shortcut In Sample Size Identification) is a software tool for the estimation of optimum sample size. The tool was developed by the Institute for the Protection and Security of the Citizen, Agriculture and Fisheries Unit (JRC).

The approach is based on taking sub-samples of the original data set and calculating mean and standard deviation for each of the sub-samples. This approach overcomes the typical limitations of conventional methods that require data-matching statistical assumptions.

Easy-to-interpret variations of means and standard deviations visually are given against the size of generated samples. Targeted at the size for which the rate of change of means becomes negligible, an automatic option for the identification of optimal sample size is delivered. An ideal application of SISSI is in supporting the sampling of plant material from field-grown crops.

SISSI is developed in Visual Basic and runs under the Windows operating systems. SISSI is available free of cost for non-profit applications.

Further reading and resources:

- Resampling-based software for estimating optimal sample size
- Software download

6.3 International Seed testing association (ISTA)

ISTA represents seed companies around the world and already has established seed testing schemes that also will be applicable to the GMO analysis. It is founded on a Performance Based Approach, under which laboratories are free to choose the methods they use. Minimum requirements for the performance of laboratories carrying out such tests are detailed in the ISTA International Rules for Seed Testing.

Further reading:

- Information Platform for GM Seed (ISTA)

7 EU experience with GMO detection techniques

Due to legislative requirements and a restrictive threshold for labelling, a tight control system for GMO detection and traceability has been well-established in the EU for several years. It benefits from the extensive research activities within the ENGL, from EU-funded traceability projects and from the anticipatory research and validation work of the CRL-GMFF and the B&GMOs Unit of JRC. Using validated methods und adjusted sampling strategies for different kinds of commodities, the established control system is able to enforce current legal tasks. However, in order to secure and to improve the practice of GMO control, EU inspection of responsible national authorities and enforcement laboratories regularly are conducted (see 7.1).

The control system is challenged by the occasional import of unauthorised GMOs. Therefore, the Commission has mandated the CRL-GMFF to coordinate emergency measures to exclude illegal imports from the EU market. This is to be executed through the rapid validation of appropriate detection procedures and the provision of control samples for unauthorised GMOs (see 7.2).

Nevertheless, the incidence of emergency cases, ongoing discussions within expert networks and the results of national inspections reveal remaining bottlenecks and gaps in the practice of GMO control (see 7.3). For this reason, research activities continue to be aimed at the improvement and harmonisation of current control systems (see chapter 8).

7.1 Inspections on GMO controls

The European Commission is responsible for ensuring that Community legislation on GMOs and derived food and feed is implemented and enforced properly. As a Commission service, the Food and Veterinary Office (FVO) plays an important role in fulfilling this task. The Office works to assure effective control systems on national levels and to evaluate the compliance with EU standards for food and feed that contain, consist of or are produced from genetically modified organisms.

In regard to GMO, the FVO evaluates, among others, the following:

- the supervision performed by the competent authority (CA) to ensure that market placement of genetically modified (GM) food and feed complies with Regulation 1829/2003 (EC) of the European Parliament and the Council.
- the application of Regulation 1830/2003 (EC) of the European Parliament and the Council, which concerns the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms;

For this purpose, mission teams of the FVO were sent on 21 visits in 18 different countries between 2001 and 2007 (for more information see Appendix IV). Addressing the control of food, feed and seed material, the objective of the missions was obtain insight into national practices of surveillance, sampling and GMO detection, as well as to identify problems or the need for improvement in current practices. Two additional FVO missions were conducted to Argentina and Brazil, countries outside Europe that are major exporters of GM agricultural products such as GM soy and GM maize.

On the missions to EU Member States, the FVO team met with the Competent Authorities of the respective country as well as with local authorities and their staff responsible for the implementation for GMO surveillance. The mission team received documents on past surveillance activities and their results, accompanied inspectors during inspections of local import or production facilities and visited GMO analysis laboratories.

Among others, the following aspects were addressed during the missions:

- national legislation on GMO and the adequate implementation of EU legislation
- the structure and organisation of responsible authorities
- the training of inspectors
- the nature and effectiveness of communication among central/federal, regional and local authorities
- inspection plans and adherence thereto
- the number of inspections
- sampling procedures – sampling frequency, sample sizes – and adherence to sampling provisions established in Commission Recommendation 2004/787/EC
- the nature of controls, e.g., control of adherence to labelling and traceability obligations (by random or systematic sampling and testing and/or document checks), and checking for unapproved GMO by sampling and testing
- the nature of companies and facilities involved, e.g., port facilities, food and feed production factories, oil mills, plant breeders and seed production facilities
- the qualification of staff members, including inspectors and laboratory staff
- the accreditation of laboratories (under ISO 17025)
- the use of validated detection methods, as well as activities regarding the development and evaluation of new methods, e.g., for the screening of unapproved GMOs
- the position of laboratories according to existing standards (ISO, CEN) and the membership of laboratories in the European Network of GMO laboratories (ENGL)
- technical equipment of laboratories
- the results of official controls, e.g., the number of GMO positive testing results and detected infringements of EU rules
- the reporting of results

Subsequently to the FVO missions, summary reports are written that, if necessary, include recommendations to the respective national authorities on the improvement of their control system and its alignment with EU requirements. Country authorities are invited to return commentary, and the mission reports as well as the comments thereto are published on the website of the EU Commission (see Appendix IV).

General outcomes

In most cases, FVO inspectors concluded that Member States have installed appropriate structures and competent staff to undertake GMO controls. However, differences exist. For example, inspectors in some Member States do not follow standardized sampling procedures. In some countries, no central sampling and control plans exist, nor has a National Reference Laboratory been designated. Some authorities were advised to extend their controls to address all EU-approved GMO as well as unapproved GMO that illegally may enter the European market. In the opinion of the inspectors, the point of entry for food and feed imports from third countries deserve more attention in several Member States. In some cases, the post-processing of GMO detection below the labelling threshold of 0.9 per cent was found to be insufficient, since it is not considered if such traces are adventitious or technically unavoidable. Furthermore, the prosecution of infringements was not found to be sufficient in all countries, as deficiencies were noted in the quantification of GMO in food and feed samples. In

singular cases, the amount of inspections and analyses of samples was insufficient and was seen to be due to limited financial and/or human resources.

