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Nordic Co-operation in Agriculture and Forestry
Agriculture and forestry in the Nordic countries are based on similar natural pre-requisites, and often face common challenges. This has resulted in a long-established tradition of Nordic co-operation in agriculture and forestry. Within the framework of the Plan of Action 1996-2000, the Nordic Council of Ministers (ministers of agriculture and forestry) has given priority to co-operation on quality agricultural production emphazising environmental aspects, the management of genetic resources, the development of regions depending on agriculture and forestry and sustainable forestry.

The Nordic Council of Ministers
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The Nordic Council
was formed in 1952 to promote co-operation between the parliaments and governments of Denmark, Iceland, Norway and Sweden. Finland joined in 1955. At the sessions held by the Council, representatives from the Faroe Islands and Greenland form part of the Danish delegation, while Åland is represented on the Finnish delegation. The Council consists of 87 elected members - all of whom are members of parliament. The Nordic Council takes initiatives, acts in a consultative capacity and monitors co-operation measures. The Council operates via its institutions: the Plenary Assembly, the Presidium and standing committees.

Contents

Preface

The project started in 2001 and was formally finalised in 2002. Due to several circumstances the writing of the report has been delayed. As a result, relevant parts of the report have been updated in order to reflect the GMO analysis situation at the beginning of 2004.

The project group has met four times, two times in 2001 and two times in 2002. During this period some of the members of the group were replaced. The following persons have participated in one or more of the meetings as members of the project group:

Haakon Sønju	Landbrukstilsynet	Norway
Øygunn Østhagen	Landbrukstilsynet	Norway
Torgun Johnsen	Landbrukstilsynet	Norway
Ulf Kjellström	Statens Utsädeskontroll	Sweden
Karin Johansson	Statens Utsädeskontroll	Sweden
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Michael Krause	Plantedirektoratet	Denmark
Peter Stephensen Lübeck	Plantedirektoratet	Denmark
Svend Pedersen (chairman)	Plantedirektoratet	Denmark

In addition, the following external persons have participated in single meetings giving lectures on specific items covered by the scope of the project:

Sampling and statistics

Kristian Kristensen, Danish Institute of Agricultural Sciences

GMO analysis methods

Folmer Eriksen and Jan Pedersen, Danish Food Directorate (now Danish Institute for Food and Veterinary Research)
Arne Holst-Jensen, The Veterinary Institute, Norway

GMO analysis companies

Michael Meldgaard, Xenogenix; Denmark
Patrik Stolt, ScanGene AB; Sweden

Identity Preservation

Thor Kristoffersen, Denofa, Norway

Biotech company

Per Henriksson, Aventis (now Bayer CropScience)

The report was prepared by Svend Pedersen with contributions from members of the project group regarding the GMO analysis situation in their respective countries. The author would like to thank Maibritt Langfeldt Sørensen from the Danish Plant Directorate for her contribution to the manuscript (figures and text regarding the international GMO analysis activities).

Summary

The global area with GM crops has been steadily increasing since the first commercial cultivation of these crops started in USA in 1996. But the European Union continues to be a region where the commercial cultivation of GM crops is very limited. In addition, probably some of the strictest regulations in the World regarding traceability and labelling of GM products have been adopted in this region.

Thus Regulation 1829/2003 on genetically modified food and feed and Regulation 1830/2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms have been in operation since 18 April 2004.

As a result, great efforts are made to develop and validate methods of analysis of GMO contents in seed, food and feed in order to be able to comply with these regulations. This report describes some of the current possibilities and limitations regarding analysis of GM contents in seed and feed.

Several incidents in recent years of adventitious presence of GM seed in conventional seed as well as incidents of accidental admixture of GM and conventional seed have highlighted the need for having reliable GMO analysis methods at hand.

Analysis for GMO contents in seed and feed is demanded by, e.g., the existence of labelling regulations, the need to control the presence of non-approved GMOs, and the need to monitor the effectiveness of regulations on co-existence of genetically modified, conventional and organic crops.

With the entering into force of Regulation (EC) 1829/2003 on genetically modified food and feed the labelling threshold for GMO content in food has been lowered from 1 % to 0.9 %. The same threshold will apply for feed. Until then there was no specific regulation on approval or labelling of genetically modified feed.

As regards seed the question of labelling thresholds for adventitious presence of genetically modified seed in conventional seed was not settled at the time of writing the present report. Since the production of seed lies before the production of food and feed, the thresholds for seed have to be lower than 0.9 %.

Labelling thresholds as diverse as 0 %, 0.9 %, 1 %, 2 %, 3 % and 5 % are in function in different countries and regions around the World. This is a situation which contributes to difficulties when products are traded across borders between countries with different thresholds. Obviously, international harmonization is needed in this field.

Labelling thresholds may be viewed as a balance between consumer requests (the lower, the better), company requests (the higher, the better), and technical capabilities (the lower, the larger the error). The current low labelling threshold in Europe is a result of political compromise, and it can be foreseen that difficulties with its enforcement may arise.

As a rule, the lower the threshold, the higher the confidence interval around the test result will be. This creates a large degree of uncertainty as to how to interpret analysis results that lie in the area close to the threshold.

Sampling and testing for the presence of GM material may be carried out at several points "from farm to fork". For instance, samples can be taken of the seed before sowing, of the plants in the field, of the crop products after harvest and at various points during further processing. Further, samples of feed and manure can be tested. For the purpose of this report only sampling and testing of seed and feed is described.

A decision on how to take samples and test for GM content will be a balance between which analysis will be most relevant and the costs of these analyses, as most analyses are still very costly.

There is a range of potential problems associated with taking samples and preparing them for analysis. In addition, at each step in this process an error is introduced. The challenge is to minimize the unavoidable sampling error.

The first sampling stage in this process is normally regarded as the most critical one as the actual distribution of the genetically modified material in the lot is not known beforehand. For seed it is reasonable to assume that the distribution of heterogeneity in the lot in most cases will be non-random, whereas in feed, which is often a mixture of different components, the distribution of GM particles in the lot would tend to be more random.

In the subsequent sampling stages, where the bulk sample is reduced in size, the distribution of genetically modified material often can be regarded as random provided that thorough mixing of the sample is done. However, through the processes of seed grinding and DNA extraction changes in the proportions of GM/non-GM units may be introduced.

The current GMO analytical methods can roughly be divided into protein-based and DNA-based methods.