7.2 Handling 'emergency issues'

Exclusion from the regional market of imports of illegal and possibly unknown GMO products.

In regard to genetically modified organisms, there have been two cases in which emergency measures* were undertaken by European authorities to prevent the potential import of unauthorised GMO to the European market. They followed the appearance of unapproved GM strains known as maize Bt10 and rice LL601. Both incidents, and the reactions of European authorities, may serve as case studies for the effectiveness of the emergency system.

CRL-GMFF supports EU policy with assistance in cases of emergency measures, e.g. rapid validation of detection procedures and provision of control samples for unauthorised GMOs on the EU market.

*** Legal Basis for emergency measures**

Regulation (EC) No 178/2002 establishes general principles and requirements of food law, as well as procedures in matters of food safety. As such, it provides the basis for the activities of reference laboratories and all other institutions that take part in the enforcement of food law.

According to recital 10, "it is necessary to adopt measures aimed at guaranteeing that unsafe food is not placed on the market and ensuring that systems exist to identify and respond to food safety problems in order to ensure the proper functioning of the internal market and to protect human health. Similar issues relating to feed safety should be addressed."

To that purpose, **article 7** establishes the **Precautionary Principle**, according to which "in specific circumstances, where …. the possibility of harmful effects on health is identified but scientific uncertainty persists, provisional risk management measures … may be adopted".

The entire Chapter IV of the regulation (Articles 50 to 57) provides the basis for setting up an improved and broadened **Rapid Alert System**, including measures for Crisis Management and Emergencies. For the notification of direct or indirect health risks deriving from food or feed, the Rapid Alert System for Food and Feed Safety (RASSF) was established as a network involving the Member States, the Commission and the EFSA. It is managed by the Commission.

Article 53 establishes that "where it is evident that food or feed originating from the Community or imported from a third country is likely to constitute a serious risk to human health, animal health or the environment," the Commission shall immediately adopt certain **emergency measures** such as the suspension of food or feed imports or laying down special conditions for import of the food or feed in question.

The Commission may adopt such emergency measures provisionally after consulting the Member States concerned and informing the other Member States. At most within 10 working days, the measures taken shall be confirmed, amended, revoked or extended and the reasons for the decision of the Commission decision shall be made public without delay.

7.2.1 LL RICE 601

On August 18, the US Department of Agriculture informed the European Commission that traces of the unauthorised, genetically modified rice strain LL RICE 601 developed by the predecessor company of Bayer CropScience (BCS) had been found in commercial rice samples in the US. They

had entered marketing and export channels for long grain rice. The Commission immediately adopted an emergency decision on August 23 to ensure that no unauthorised rice entered the European market (Commission Decision 2006/578/EC). To that purpose, shipments with long grain rice would be permitted for import into the EU only if they had been certified by an accredited laboratory to be free of LL601. In addition, to verify the absence of LL RICE 601 in rice products already on the market, appropriate control measures such as random sampling and analysis were to be undertaken at national levels. These provisional emergency measures were confirmed by the Standing Committee for Food Chain and Animal Health (SCFAH) two days later and resulted in the Commission Decision 2006/601/EC, which replaced the first Decision. A list is available of thirteen types of rice products within the scope of the measures.

Detection methods

At the time of enactment of the provisional emergency measures, Bayer CropScience made available two methods for detection of genetically modified rice LL RICE 601. These methods previously had been validated by the US Grain Inspection Administration (GIPSA) in collaboration with the Community reference laboratory. On August 31, only 8 days after being provided with the two PCR-based detection methods, the JRC announced that it had validated the methods. The validation reports were published on the JRC website on the first day of September. Control samples were distributed to the members of the European Network of GMO Laboratories.

As a representative of the Joint Research Centre (JRC) clarified to the Standing Committee on the Food Chain and Animal Health (SCFAH) on September 11, one of the two methods is a **construct specific** (35 S-bar) method, suitable for screening, while the second one is **event specific** for LL601. While useful for pre-screening, the construct-specific 35 S-bar method may give rise to false positive results for other (authorised) LL constructs such as Bt-176 maize or LL rapeseed. This particularly may occur in mixed foods. In order to exclude such false positives, the JRC recommended the combination of the construct and the event specific methods. At the same time, the JRC advised against the use of the less specific 35S generic method, due to its reaction to the presence of a variety of GMOs.

Sampling was directed to be undertaken in accordance with the relevant Recommendation 2004/787/EC. This document establishes, among other specifications, sampling sizes in relation to the size of lots to be tested. The size of laboratory samples was set at 2.5 kg.

The 35 S-bar method also detects two other unapproved rice strains (LL RICE 604 and LL RICE 62) which, subsequently, also were detected in European rice samples.

Implementation

In regard to the implementation of the emergency measures, customs authorities, entry-point administrators and operators rapidly were informed of the Commission Decision. They ensure that the long grain rice products in question are accompanied by certificates proving them to be free of LL RICE 601. However, according to protocols validated by the JRC, the actual testing in Member States began with possible delays of up to two weeks due to the necessity of equipping laboratories specifically for this type of analysis . Since some Member States during this time already had begun testing on the basis of the non-specific 35S method, the possibility of ‘false-positive’ results must be considered.

In response to findings of LLRICE 601 in shipments of US long grain rice despite the certification of this rice as free from unauthorised GMO, the Commission amended its original Decision. Thereafter, strict counter testing of all US long grain rice imports was enacted instead of only the probing and analysis of random samples (Commission Decision 2006/754/EC of 6 November 2006).

Review

On 16 January 2007, the Standing Committee on the Food Chain and Animal Health was informed that, since the disclosure of unauthorised LL RICE 601 in US imports, Member States had taken more than 1500 official samples upon import as well as from products that already were on the market. Initially, a significant number of positive results was reported. Affected lots were withdrawn from the market. However, since the enactment of Decision 2006/754/EC, which imposes mandatory counter-testing of every imported lot of long-grain rice, imports and the resulting incidence of positive test results virtually have ceased.

Further reading and resources:

CRL-GMFF webpages and documents concerning LL601 Rice

- CRL-GMFF: LLRICE601 updates (validated detection methods)
- Report on the verification of an event-specific detection method for identification of rice GM-event LLRICE601 using a real-time PCR assay (CRL)
- Addendum to the Report on the Verification of an event-specific Detection Method for Identification of Rice GM-event LLRICE601 Using a Real-time PCR Assay (CRL)

Rapid Alert System for Food and Feed (RASFF)

- RASFF Annual Report 2006; Health and Consumer Protection Directorate-General of the European Commission

Summary records of the Standing Committee on the Food Chain and Animal Health (SCFCAH)

- Summary Record SCFCAH, 25 August 2006
- Summary Record SCFCAH, 11 September 2006
- Summary Record SCFCAH, 23 October 2006
- Summary Record SCFCAH, 16 January 2007
- Summary Record SCFCAH, 2 March 2007
- Summary Record SCFCAH, 20 March 2007
- Summary Record SCFCAH, 11 May 2007

7.2.2 Bt10 maize

On March 22, the European Commission was informed by the US mission to the European Union that, in the United States from 2001 to 2004, the Syngenta company inadvertently had marketed the genetically modified maize known as Bt10. In Europe and the USA, approval for Bt10 maize existed neither for planting nor for food and feed. However, maize seeds containing Bt10 may have been planted on approximately 37,000 hectares and approximately 1,000 tons of feed products containing

Bt10 traces may have entered the European feed chain. On April 18, the Commission adopted the Decision 2005/317/EC to implement emergency measures, requiring all import shipments from the USA with corn gluten feed or brewers' grain to be certified as free of Bt10 maize.

Detection method

On April 22, the JRC published the validation report for an event specific detection method for Bt10 maize. Originally proposed by Syngenta for the testing of imports, the method then was validated by Genescan and, subsequently to in-house laboratory testing, was certified by CRL-GMFF (JRC) to become the official EU method for the detection of Bt10. The validation study assessed crucial performance characteristics of the method, including molecular specificity, limit of detection and repeatability of measurements. In October 2006, the Standing Committee on the Food Chain and Animal Health was informed that in previous months Syngenta and the Community Reference Laboratory had performed further analysis on the molecular structure of Bt10. The status of the knowledge at that time led to the conclusion that the validated, event-specific method was appropriate for the implementation of the emergency measures regarding Bt10.

Review of implementation of measures

On 24 May 2005, a shipment contaminated with Bt10 arrived in Ireland. The positive test result became available during the first weeks of implementation of the emergency measure while the vessel already was en route to the EU. This posed no infringement of the Decision and allowed Irish authorities to take measures in order to prevent the placement of the product on the market.

According to a note provided to the members of the Standing Committee on the Food Chain and Animal Health on 27th October 2005, approximately 1400 analytical tests were conducted between April and the end of September 2005 on corn gluten feed in the USA. The tests served to demonstrate the absence of Bt10 in products intended for export to the EU. Approximately the same number of tests was reported from EU Member States and largely was performed on food and feed products that already were on the market. The presence of Bt10 maize was indicated by none of these tests and this information was considered to prove the effectiveness of the emergency measures.

On 16th January 2007, the Commission and Member States voted in favour of lifting the emergency inspection measures for corn gluten feed and brewers' grain. The respective Commission Decision became effective on March 7, 2007.

The cases of Bt10 and LL RICE 601 provide clear examples of the contribution of the outstanding scientific capacity of the JRC in response to an unforeseen emergency policy situation.

Further reading and resources:

CRL-GMFF webpages and documents concerning Bt10 maize

- CRL-GMFF: Bt10 updates (validated detection methods)
- Scientific Report on a PCR assay for detection of maize transgenic event BT10 - Version 1 (22/04/2005)
- Scientific Report on a PCR assay for detection of maize transgenic event BT10 - Version 2 (13/07/2005)
- Scientific Report on a Detection Method for Event Bt10 using a qualitative PCR assay - Protocol for verification of positive results by restriction analysis (23/06/2005)
- Scientific Report on the in-house Validation of a detection method for event BT10 maize using a qualitative PCR assay - Version 1 (22/04/2005)
- Scientific Report on the in-house Validation of a detection method for event BT10 maize using a qualitative PCR assay - Version 2 (13/07/2005)
- Summary report on scientific data obtained at the JRC-GMO-CRL and an analysis of the data on Bt-10, obtained by Syngenta (1/12/2006)

Summary records of the Standing Committee on the Food Chain and Animal Health (SCFCAH)

- Summary Record SCFCAH, 27 October 2005
- Summary Record SCFCAH, 3 March 2006
- Summary Record SCFCAH, 23 October 2006
- Summary Record SCFCAH, 16 January 2007

7.3 The bottlenecks of GMO detection and its harmonisation

On the international level, problems in GMO control mainly are caused by the lack of harmonisation of GMO detection and by the lack of synchronicity between different countries and regions in regard to GMO approval processes. A selection of current problems of GMO detection and control is outlined below:

- According to Regulation 1829/2003 (EC), authorised GMOs must possess a corresponding and validated detection method. The detection of unauthorised or unknown GMOs is made difficult by the lack of molecular knowledge of their genetic contents. However, precisely this lack of knowledge and the illegality of such unapproved or unknown GMOs on all levels in Europe (and in most other countries) necessitate their detection.
- Different interpretations of analytical results are to be expected due to different testing regimes in different countries. For example, the use of different units of measurements (e.g., mass percentage and DNA copy number) can cause inequality of test results. The decision by the EC to recommend the use of DNA ratios to express GMO quantity was an important

step towards coherence with the legal requirements. ENGL has confirmed this approach in a recent explanatory document stating that DNA haploid genome copy number ratios are the only universally applicable unit to measure and express contamination levels. However, this unit has not been fully implemented to date within the European Union and beyond.