The protein-based methods are the fastest, cheapest and the most simple to perform. The methods are based on the development of antibodies that are specific against new proteins that are produced in the GM plants. The currently commercially available methods for analysis for GM plants have been developed for B.t. toxins, which result in insect resistance, and for herbicide tolerance. As some of the proteins are common in different GM plants, the methods can only be used for detection of the GM characteristic but not for identification of the individual GMOs.

The most often used DNA-based methods are the so-called PCR (Polymerase Chain Reaction) methods, which can be used for both qualitative (detection and identification) and quantitative analyses.

With a PCR test the presence of the inserted gene itself is studied. If one examines the transition between the inserted gene and the plant’s own DNA, the individual GM plant (“transformation event”) can unambiguously be identified. A PCR-test is performed with the use of pairs of primers which are small pieces of DNA specific for the DNA to be analysed.

In the PCR methods there are limits of detection and quantification of GMOs, respectively. The detection limit is the smallest amount of GM DNA, which is measurable.

The quantification limit is the smallest amount of GM DNA, which is necessary for measuring the actual content of GM DNA.

Determination of whether the GMO content complies with labelling provisions demands the use of quantitative methods. Quantification of the GMO content will typically be preceded by the identification of the GMO(s) present.

Some plant species (e.g. wheat) have a large genome (large amount of chromosomal DNA), that sets a limit on the minimum quantity of GM DNA that can be analysed, because there are limits to the amount of DNA that can be present in the PCR reaction.

There are several ways to calculate the GMO level in a sample. For seeds, e.g., the GMO content can be calculated per haploid genome, as seeds by number or seeds by mass. It is still being discussed whether to express GMO content at the seed or DNA level. In order to seek comparability with the way GMO content is expressed in foods and feeds it may be argued to express the GMO content in seed at the DNA level (GMO-DNA content as a percentage of total DNA content).

There is concern that the expression of GMO content can lead to different conclusions depending on expression as % seeds or % DNA content. As mentioned in an example with maize, there are situations where, e.g., seed would have to be labelled for GMO content whereas the flour resulting from grinding the same seed would not. As long as no consensus has been reached on this issue, this contributes to confusion regarding how to interpret GMO analysis results.

A situation which is becoming more and more common is the occurrence of more than one transformation event in the same plant. This is the case, e.g., for GM hybrid oilseed rape and for several GM maize hybrids currently awaiting marketing approval in the European Union. Unless a specific marker is introduced in the hybrid between two GM plants, it is not possible to determine whether a given sample contains the hybrid or a mixture between the two plants. The only possibility would be to analyse single grains which is not feasible for large samples. This problem is currently unsolved.

The relatively recently developed “micro-array” technique is suitable for screening and identifying many GM plants in a single test. In this way, it will be possible to test for the presence of all GM plants that are approved in the EU at the same time. Currently, there is an EU project running with a view to developing these methods for testing the GMO content in foods.

A number of alternative methods or techniques for testing of GMO content have been developed. These include germination tests, tetrazolium tests, insect resistance bioassays, chromatography, near infrared spectroscopy, microfabricated devices and nanoscale analysis.

In the future, it may be expected that specific information is inserted GM plants which would make it easier to identify them. An example is a so-called bar-coding technique for which a patent recently was granted.

For GMO labelling thresholds to be enforced there is a requirement to use validated methods so that one can be sure that the results of a GMO analysis is the same independent of the laboratory having performed the analysis. In addition, the laboratory should also be accredited according to international standards.

According to the EC regulation 1829/2003 on genetically modified food and feed the Community Reference Laboratory will play a central role in the validation of GMO analysis methods in future. The European Commission's Joint Research Centre will act as the Community Reference Laboratory and will be assisted by a consortium of national reference laboratories (the European Network of GMO Laboratories; ENGL).

Activities related to the GMO analysis area are also carried out in several other international forums such as OECD, ISTA, Codex Alimentarius, CEN and others.

As a way to illustrate the possible variation in results when a given sample is sent to different laboratories for analysis of GMO content, a simple ring test experiment was carried out among the members of the project group. This was to show the analysis situation at a moment when there was no standards existing yet as to how to perform the analysis. In other words, each laboratory used its own methods, only the samples were presumed to be identical.

The material to be analysed in the ring test was maize meal imported to Iceland from USA, soy meal imported to Denmark from USA, and oilseed rape seed from Sweden made up as mixtures of conventional seed and GM seed.

Even though there were no conditions set as to which methods for sample preparation or analysis should be used, the results of the ring tests illustrates how results may vary depending on which laboratory performs the analysis. In some cases the result may lie above the labelling threshold and in others below. This is a problem which could be predicted to give rise to difficulties, if controversies regarding the observation of labelling thresholds are to be decided in court.

The final chapter on GMO analysis activities in the Nordic countries illustrates the extent of such activities in the individual countries regarding seed and feed. Apart form Iceland, all Nordic countries analyse seed and feed samples for GMO contents. However, with the adoption of the EU regulations on GM food and feed as well as on traceability and labelling of GMOs into Icelandic law, GMO analysis activities are expected to commence in Iceland too in 2004.

The kinds of laboratories that carry out GMO analyses vary between countries. In Norway and Finland all GMO analyses are performed by national laboratories, in Denmark and Sweden both national and commercial laboratories are used, whereas in Iceland the future GMO analyses is expected to be performed by a commercial laboratory.

Dansk sammendrag

Det globale areal med GM afgrøder er vokset støt siden den første kommercielle dyrkning af disse afgrøder startede i USA i 1996. Men den Europæiske Union er fortsat et område med meget begrænset dyrkning af GM afgrøder. Herudover er nogle af de måske mest restriktive regler i Verden med hensyn til sporbarhed og mærkning af GM produkter blevet indført i denne region.

Således har Forordning 1829/2003 om genetisk modificerede fødevarer og foderstoffer samt forordning 1830/2003 om sporbarhed og mærkning af genetisk modificerede organismer og sporbarhed af fødevarer og foderstoffer fremstillet af genetisk modificerede organismer været i funktion siden den 18. april 2004.

Som resultat heraf bliver der gjort store bestræbelser på at udvikle og validere metoder til analyse af GMO indhold i frø, fødevarer og foder med henblik på at følge reglerne i forordningerne. Denne rapport beskriver nogle af de aktuelle muligheder og begrænsninger med hensyn til analyse af GMO-indhold i frø og foder.

Der har i de senere år været flere tilfælde med utilsigtet forekomst af GM frø i konventionelt frø samt tilfælde med utilsigtet sammenblanding af GM og konventionelle frø, som har understreget behovet for at have pålidelige GMO-analysemetoder til rådighed.