- Reference material is not available for all GMO events on the global market. The matrix Certified Reference Materials (CRMs) have demonstrated high stability and are available in appropriate concentration levels for the monitoring of the European legislation. However, the "classical" plant-derived CRMs may display such disadvantages as expense and limited availability for specific ranges of concentration. They also may contain traces of other GMOs and the applicability of these CRMs for highly processed food and feed samples must be evaluated case by case.

- Challenges to GMO detection also include the detection of transgenic material in crops with varying chromosome numbers and in crops with a large genome (e.g., wheat). This restricts the minimum quantity of GM DNA that can be analysed, due to the limitations of DNA quantities in PCR analysis.

- There is an inconsistent legal status of products containing 'trace botanical impurities' derived from GMO. Regulation 1829/2003 determines a threshold of 0.9% for adventitious presence of material derived from GMOs.

  (a) When a cargo contains mixed GMOs (for example, maize and soybean), the 0.9% threshold applies for each species. In this case, the maize may contain 0.3% authorised GMO and the soybean 1.2% authorised GMO. Such cargo thus would need labelling concerning the GM soybeans.

  (b) The term 'trace botanical impurities' refers to cases in which, for example, maize commodities (GMO content below 0.9 %) are mixed with traces (0.01 %) of pure GMO soybeans. According to Regulation 1829/2003, such a shipment is defined as 100% GMO and must be labelled accordingly.

- An increasingly immanent situation is the occurrence of more than one transformation event in the same plant ('stacked' events). Unless a specific marker is introduced into the hybrid plant, the determination of whether a sample solely contains the hybrid itself or a mixture of two different GM plants is almost impossible when conducted on material other than seeds or grains. The current available detection methods do not solve the problem of stacked genes and the only available approach in such cases at the moment is the analysis of single grains.

- Generally, a considerable need exists for rapid and economic detection methods, which would not only benefit the EU control system but also particularly would enable developing countries to establish effective GMO control measures.

The development of new, improved and innovative detection methods is a goal of the B&GMOs Unit, the ENGL and several EC-funded research and development programmes on GMO traceability and detection. These programmes also have produced concepts that are more efficient and more economical.

The next chapter provides a selection of related research topics that address the challenges of GMO detection mentioned above.

Further reading:

- Challenges to Achieve a Coherent GMO Legislation (DG Environment)
- Explanatory Document on the use of “percentage of GM-DNA copy numbers in relation to target taxon specific DNA copy numbers calculated in terms of haploid genomes” as a general unit to express the percentage of GMOs (ENGL, 2007)
- Coherence Between Legal Requirements and Approaches for Detection of Genetically Modified Organisms (GMOs) and their Derived Products. A. Holst-Jensen, M. De Loose and G. Van den Eede (2006)
- Control of GMO Content in Seed and Feed - possibilities and limitations. Nordic Council of Ministers, Copenhagen 2004

8 The next generation of detection methods

The B&GMOs Unit (JRC) and several EU-funded projects address a range of topics that are aimed at improving the current system of GMO control and at filling the encountered gaps by contributing new approaches and techniques for GMO detection.

As part of its research and development programmes FP5 and FP6, European Commission has financed several research and development projects for the detection of genetically modified organisms (GMOs). These include:

QPCRGMOFOOD: Reliable, standardised, specific, quantitative detection of genetically modified foods. Coordinator: Dr. Arne Holst-Jensen

DNA-TRACK: Traceability of DNA fragments throughout the food chain by DNA/PNA technologies. Application to Novel Foods. Coordinator: Nelson Marmiroli.

GMOCHIPS: Development of biochips to detect Genetically Modified Organisms (GMOs) in food. Coordinators: J. Remacle and Y. Bertheau.

Co-Extra: GM and non-GM supply chains: their CO-EXistence and TRAceability. Coordinator: Y. Bertheau.

ENTRANSFOOD: European network safety assessment of genetically modified food crops.

Most of the members of these EC GMO-traceability programmes, as well as the national competent authorities of all member states, collaborate as part of the European Network of GMO Laboratories (ENGL), which is under the chairmanship of EC Joint Research Centre's Institute for Health and Consumer Protection (IHCP).

A selection of research activities of the JRC and ENGL, as well as EU-funded research projects, is given below.

8.2 GMO testing: the modular approach

Reduction of costs, enhanced efficiency and simplification of validation

Traditionally, a complete detection method has been validated for only one particular GMO (and food/feed matrix) at a time. However, since several sub-tasks of GMO detection may be applied to a variety of GMOs, the idea of a modular approach to GMO testing is of interest. In such an approach, each procedure (for example, PCR reaction or DNA extraction) may be validated independently and used subsequently in a variety of detection tests. Presently, this modular approach is being standardised and adopted by the collaborative testing laboratories of the JRC and ENGL. In addition to the reduction of costs, its advantages include enhanced efficiency and the simplification of the task of validating tests for many different GMOs using different matrices. Such an approach also may be considered for validation in other areas of detection addressing such issues as food pathogens, mycotoxins and allergenic organisms.

Further reading:

- Co-Extra: Improving PCR based detection methods
- The Modular Analytical Procedure and Validation Approach and the Units of Measurement for Genetically Modified Materials in Foods and Feeds. A. Holst-Jensen, K. G. Berdal (2004)
- Critical points of DNA quantification by real-time PCR – effects of DNA extraction method and sample matrix on quantification of genetically modified organisms. K. Cankar, D. Štebih1, T. Dreo, J. Žel and K. Gruden (2006)

8.3 GMO screening: the application of DNA chips

Improving laboratory economy and testing for a large numbers of target gene sequences in one step

A new method of multiplex screening (called Dual Chip®) has been developed by the Co-Extra project and the preceding GMO Chips project. This method will be commercialized by Eppendorf Array Technologies (Belgium). The aim of the project was to provide methods with which national regulatory laboratories may infer the identity of a GMO that is likely to be found in a given sample. Necessitating a rapid screening method, such inferences allow great economy of time and effort. Multiple specific DNA capture probes, corresponding either to GMO elements, to species-specific targets or to control targets are mounted on glass slides. Through a colorimetric reaction of DNA hybridization and subsequent statistical analysis, various DNA elements present in a sample are detected and indicate the identity of the GMO. Target DNA can be detected to a level of 0.1% and the suitability of the method recently was validated by a collaborative ring trial organised by the EC's JRC. In addition to improving laboratory economy, the method is able to accommodate large numbers of target gene sequences. This trait is of particular interest, due to the expected increase in approved and non-approved GMO traits and new GMO crop species. The micro-array method is one of the applications of the "matrix approach" (see 8.5). The use of sub-sampling strategies also may allow the use of qualitative methods such as micro-arrays to determine if the GMO content of a sample exceeds a labelling threshold.

Further reading and references:

- Validation Report: Microarray Method for the Screening of EU Approved GMOs by Identification of their Genetic Elements (CRL-GMFF)
- Biochips: A powerful tool for multiple and fast analysis of genes and DNA sequences
- Project summary of GMOchips (EU-funded project)
- DNA-Track (EU-funded project)

8.4 The economics of GMO traceability and detection

Development of methods enabling cost reduction

The financial resources of many European and non-EU countries often are strained by the computer-controlled machines, expensive reagents and highly trained personnel necessary for the quantitative testing of GMOs. Such burdens often preclude the participation of developing countries in testing. Activities of the B&GMOs Unit of the JRC-IHCP and the Co-Extra programme are aimed towards the development of methods of cost reduction by using new methods and different apparatus as well as alternative chemistries and screening methods. Statistical methods also are used to reduce the uncertainty involved in measurement. The hidden costs and advantages of GMO traceability are being determined by a cost-benefit analysis. Possible applications also are being considered for the same techniques in other fields of detection, such as those addressing pathogens and allergens. Within the Co-Extra programme, the positive impact of general requirements of traceability currently are being evaluated with regard to GMO traceability economics and to other areas of mandatory traceability and labelling.

Further reading:

- Development and evaluation of methods for GMO analysis (B&GMOs Unit, JRC)
- Improving PCR based detection methods (EU-funded project Co-Extra)

8.5 The detection of unknown GMOs in the supply chain

Accelerating emergency measures against illegal GMOs on the market

Two concepts currently under development in the Co-Extra programme are the ‘differential PCR’ and the ‘matrix’ approaches. Both are aimed at the determination of the probable presence of unknown GMOs. Differential PCR quantitatively induces the ratios of different genetic elements in sample DNA which then are compared with expected ratios for known GMOs. The presence of an unknown GMO is indicated if the statistical result differs from zero. The matrix approach tests simultaneously for the presence of a large number of possible DNA fragments and compares the resulting combinations to a database of known GMOs. A possible unknown GMO is indicated by the presence of unusual combinations of DNA targets (see above micro-array application of the “matrix approach”). This approach may be extended by the inclusion of a screening microarray that permits the detection of several thousand genetic elements. Such elements may include those that have not yet been used in an authorised or registered GMO. Application of this screening microarray also may facilitate further characterisation of the GMO in order to enable immediate preliminary risk assessment of the GMO.

Further reading:

- What is the future of GMO detection? A freely speaking scientist 's opinion (EU-funded project Co-Extra)
- Design of a DNA chip for detection of unknown genetically modified organisms (GMOs). H. Nesvold, A. Kristoffersen, A. Holst-Jensen and K. G. Berdal (2005)

8.6 Novel approach for direct target quantification

Reducing the error rate of currently used PCR methods

The B&GMOs Unit is seeking innovative DNA detection methods to reduce the error rate of currently used PCR methods. Today, quantification approaches imply an amplification of specific GMO DNA sequences by PCR. This leads to indirect GMO quantification and introduces uncertainty due to the intrinsic PCR error. The B&GMOs Unit currently is evaluating the suitability of a novel direct DNA labelling system for detecting and quantifying DNA targets without the need of the amplification step. The Unit also is studying the reliability of the system for direct target measurements.

Further reading:

Development and evaluation of methods for GMO analysis (B&GMOs Unit, JRC)

8.7 Development of cloned DNA control samples

Overcoming disadvantages of plant derived Certified Reference Materials (CRMs)

Positive and negative control samples and reference material of GMOs for PCR-based detection methods are a legal prerequisite for the authorization process (EC Regulation 1829/2003) and for compliance with the enforced threshold for labelling in the EU. Proper quantitative controls are crucial for the calibration of instruments and for quantitative detection approaches.

Plant-derived Certified Reference Materials (CRMs) may display certain disadvantages, such as being expensive and available only for limited ranges of concentration. Alternative CRMs may be less expensive, available in all concentration ranges (0-100%) and easier to control in regard to purity. Furthermore, GMOs may continue to contaminate samples for many years after their cultivation has ceased and, while plant-derived CRMs may be unavailable after the termination of cultivation, alternative CRMs may be made available for an unlimited period of time.