Analyse af GMO-indholdet i frø og foder er påkrævet bl.a. på grund af de gældende mærkningsregler, behovet for at kontrollere for tilstedeværelse af ikke-godkendte GMO'er, samt behovet for at monitere virkningen af regler om sameksistens mellem genetisk modificerede, konventionelle og økologiske afgrøder.

Med ikrafttrædelsen af Forordning (EF) 1829/2003 om genetisk modificerede fødevarer og foderstoffer blev tærskelværdien for mærkning af utilsigtet GMO-forekomst i fødevarer sænket fra 1 % til 0,9 %. Den samme tærskelværdi gælder for foderstoffer. Indtil da fandtes der ingen specifikke regler for godkendelse eller mærkning af genetisk modificeret foder.

Med hensyn til frø var der endnu ikke blevet fastsat tærskelværdier for utilsigtet forekomst af GM frø i konventionelt frø på tidspunktet for færdiggørelsen af denne rapport. Eftersom produktionen af frø ligger forud for produktionen af fødevarer og foder, er tærskelværdierne for frø nødt til at være lavere end 0,9 %.

I forskellige dele af Verden gælder så forskellige tærskelværdier for mærkning som 0 %, 0,9 %, 1 %, 2 %, 3 % og 5 %. Denne situation bidrager til at gøre det vanskeligt, når diverse produkter forhandles tværs over landegrænser, hvor forskellige tærskelværdier er gældende. Der mangler oplagt international harmonisering på dette område.

Tærskelværdier for mærkning kan ses som en balance mellem forbrugerønsker (jo lavere, desto bedre), firmaønsker (jo højere, desto bedre) og de tekniske muligheder (jo lavere, desto højere usikkerhed). Den nuværende lave tærskelværdi for mærkning i Europa er resultatet af et politisk kompromis, og det må forudses, at der kan opstå vanskeligheder i forbindelse med overholdelsen af den.

Det gælder således generelt, at jo lavere tærskelværdi, desto højere vil usikkerhedsintervallet omkring analyseresultatet være. Dette skaber en høj grad af usikkerhed med hensyn til tolkningen af analyseresultater, der ligger tæt på tærskelværdien.

Prøvetagning og analyse for forekomst af GM materiale kan foretages adskillige steder på vejen "fra jord til bord". Der kan f.eks. udtages prøver af frø før udsåning, af planterne på marken, af afgrøden efter høst samt på forskellige punkter under den videre forarbejdning. Herudover kan der foretages analyser af foder og gødning.

Eftersom analyserne stadigvæk er temmelig dyre, vil en beslutning om, hvordan prøverne skal udtages og analyseres for GMO-indhold være en afvejning mellem, hvilke analyser, der vil være mest relevante at udføre, og omkostningerne ved disse.

Der eksisterer en række potentielle problemer i forbindelse med udtagelsen af prøver og forberedelsen af disse til analyse. Herudover skal der regnes med en fejl på hvert trin i processen. Udfordringen er at minimere de uundgåelige prøvetagningsfejl.

Det første prøvetagningstrin i denne proces regnes normalt for at være det mest kritiske, idet den faktiske fordeling af genetisk modificeret materiale i partiet ikke kendes på forhånd. For frø er det rimeligt at antage, at fordelingen af heterogenitet i partiet i de fleste tilfælde ikke vil være tilfældig, hvorimod der i foder, hvor der ofte er sket en fysisk opblanding af forskellige komponenter, i højere grad vil være en tilfældig fordeling af GM partikler.

I de efterfølgende prøvetagningsstadier, hvor partiprøven reduceres i størrelse, kan fordelingen af genmodificeret materiale ofte betragtes som tilfældig, forudsat at der sker en grundig blanding af prøven. Der kan dog ske ændringer i fordelingen af GM og ikke-GM-partikler under formalingen af frø og under DNA-ekstraktionen i forbindelse med forberedelsen af den endelige analyseprøve.

De nuværende GMO-analysemetoder kan groft opdeles i proteinbaserede og DNA-baserede metoder.

De proteinbaserede metoder er de hurtigste, billigste og mest simple at udføre. Metoderne er baserede på udviklingen af antistoffer, der er specifikke over for de nye proteiner, som produceres i GM-planterne. Der er indtil nu udviklet kommercielt tilgængelige metoder til analyse for indhold af B.t.-toksiner, som medfører insektresistens, og for herbicidtolerance. Da visse af proteinerne er fælles for flere GM planter, kan metoderne kun benyttes til detektion af GM-egenskaben men ikke til identifikation af individuelle GMO'er.

De mest anvendte DNA-baserede metoder er de såkaldte PCR (Polymerase Chain Reaction)-metoder, som kan anvendes til såvel kvalitative (detektion og identifikation) som kvantitative analyser.

Med en PCR-test er det tilstedeværelsen af selve det indsatte gen, som analyseres. Ved at analysere overgangen mellem det indsatte gen og plantens eget DNA kan den individuelle GM-plante ("transformationsbegivenheden") entydigt identificeres. En PCR-test udføres ved anvendelse af primer-par, som er små stykker DNA, der er specifikke for det DNA, som skal analyseres.

I PCR-metoderne er der grænser for henholdsvis detektion og kvantificering af GMO'er. Detektionsgrænsen er den mindste mængde GM-DNA, som er målbart. Kvan-

tificeringsgrænsen er den mindste mængde GM-DNA, som er nødvendigt for at måle det faktiske indhold af GM-DNA.

Afgørelse af, om GMO-indholdet svarer til mærkningskravene, kræver anvendelse af kvantitative metoder. Forud for kvantificering af GMO-indholdet vil der typisk være sket en identifikation af GMO'en (eller GMO'erne), som er til stede.

Visse plantearter (f.eks. hvede) har store genomer (en stor mængde kromosomalt DNA), som sætter en grænse for den mindste mængde GM-DNA, der kan analyseres, fordi der er grænser for, hvor meget DNA, der kan være til stede i PCR-reaktionen.

Der er flere måder at regne GMO-indholdet i en prøve ud på. For frø kan GMO-indholdet f.eks. udregnes pr. haploidt genom, som frø pr. antal, eller som frø pr. vægt. Det er endnu uafklaret, om GMO-indholdet bør udtrykkes på frø- eller DNA-niveau. Med henblik på at opnå størst mulig sammenlignelighed med den måde, hvorpå GMO-indholdet udtrykkes i fødevarer og foder, kan der argumenteres for, at GMO-indholdet i frø udtrykkes på DNA-niveau (GM-DNA-indhold som procent af totale DNA-indhold).

Der er endvidere bekymring over, at måden, hvorpå GMO-indholdet udtrykkes, kan medføre forskellige konklusioner afhængigt af, om indholdet udtrykkes på frøniveau eller på DNA-niveau. Som beskrevet i et eksempel med majs vil der være situationer, hvor f.eks. frø ville skulle mærkes for GMO-indhold, medens det mel, som stammer fra formalingen af de samme frø, ikke ville skulle mærkes. Så længe der ikke er opnået enighed om dette spørgsmål, vil det bidrage til forvirring med hensyn til, hvordan GMO-analyseresultater skal tolkes.

En situation, som bliver mere og mere almindelig, er tilstedeværelsen af mere end én transformationsbegivenhed i den samme plante. Dette er f.eks. tilfældet for GM-hybridraps samt for flere GM-majshybrider, som afventer tilladelse til markedsføring i EU. Med mindre der indsættes en specifik markør i en hybrid mellem to GM-planter, er det ikke muligt at afgøre, om en given prøve indeholder hybriden eller en blanding mellem de to planter. Den eneste mulighed for at afgøre dette spørgsmål ville være at analysere enkeltkerner, hvilket ikke er praktisk muligt for store prøver. Dette problem er endnu ikke løst.

Den relativt nyudviklede "micro-array"-teknik er brugbar til screening og identificering af mange GM-planter i en enkelt analyse. Med denne teknik vil det være muligt at teste for tilstedeværelse af alle de GM-planter, der er godkendt i EU, på én gang. Der eksisterer et EU-projekt, som har til formål at udvikle denne metode til analyse af GMO-indholdet i fødevarer.

Der er blevet udviklet en række alternative metoder og teknikker til analyse for GMO-indhold. Disse omfatter spiringstests, tetrazolium tests, bioassays for insektresistens, kromatografi, nær infrarød spektroskopi, mikrofremstillede apparater og nanoskala-analyser.

I fremtiden kan det forventes, at der indsættes specifik information i GM-planter, som gør det lettere at identificere dem. Et eksempel herpå er den såkaldte "bar-kodningsteknik", som for nylig opnåede patentbeskyttelse.

For at kunne håndhæve GMO-tærskelværdier for mærkning er det nødvendigt at anvende validerede metoder, således at man kan være sikker på, at resultatet af en GMO-analyse er det samme uafhængigt af, hvilket laboratorium, der har udført analysen. Herudover bør laboratoriet være akkrediteret i henhold til internationale standarder.

Ifølge Forordning 1829/2003 om genetisk modificerede fødevarer og foderstoffer vil EF-referencelaboratoriet fremover spille en central rolle i valideringen af GMO-analysemetoder. Europakommissionens Fælles Forskningscenter vil fungere som EF-referencelaboratoriet, og vil blive bistået af et konsortium af nationale referencelaboratorier (Det Europæiske Netværk af GMO-laboratorier; ENGL).

Der udføres yderligere forskellige aktiviteter med relation til GMO-analyseområdet i adskillige internationale fora som OECD, ISTA, Codex Alimentarius, CEN, med flere.

For at illustrere den mulige variation i analyseresultat, når en given prøve sendes rundt til forskellige laboratorier til analyse af GMO-indhold, udførte projektgruppens medlemmer et simpelt ringanalyseeksperiment. Formålet var at belyse analysesituationen på et tidspunkt, hvor der endnu ikke var udviklet standarder for, hvordan analysen skulle udføres. Med andre ord anvendte hvert laboratorium sine egne metoder, idet udelukkende prøverne blev antaget at være identiske.

Materialet, der skulle analyseres i ringtesten var majsmel importeret til Island fra USA, sojamel importeret til Danmark fra USA, og rapsfrø fra Sverige bestående af blandinger af konventionelle frø og GM-frø.

Selvom der ikke var fastsat nogen betingelser for, hvilke metoder der skulle anvendes til prøveforberedelse eller analyse, illustrerer resultaterne af ringanalysen, hvordan resultater kan variere afhængigt af, hvilket laboratorium, der udfører analysen. Dette er et problem, som vil kunne forudses at give anledning til vanskeligheder, hvis kontroverser vedrørende overholdelse af tærskelværdier for mærkning skal afgøres i retten.

Det afsluttende kapitel om GMO-analyseaktiviteter i de nordiske lande illustrerer omfanget af sådanne aktiviteter i de enkelte lande for så vidt angår frø og foder. Bortset fra Island analyserer alle de nordiske lande frø- og foderprøver for GMO-indhold. Imidlertid forventes det, at også Island starter GMO-analyseaktiviteter i 2004 i forbindelse med ikrafttrædelsen af EU-forordningerne om GM fødevarer og foder samt om sporbarhed og mærkning af GMO'er.

Der er variation mellem landene med hensyn til, hvilken type laboratorier, der udfører GMO-analyser. I Norge og Finland udføres alle GMO-analyser af statslige laboratorier, i Danmark og Sverige anvendes såvel statslige som private laboratorier, medens de fremtidige GMO-analyser i Island forventes at blive udført af et privat laboratorium.

1 Introduction

Several incidents in recent years of adventitious presence of GM seed in conventional seed or incidents of accidental admixture of GM and conventional seed have highlighted the need for having reliable GMO analysis methods at hand. In addition, several pieces of legislation in the European Union and in the Nordic countries now demand labelling thresholds to be observed.

In 2000, several incidents of adventitious presence of GM seed in conventional oilseed rape varieties imported from Canada was revealed in a number of European countries, including Sweden and Denmark.

This led the European Union to adopt a plan for testing of selected seed lots of conventional varieties to determine the presence of GMO impurities.

Even in USA, where labelling of GMO content in food is not required, there have been incidents which have demanded the application of GMO analysis methods. The incident with the till now farthest reaching consequences was the discovery in 2000 of admixture into food products of the StarLink (Cry9C) maize which had only been approved for animal feed use.

In 2003, traces of StarLink maize still showed up in about 1 percentage of the samples tested by the Grain Inspection, Packers and Stockyards Administration (GIPSA) in USA.

In 2001 the company Monsanto initiated a “biotechnology consultation process” with the U.S. Food and Drug Administration because of the finding that the genetically modified oilseed rape line “GT200”, which was not intended for commercialisation, “has the potential to be present at low adventitious levels in commercial canola varieties”.

Another example is the accidental admixture in 2002 of maize genetically modified by the company ProdiGene to make a pharmaceutical product into harvested soybeans intended for human consumption.