The B&GMOs Unit and ENGL currently are advising the EC on alternatives to plant-derived CRM. Plasmids have been demonstrated to represent a cheap and reliable alternative to such CRMs. Cloned GMO-DNA as positive and negative control samples and reference material will be defined for the detection and quantification of GM products and for distribution to ENGL and to testing laboratories. A first set of plasmids bearing the DNA sequence targeted by validated method for the event-specific quantification is ready for market placement by the IRMM. The material can be used primarily for calibration purposes.

Further reading:

- Development and evaluation of methods for GMO analysis (B&GMOs Unit, JRC)
- Development and application of a novel class of real-time PCR standards based on cloned sequences for GMO quantification (JRC)
- Development and applications of real-time PCR standards for GMO quantification based on tandem-marker plasmids. E. Mattarucchi, F. Weighardt, C. Barbati, M. Querci, and G. Van den Eede (2005)
- Event-specific plasmid standards and real-time PCR methods for transgenic Bt11, Bt176 and GA21 maize and transgenic GT73 canola. I. Taverniers, P. Windels, M. Vaitilingom, A. Milcamps, E. Van Bockstaele, G. Van den Eede, and M. de Loose (2005)
- A Real-Time PCR Based Approach for the Quantification of the pat Gene in the T25 Zea mays Event. F. Weighardt, C. Barbati, C. Paoletti, M. Querci, S. Kay, M. De Beuckeleer, and G. Van den Eede (2004)
- Development and applications of real-time PCR standards for GMO quantification based on tandem-marker plasmids. E. Mattarucchi, F. Weighardt, C. Barbati, M. Querci, and G. Van den Eede (2005)

8.8 ELISA Reverse method and device (ELISA-R m&d)

For a simultaneous screening of a large number of samples

In collaboration with Italian universities and institutions, the B&GMO Unit of IRC has developed innovative ELISA test systems for the detection and quantification of GMO containing the endotoxin Cry1Ab present in MON810 and Bt11 genetically modified (GM) maize lines and containing CP4EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) present in soy. Its application is proposed in cases in which a large number of samples must be screened simultaneously or when the simultaneous detection of different proteins is required. The last method mentioned currently is the only internationally accepted protein-based detection method and is part of ISO standard 21572. For example, a protocol to quantify Cry1Ab protein in GM maize lines (MON810 and Bt11) with a limit of detection of 0.0056% (w/w) and a limit of quantification of 0.0168% (w/w) has been developed.

Further reading:

- Development and evaluation of methods for GMO analysis (B&GMOs Unit, JRC)
- Application of the ELISA Reverse Method and device to quantify CP4EPSPS protein in GM RUR Soya. M. Ermolli, A. Prospero, B. Balla, M. Querci, A. Mazzeo, and G. Van den Eede (2006)
- ELISA Reverse m&d for Multiplex Detection and Quantification of Target Proteins in Food Analyses. A. Prospero (2006)

8.9 High-throughput immunoassay

The first application of a quantitative and protein-based high-throughput system

A covalent microsphere immunoassay using fluorescent beads coupled to a specific antibody was developed for the quantification of the endotoxin Cry1Ab present in the GM maize lines known as MON810 and Bt11. The limits of detection and quantification equal 0.018% and 0.054% (w/w) respectively. The present study describes the first application of quantitative high-throughput immunoassays in GMO analysis.

Further reading:

- First Application of a Micro-sphere Based Immunoassay to GMO Detection: Quantification of Cry1Ab Protein in GM Maize. A. Fantozzi, M. Ermolli, M. Marini, D. Scotti, B. Balla, M. Querci, S. Langrell, G. Van den Eede (2007)
- Development and evaluation of methods for GMO analysis (JRC)

Annex I - Internet Guide to European bodies and research projects related to detection of GMOs in the supply chain (selection)

Higher-level EU institutions

- European Commission - Joint Research Centre (JRC)
- European Food Safety Authority (EFSA)
- The Institute for Reference Materials and Measurements (IRMM)
- European Committee for Standardization (CEN)

Institution and networks for the development, validation and harmonisation of GMO detection methods

- Biotechnology and GMOs Unit (JRC)
- Community Reference Laboratory (JRC)
- European Network of GMO Laboratories (ENGL)
- Institute for Reference Materials and Measurements (IRMM)

Training activities on GMO detection

- Training courses of B&GMOs Unit (JRC)

Databases / data collections

- GMO Methods Database (B&GMO Unit, JRC)
- Guidance documents for the validation of GMO detection methods (CRL-GMFF, JRC)
- Validated GMO detection methods (CRL-GMFF, JRC)
- Information on notifications about deliberate field trials and placing on the market of genetically modified organisms (B&GMO Unit, JRC)
- Regulatory Information Systems on GMOs (B&GMO Unit, JRC)
- Document collection of B&GMOs Unit (JRC)

Software tools for validation of GMO detection methods and sampling

- AMPE: Analytical Method Performance Evaluation (IRC)
- Sampling Software: KesTE / CoDE (JRC)

Relevant EU research projects

- CO-EXTRA: GM and non-GM supply chains: their CO-EXistence and TRAceability
- DNA-TRACK: Traceability of DNA fragments throughout the food chain by DNA/PNA technologies. Application to Novel Foods
- ENTRANSFOOD: European network on safety assessment of genetically modified food crops
- QPCRGMOFOOD: Reliable, standardised, specific, quantitative detection of genetically modified food

Annex II – Publications and Poster

- General publications on GMO detection, quantification and validation
- PCR-based GMO detection methods, DNA standard material and reference material
- DNA-based Microarrays
- Protein-based GMO detection methods
- Harmonisation and validation of detection methods
- Sampling

General publications on GMO detection, quantification and validation

Analytical methods for Detection and Determination of Genetically Modified Organisms (GMO's) in Agricultural Crops and Plant-derived Food Products.
E. Anklam, F. Gadani, P. Heinze , H. Pijnenburg, and G. Van den Eede
European Food Research and Technology (2002) 214:3-26