The importance of the ability to analyse for the presence of GMOs in various types of materials is made topical by the entering into force in the European Union of Regulation 1829/2003 on genetically modified food and feed and Regulation 1830/2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms. Both regulations will apply from 18 April 2004.

This report will only briefly describe the various GMO analysis techniques to give an overview of the current possibilities of analysis. Regarding the more technical descriptions of the techniques, several scientific articles – including a number of review articles – are available.

Instead the report will try to emphasize some of the problems related to analysis of GMO contents. A central issue in this context is the possibilities – or maybe rather the lacking possibilities – for making precise measurements of low GMO contents. This is a

problem of special importance when low thresholds for labelling of GMO content are established in various pieces of legislation.

In a special section the results of a small GMO ring analysis experiment carried out by the members of the project group are presented.

The final part of the report contains an overview of the current GMO analysis situation regarding seed and feed in the individual Nordic countries. In addition, results of GMO controls in the respective countries from the period 2001-2003 are presented here.

2 Sources of GMO admixtures

2.1 Where are GM crops grown

In 2003, about 99 % of the global genetically modified crop area was divided among USA, Canada, Argentina, Brazil, China and South Africa. The dominant genetically modified crops are currently soybean, maize, cotton and oilseed rape.

In 2003 the commercial cultivation of genetically modified soybean in Brazil was legalised for a limited time period. Until then extensive areas of GM soybean were illegally grown in the southernmost province of the country.

Table 1 shows the area distribution as well as which genetically modified crops were grown in individual countries in 2003. The total global area was estimated to be 67.7 mio. hectares (James 2003).

Country	Area (ha)	Genetically modified crops
USA	42.8 mio.	Soybean, maize, cotton, oilseed rape, tomato, squash, papaya
Argentina	13.9 mio.	Soybean, maize, cotton
Canada	4.4 mio.	Oilseed rape, maize, soybean
Brazil	3.0 mio.	Soybean
China	2.8 mio.	Cotton
South Africa	400,000	Maize, cotton
India	125,000	Cotton
Australia	100,000	Cotton
Romania	70,000	Soybean
Uruguay	60,000	Soybean
Mexico	40,000	Cotton, soybean
Spain	32,000	Maize
Philippines	2,000	Maize
Colombia	5,000	Cotton
Bulgaria	5,000	Maize
Honduras	2,000	Maize
Germany	500	Maize
Indonesia	500	Cotton

Table 1. Global distribution of genetically modified crops in 2003 (Sources: 1) James, C. 2003. Global Status of Commercialized Transgenic Crops: 2003. ISAAA Briefs No. 30: Preview. 2) Trans-Gen-Informationsdienst, www.transgen.de).

In addition to the officially stated area with GM cotton, genetically modified tomatoes, pepper and petunia is believed to be cultivated in China.

Spain has until now been the only country within the European Union where commercial cultivation of genetically modified crops has taken place. Between 1998 and 2002, between 12,000 and 25,000 ha of a single genetically modified maize variety ("Compa Cb") has been cultivated each year. This area increased to 32,000 ha in 2003. The variety is derived from the transformation event "Bt176" and is resistant to attacks from the European Corn Borer.

In 2003 and 2004 five and nine new GM maize varieties, respectively, have entered the Spanish list of varieties. The varieties are derived from the transformation events "Bt176" or "MON810", and they are all resistant to the European Corn Borer.

The area with GM maize in Germany is cultivated according to a special arrangement where cultivation of GM plants which are not yet approved to be put on the variety list is allowed on a limited area. The harvest is cut and used as animal feed (silage).

2.2 Possible sources of admixture

There are in principle two sources of admixture, i.e. from GMOs already commercialized and from GMOs that are still only being tested in field trials.

2.2.1 Commercialized GMOs

GMOs can be dispersed by pollination from GM plants to conventional plants or by admixture of seed during harvest or subsequent handling of the harvested products. The GMOs in question can be either approved or non-approved in Europe. For example, some GM crops grown in USA and Canada are not approved in the EU.

As part of the approval process in the EU, companies commercializing the GMOs have to supply information on specific detection methods as part of the approval process. Also it should, in principle, be possible to acquire the needed DNA primer sequences from the relevant companies when the possible presence of non-approved GM plants has to be controlled.

2.2.2 GMOs from experimental releases

Some GM plants that are field tested in Europe have already been commercialized in other countries. Since these plants are well characterized at the molecular level, their presence can easily be detected. For these plants can be expected that event-specific methods have been developed.

GM plants that are field tested in an earlier stage of development are not necessarily that well characterized at the molecular level. Still, according to the EU deliberate release directive (2001/18) the developers of these plants have to deliver a description of techniques for detection and identification of the plants in their field trial applications.

3 Terminology/definitions

3.1 Detection

GMO detection methods are used to detect the presence of GMOs. An example is DNA screening methods where DNA elements common to several GMOs (e.g., the 35S promoter) are detected. These methods give no information as to which particular GMO is present and in what quantity. Accordingly, these methods are primarily used as a first test after which it is decided whether to continue with identification and/or quantification depending on the result.

3.2 Identification

Methods for GMO identification can be either trait specific, construct specific or event specific.

3.2.1 Trait specific methods

With trait specific methods the presence of, e.g., a specific herbicide tolerance trait is tested. Examples of this type of tests is immunological tests and germination tests (see below). The same trait may thus be present in several individually developed GM plants. Accordingly, the specificity of this kind of test is limited to the identification of, e.g., glyphosate tolerant plants.

3.2.2 Construct specific methods

These methods can identify the presence of specific constructs, i.e. promoter, gene and terminator (plus other potential sequences such as transit peptide sequences). However, since a specific construct can be present in several GM plants developed individually, such methods are still not completely specific.

3.2.3 Event specific methods

Ultimate specificity is obtained by the use of event specific methods. These methods identify the presence of the specific DNA sequences that span the junction between the plant host DNA and the inserted DNA. Since the site of integration of the gene construct in the plant genome is unique, the use of an event specific method will specifically identify the GMO in question.

3.3 Quantification

Determination of whether the GMO content complies with labelling provisions demands the use of quantitative methods. Quantification of the GMO content will typically be preceded by the identification of the GMO(s) present.

A typical procedure for testing the GMO content of a given sample is illustrated in figure 1.

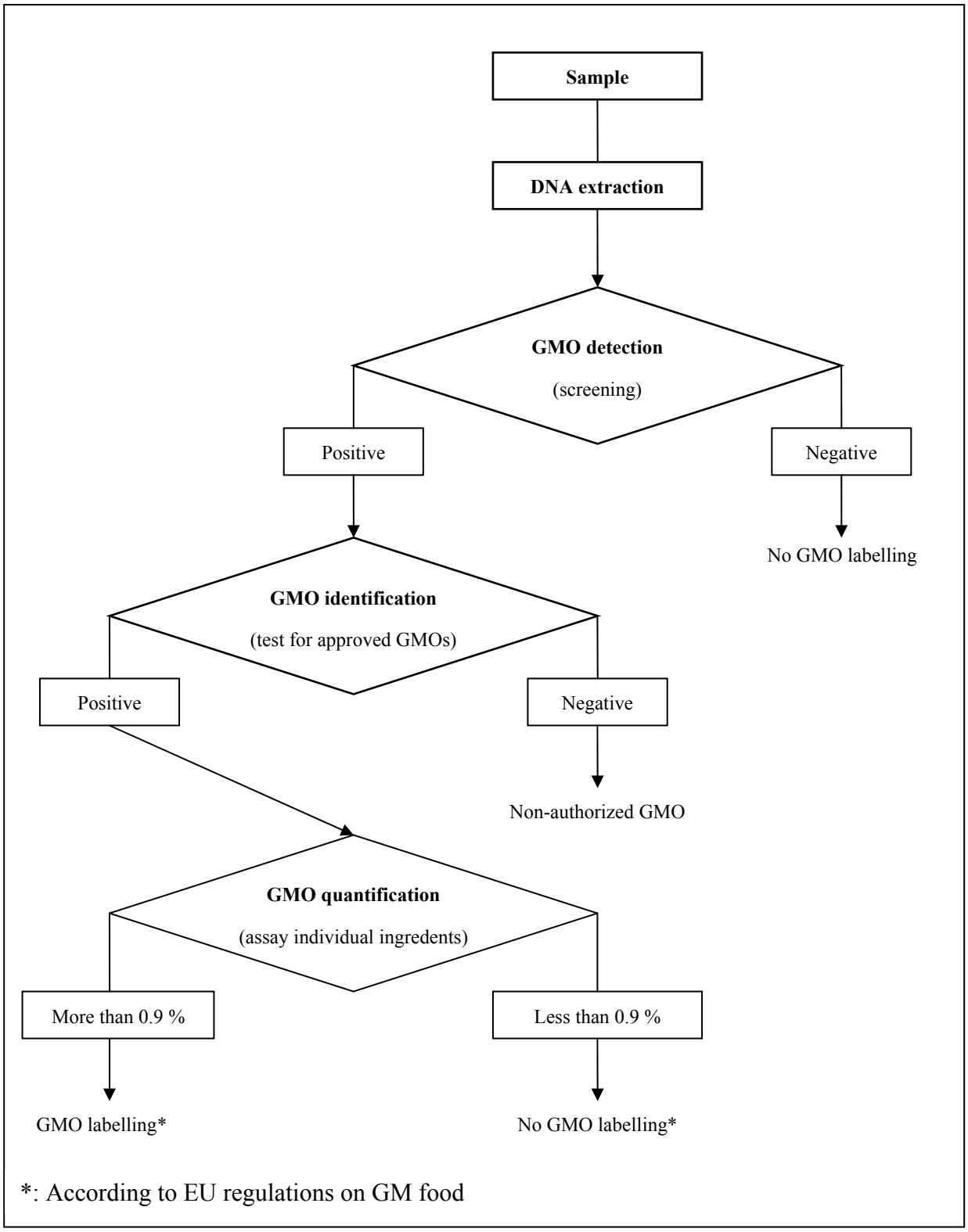


Figure 1. Procedure for testing a sample for GMO content (adapted from Hübner et al. 1999).

4 Analysis and control requirements in relation to seed and feed

Analysis for GMO contents in seed and feed is demanded by, e.g., the existence of labelling regulations, the need to control the presence of non-approved GMOs, and the need to monitor the effectiveness of co-existence regulations. Below is a short presentation of some of the control requirements and some difficulties associated with them.

4.1 Approved GMOs

For GMOs that are approved for commercialisation in the Nordic countries the primary need for GMO analysis will be to determine whether relevant labelling thresholds are observed.

In addition there is a need to monitor the possible dispersal of the GM plants into the environment as well as monitoring the effectiveness of possible cultivation distances between GM and non-GM crops.

4.2 Non-approved GMOs

Several of the GM plants that are commercially grown in, e.g., USA and Canada are not approved for commercialisation in Europe.

The possibility of analysing for the presence of non-approved GMOs depends on the availability of primers that are specific for the GMOs in question. It might, however, be envisaged that it would be difficult to acquire information on the relevant primer sequences for GMOs that are not even marketed in a given region.

4.3 Non-approved GMOs mixed with approved GMOs

A complicated situation occurs when approved and non-approved GMOs are mixed in a given sample. Then it may only be possible to analyse for the content of approved GMOs (assuming that all relevant primer sequences for these GMOs are actually available) whereas the content of non-approved GMOs may remain unknown.

In some cases, relevant primer sequences for the purpose of analysing specific GM plants are published in the scientific literature but it remains fortuitous for which GM plants such sequences can be found.

4.4 Several transformation events in the same GMO

A situation which is becoming more and more common is the occurrence of more than one transformation event in the same plant. This is the case, e.g., for GM hybrid oilseed rape and for several GM maize hybrids currently awaiting marketing approval in the European Union. Unless a specific marker is introduced in the hybrid between two GM

plants, it is not possible to determine whether a given sample contains the hybrid or a mixture between the two plants. The only possibility would be to analyse single grains which is not feasible for large samples. This problem is currently unsolved.

4.5 Conventional contra organic seeds/feedstuffs

According to the EU regulation on organic farming it is not allowed to use genetically modified organisms in organic farming. However, the regulation allows for the setting of a threshold for unavoidable presence of GMOs in organic products. Until now such a threshold has not been set.

In the Commission Recommendation of 23 July 2003 on guidelines for the development of national strategies and best practices to ensure the co-existence of genetically modified crops with conventional and organic farming it is stated that in the absence of such thresholds the general thresholds will apply. This means that so far the method requirements are the same for conventional and organic products. The only difference is that for conventional products with GMO contents above the threshold the product may still be sold on condition that they are labelled, whereas organic product with GMO contents exceeding the threshold must not be sold as organic.