Review of GMO Detection and Quantification Techniques
L. Bonfini, H. Petra, S. Kay, and G. Van den Eede
EUR 20384 EN (2002)

The limits of GMO detection.
S. Kay and G. Van den Eede
Nature Biotechnology Vol 19 No 5 p 405 (2001)

Food Products Identity: the Need for a High Through-Put Approach in Food Analysis
A. Fantozzi, M. Marini, M. Ermolli, G. Van den Eede
1st International Symposium on "Recent Advances in Food Analysis" - Prague 5-7 November 2003

Food products identity: the need for a high through-put approach for GMO screening
A. Fantozzi, M. Marini, M. Ermolli, and G. Van den Eede
7th Food Authenticity and Safety International Symposium - Nantes, 15-17 October 2003

PCR-based GMO detection methods, DNA standard material and reference material

Event-specific plasmid standards and real-time PCR methods for transgenic Bt11, Bt176 and GA21 maize and transgenic GT73 canola.
I. Taverniers, P. Windels, M. Vaitilingom, A. Milcamps, E. Van Bockstaele, G. Van den Eede , and M. de Loose
Journal of Agricultural and Food Chemistry (2005) 53: 3041–3052

A Real-Time PCR Based Approach for the Quantification of the pat Gene in the T25 Zea mays Event.
Journal of the AOAC International 87(6): 1342-1355 (2004)
F. Weighardt, C. Barbati, C. Paoletti, M. Querci, S. Kay, M. De Beuckeleer, and G. Van den Eede

Development and applications of a novel class real-time PCR standards based on cloned sequences for GMO quantification.
C. Barbati, F. Weighardt, E. Mattarucchi, M. Querci, and G. Van den Eede
6th Italian workshop in Real time PCR, Arrays Quantitative PCR and mutation detection - Florence, 18 November 2003

Real Time PCR based GMO quantification: limits and accuracy
C. Barbati, F. T. Weighardt, S. Kay, C. Paoletti, M. Querci, and G. Van den Eede
Third CSL/JIFSAN Symposium on Food Safety and Nutrition" at the Central Science Laboratory, York, UK

Production of Reference Materials for the Detection of GMOs.
S. Trapmann, H. Schimmel, P. Brodmann, G. Van den Eede, K. Vorburger and J. Pauwels
Journal of the AOAC International, Vol. 85, No. 3 (2002) pp. 775-779

Development and applications of real-time PCR standards for GMO quantification based on tandem-marker plasmids.
E. Mattarucchi, F. Weighardt, C. Barbati, M. Querci, and G. Van den Eede
European Food Research and Technology 211: 511-519 (2005) ISSN: 1438-2385

DNA-based micro arrays

Microarray Method for the Screening of EU Approved GMOs by Identification of their Genetic Elements. Report of validation coordinated by the Community Reference Laboratory for GM Food and Feed of the Joint Research Centre, Institute for Health and Consumer Protection, Biotechnology and GMOs Unit
S. Hamels, S. Leimanis, M. Mazzara, G. Bellocchi, N. Foti, W. Moens, J. Remacle and G. Van den Eede

Protein-based GMO detection methods

First Application of a Micro-sphere Based Immunoassay to GMO Detection: Quantification of Cry1Ab Protein in GM Maize.
A. Fantozzi, M. Ermolli, M. Marini, D. Scotti, B. Balla, M. Querci, S. Langrell, G. Van den Eede
Journal of Agricultural and Food Chemistry, Vol. 55 (2007), 1071-1076

Development of an innovative immunoassay for CP4EPSPS and Cry1AB GM proteins detection and quantification.
M. Ermolli, A. Prospero, B. Balla, M. Querci, A. Mazzeo, and G. Van den Eede
Food Additives and Contaminants. Volume 23, Number 9/September 2006: 876-882

Food safety: screening tests used to detect and quantify GMO proteins.
M. Ermolli, A. Fantozzi, M. Marini, D. Scotti, B. Balla, S. Hoffmann, M. Querci, C. Paoletti, and G. Van den Eede
Accreditation and Quality Assurance - ACQUAL 11(1-2): 55–57. (2006)

Application of the ELISA Reverse Method and and device to quantify CP4EPSPS protein in GM RUR Soya. The Safe Consortium.
M. Ermolli, A. Prospero, B. Balla, M. Querci, A. Mazzeo, and G. Van den Eede
The First Safe International Congress on Food Safety, Budapest Congress Centre, Budapest (Hungary), 11-14 June 2006

ELISA Reverse m&d for Multiplex Detection and Quantification of Target Proteins in Food Analyses.
A. Prospero.
University of Teramo, 11th Workshop on the Developments in the Italian PhD Research on Food Science and Technology, University of Teramo, Mosciano Sant'Angelo, 27-29 September 2006

An Innovative High-Throughput Assay for the Detection and the Quantification of GMOs Proteins and Sequences in Food Samples.
M. Ermolli
Facoltà di Agraria - Dipartimento di Scienze degli Alimenti
The 10th Workshop on the Developments in the Italian PhD Research in Food Science and Technology, Foggia (Italy), 7-9 September 2005

Protein Based Methods in GMO Detection and Quantification in Food.
M. Ermolli, A. Prospero, M. Querci, and A. Mazzeo
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Annex III – Register of validated GMO detection methods

(as of December 2007)