5 Thresholds and labelling regulations

5.1 GMOs in conventional seeds and feedstuffs

In the EU the labelling regulations have recently been changed. With the entering into force of the Novel Foods Regulation in 1997 (Regulation (EC) 258/97 on Novel Foods and Novel Food Ingredients) genetically modified foods had to be labelled. Regulation (EC) 49/2000 introduced a 1 % threshold for the adventitious presence of DNA or protein (per ingredient) in conventional food resulting from genetic modification below which labelling was not required. With the entering into force of Regulation (EC) 1829/2003 on genetically modified food and feed the labelling threshold for GMO content in food is lowered to 0.9 %. The same threshold will apply for feed. Until then there was no specific regulation on approval or labelling of genetically modified feed.

In Norway the existing labelling threshold is 2 % per ingredient for food and feed. It is expected, however, that this threshold will be changed to 0.9 % in order to comply with the EU regulations.

In Iceland no threshold for GMO labelling has as yet been established but it is expected that EU labelling regulations will be adopted in Icelandic legislation in 2004.

As regards seed the question of labelling thresholds for adventitious presence of genetically modified seed in conventional seed was not settled at the time of writing the present report. Since the production of seed lies before the production of food and feed, the thresholds for seed have to be lower than 0.9 %. In the period 2000-2003 the EU Standing Committee on Seed and Propagating Material have discussed a Commission working paper proposing the thresholds shown in table 2.

Crop	**Labelling threshold**
Swede rape	0.3 %
Maize, beet, potato, cotton, tomato, chicory	0.5 %
Soy bean	0.7 %

Table 2. Labelling thresholds for seed proposed in working paper by the European Commission.

The different thresholds reflect differences in reproduction systems between the plant species concerned. However, certain EU member countries advocate for the thresholds to be as low as technically possible.

According to the EU seed legislation seed lots from genetically modified varieties shall be specifically labelled to indicate that the variety has been genetically modified. In addition, genetically modified varieties shall be clearly indicated as such in the official catalogues of varieties and in sales catalogues.

Contrary, this is not the case for the OECD lists of varieties (the OECD Seed Schemes) where there is no labelling of genetically modified varieties. However, this issue is currently being discussed in the OECD "Working Group on Genetically Modified Seed Issues". Some member countries feel that genetically modified varieties should be labelled on the list, whereas others do not. No agreement has yet been found on this matter.

Still, the International Seed Federation (ISF) has issued a "Position on OECD List of Varieties" (June 2003), where they state that "in order to take into account commercial practises and to facilitate the communication between National Designated Authorities, ISF will now accept the indication that a variety is a GM variety on the OECD list of varieties, for internal governmental use. ISF remains, however, strongly opposed to the compulsory labelling of GM varieties for international seed certification and marketing, although ISF members will comply with compulsory labelling requirements in individual countries" (www.worldseed.org).

5.2 GMOs in organic seeds and feedstuffs

As mentioned above, the EU Regulation (EC) 1804/1999 amending the organic farming regulation 2092/91 allows for the setting of a specific threshold for the unavoidable presence of GMOs in organic products. Since no such threshold has yet been set it is the opinion of the EU Commission that the general thresholds for adventitious presence of GMOs also apply to organic products (Commission Recommendation of 23 July 2003 on guidelines for the development of national strategies and best practices to ensure the co-existence of genetically modified crops with conventional and organic farming).

5.3 Non-approved GMOs

According to the EU regulation on genetically modified food and feed there is a transitional threshold for adventitious or technically unavoidable presence of genetically modified material of 0.5 %. The presence is allowed provided that the material has received a favourable scientific risk assessment by the Scientific Committees or the European Food Safety Authority before the date of application of the regulation and that detection methods are publicly available. The application of the threshold is limited to three years. It should be noted that this threshold is not a labelling threshold but only a threshold for the allowed presence of the relevant GMOs.

5.4 Thresholds around the World

Table 3 shows the current labelling thresholds for food products in various countries and regions in the World.

Country/region	Status of labelling	Labelling threshold
China	Mandatory	0 %
European Union	Mandatory	0.9 %
Australia	Mandatory	1 %
New Zealand	Mandatory	1 %
Saudi Arabia	Mandatory	1 %
Israel	Draft	1 %
Norway	Mandatory	2 % (to be changed to 0.9 %)
Switzerland	Mandatory	0.5 % for seeds 1 % for food 3 % for feed
South Korea	Mandatory	3 %
Malaysia	Draft	3 %
Brazil	Mandatory	1 %
Japan	Mandatory	5 % (selected products)
Hong Kong	Draft	5 %
Taiwan	Draft	5 %
Thailand	Draft	5 %
Russia	Mandatory	5 % (to be changed to 0.9 %)
Argentina	None required	-
Iceland	None required	- (to be changed to 0.9 %)
USA	Voluntary	-
Canada	Voluntary	-

Table 3. GMO labelling thresholds for food products in various countries and regions in the World (Source: GM Crops? Coexistence and Liability. Report by the Agricultural and Environment Biotechnology Commission, UK, November 2003).

As can be seen from the table labelling thresholds as diverse as 0 %, 0.9 %, 1 %, 2 %, 3 % and 5 % are in function in different countries and regions around the World. This is a situation which contributes to difficulties when products are traded across borders between countries with different thresholds. Obviously, international harmonization is needed in this field.

5.5 Analytic thresholds

In the DNA-based GMO testing methods (the PCR methods), there are limits of detection and quantification of GMOs, respectively. The detection limit is the smallest amount of GM DNA, which is measurable. The quantification limit is the smallest amount of GM DNA, which is necessary for measuring the actual content of GM DNA.

The theoretical limit for detection of GMOs by the PCR method is often stated as 0.01 % or less. In practice, the average detection limit will often be near 0.1 % because of sampling and measuring uncertainty.

The EU Scientific Committee on Plants has also in its statement of 7 March 2001 on the adventitious presence of GM seed in conventional seed, declared that the technical limit for detection is 0.1 % for routine tests.

In addition, some plant species (e.g. wheat) have a large genome (large amount of chromosomal DNA), that sets a limit on the minimum detectable quantity of GM DNA, because there are limits to the amount of DNA that can be present in the PCR reaction.

The relationship between the size of the genome and the detection and quantification limits are shown in table 4. The figures are stated for PCR tests in which 100 ng of DNA are used in the PCR reaction on the assumption that there must be 10 GM DNA copies available for detection and 100 GM-DNA copies for quantification. The stated values apply under optimum analytical conditions and will often be higher due to the uncertainty factors mentioned above.

Plant	Size of genome (1 C value)	Detection limit	Quantification limit
Oilseed rape	1.15 pg	0.01 %	0.12 %
Maize	2.73 pg	0.03 %	0.27 %
Soy	1.14 pg	0.01 %	0.11 %
Wheat	17.33 pg	0.17 %	1.73 %

Table 4. Practical limits of detection and quantification of GM DNA in different plant species.