1. Validated methods by CRL-GMFF (for GMO detection and DNA extraction)

2. Method validations in process (published detection methods proposed by the applicant)

1. Validated methods by CRL-GMFF (for GMO detection and DNA extraction)

Event	Unique identifier	Applicant	Validation Reports	Validated Method
1507 Maize	DAS-01507-1	Pioneer Hi-Bred, Dow Agrosciences, Mycogen Seeds	Click here	Method description DNA Extraction
1507 x 59122 Maize	DAS-01507-1 x DAS-59122-7	Mycogen Seeds, c/o Dow AgroSciences LLC	Click here	Method description (1507) Method description (59122)
1507 x NK603 Maize	DAS-01507-1 x MON-00603-6	Pioneer Hi-Bred, Mycogen Seeds	Click here	Method description (NK603) Method description (1507)
3006-210-23/281-24-236 Cotton	DAS-24236-5 x DAS-21023-5	Dow AgroSciences	Click here	Method description (281-24-236) Method description (3006-210-23) DNA extraction
59122 Maize	DAS-59122-7	Pioneer Hi-Bred; Mycogen Seeds c/o Dow AgroSciences	Click here	Method description DNA Extraction
A2704-12 Soybean	ACS-GM005-3	Bayer CropScience	Click here	Method description DNA Extraction
Bt10 Maize	-	-	Click here	Method description
Bt11 Field Maize	SYN-BT011-1	Syngenta Crop Protection	Click here	Method description DNA Extraction
Bt11 Sweet maize	SYN-BT011-1	Syngenta Seeds	Click here	Method description
Carnation (Dianthus caryophyllus L.) line 123.2.38	FLO-4Ø644-4	Florigene Ltd.	Click here	Method description
EH92-527-1 Potato	BPS-25271-9	BASF Plant Science Holding Gmbh	Click here	Method description DNA Extraction
GA21 Maize	MON-00021-9	Monsanto	Click here	Method description
GA21 Maize	MON-00021-9	Syngenta Crop Protection	Click here	Method description DNA Extraction
GT73 Rapeseed	MON-00073-7	Monsanto	Click here	Method description DNA Extraction
LLCOTTON25	ACS-GH001-3	Bayer CropScience	Click here	Method description DNA Extraction
LLRICE601			LLRice601 update	LLRice601 update
LLRICE62 Rice	ACS-OS002-5	Bayer CropScience	Click here	Method description DNA Extraction
MIR 604 Maize	SYN-IR604-5	Syngenta	Click here	Method description DNA Extraction

Event	Unique identifier	Applicant	Validation Reports	Validated Method
MON 04032-6 Soybean	MON-04032-6	Monsanto	Click here	Method description DNA Extraction
MON 863 x MON 810	MON-00863 x MON00810-6	Monsanto	Click here	Method description (MON 810) Method description (MON 863)
MON 863 x MON 810 x NK603 Maize	MON-00863-5 x MON-00810-6 x MON-00603-6	Monsanto	Click here	Method description (MON 810) Method description (NK603) Method description (MON 863)
MON 863 x NK603 Maize	MON-00863-5 x MON-00603-6	Monsanto	Click here	Method description (NK603) Method description (MON 863)
Mon863 Maize	MON-00863-5	Monsanto	Click here	Method description
Ms8 Rapeseed	ACS-BN005-8	Bayer CropScience	Click here	Method description DNA Extraction
Ms8xRf3 Rapeseed	ACS-BN005-8 x ACS-BN003-6	Bayer CropScience	Click here	Method description (Ms8) Method description (Rf3)
NK603 Maize	MON-00603-6	Monsanto	Click here	Method description
NK603 x MON 810 Maize	MON-00603-6 x MON-00810-6	Monsanto	Click here	Method description (MON 810) Method description (NK603)
Rf3 Rapeseed	ACS-BN003-6	Bayer CropScience	Click here	Method description DNA Extraction
RUR H7 Sugar beet	KM-000H71-4	KWS SAAT AG. Monsanto	Click here	Method description DNA Extraction
T25 Maize	ACS-ZM003-2	Bayer CropScience	Click here	Method description
T45 Rapeseed	ACS-BN008-2	Bayer CropScience	Click here	Method description DNA Extraction

2. Method validations in process

Published detection methods proposed by the applicant

Event	Unique identifier	Applicant	Methods proposed by the applicant
MON 1445 Cotton	MON-01445-2	Monsanto	Click here
MON 15985 Cotton	MON-15985-7	Monsanto	Click here
MON 531 Cotton	MON-00531-6	Monsanto	Click here
Novo Yeast Cream		Novo Nordisk A/S	Click here
PL73 Brevibacterium		Ajinomoto Eurolysine SAS	Click here

Annex IV - FVO missions regarding national GMO controls on food, feed and seeds

Period	Country	Inspection number	FVO reports and comments from national authorities (pdf)
04/2007	Romania	7186/2007	FVO report Comments Romania
03/2007	Brazil	7180/2007	FVO report Comments Brazil
12/2006	Argentina	8118/2006	FVO report Comments Argentina
06/2006	United Kingdom	8116/2006	FVO report Comments United Kingdom
05/2006	Hungary	8109/2006	FVO report Comments Hungary
05/2006	Czech Republic	8110/2006	FVO report Comments Czech Republic
05/2006	France	8086/2006	FVO report Comments France
03/2006	Poland	8106/2006	FVO report Comments Poland
03/2006	Germany	8105/2006	FVO report Comments Germany
03/2006	Slovenia	8104/2006	FVO report Comments Slovenia
02/2006	Belgium	8102/2006	FVO report Comments Belgium
02/2006	Slovak Republic	8100/2006	FVO report Comments Slovak Republic
10/2005	Portugal	7669/2005	FVO report Comments Portugal
09/2005	The Netherlands	7666/2005	FVO report Comments of the Netherlands
06/2005	Italy	7653/2005	FVO report Comments Italy
03/2005	Spain	7632/2005	FVO report Comments Spain
10/2003	United Kingdom	9249/2003	FVO report Comments United Kingdom
06/2003	Austria	9141/2003	FVO report Comments Austria
03/2003	Spain	9103/2003	FVO report Comments Spain
04/2002	Sweden	8605/2002	FVO report Comments Sweden
10/2001	Germany	3233/2001	FVO report Comments Germany

Source: http://ec.europa.eu/food/fvo/ir_search_en.cfm