As can be seen from the table, under the stated assumptions it is not technically possible to quantify DNA contents below 1.73 % in wheat. In this case there is a clear discrepancy between the EU labelling threshold of 0.9 % and what is technically possible.

The relationships mentioned apply to tests of the GM content in seed, which are relatively simple to carry out. In tests of admixtures the detection and quantification limits are increased because the measurable DNA is diluted. In tests of processed material it must be taken into account that the nature of DNA might change during processing, which increase the uncertainty, and hence the detection and quantification limits are proportionally increased.

The problem with enforcing low labelling thresholds in various legislations is highlighted by the results from proficiency studies conducted by, e.g., the Grain Inspection, Packers and Stockyards Administration in USA. As a rule, the results show that the lower the threshold, the higher the confidence interval around the test result will be. This creates a large degree of uncertainty as to how to interpret analysis results that lie in the area close to the threshold. In addition, results of such studies show a high degree of variation between laboratories.

Labelling thresholds may be viewed as a balance between consumer requests (the lower, the better), company requests (the higher, the better), and technical capabilities (the lower, the larger the error) (Guy Van den Eede, EU Joint Research Centre, cited in Anal. Chem. Vol. 75, Issue 17, 2003). The current low labelling threshold in Europe is a result of political compromise, and it can be foreseen that difficulties with its enforcement may arise.

In addition there are several ways to calculate the GMO level in a sample. For seeds, e.g., the GMO content can be calculated per haploid genome, as seeds by number or seeds by mass. However, it is not trivial how the GMO content is actually calculated. As an example, if a maize seed lot containing 1 % GM seeds with the GM trait in a hemizygous state were grinded, only 0.29 % of the DNA in the flour would contain the GMO allele (figure 2). In other words, according to EU regulations the seed would have to be labelled as containing GMOs, whereas the flour originating from the same seed would not.

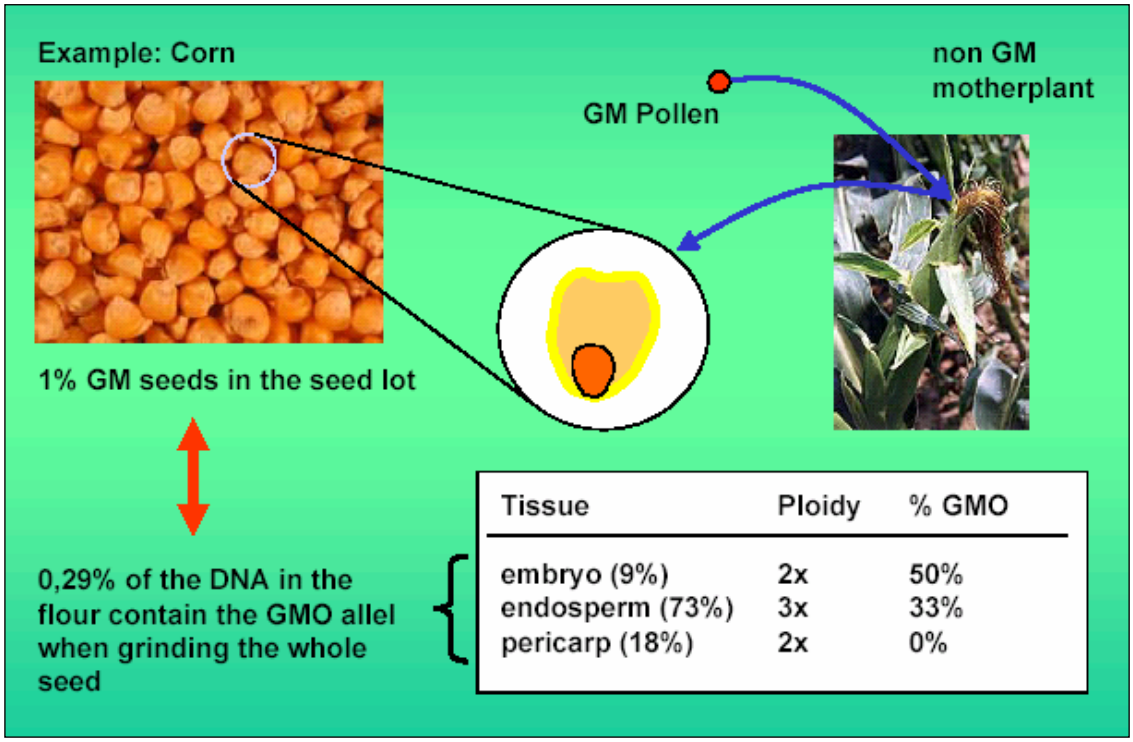


Figure 2. Difference between the proportion of GM seeds in a maize seed lot and the GMO content in the flour made from this seed lot (slide from the presentation "Sampling for GMO detection in the case of heterogeneous seed lots" by Michael Kruse, University of Hohenheim, Germany, at the Adventitious Presence (AP) Symposium and Workshop, June 13, 2002, Sioux Falls, SD, USA. Reproduced with permission from Michael Kruse).

For seeds, it is still being discussed whether to express GMO content at the seed or DNA level. In order to seek comparability with the way GMO content is expressed in foods and feeds it may be argued to express the GMO content in seed at the DNA level (GMO-DNA content as a percentage of total DNA content).

5.6 Unique identifiers

In February 2002 OECD published the "OECD Guidance for the Designation of a Unique Identifier for Transgenic Plants". The purpose of adopting a system of unique identifiers is to promote international harmonization of identification of genetically modified plants, e.g., on product labels in order to facilitate the identification of genetically modified plants in international trade. They will also serve as common entry points in various databases that are used to, e.g., register these plants.

Contained in the unique identifier code is information on the company responsible for developing the transgenic plant as well as information on the transformation event. As an example, the transgenic oilseed rape event "GT73" developed by the company Monsanto has been assigned the unique code "MON-00073-7" (where the last digit serves as a verification digit). Other examples can be viewed at http://www1.oecd.org/scripts/biotech/.

In the context of the EU Regulation on traceability and labelling of GMOs the OECD unique identifier system has been adopted in the EU legislation as “Commission Regulation (EC) 65/2004 establishing a system for the development and assignment of unique identifiers for genetically modified organisms”.

6 Methods of sampling and analysis

Several recent review articles contain detailed descriptions of the current methods of sampling and analysis of GMO contents (e.g., Anklam et al. 2002a; Bonfini et al. 2002; Holst-Jensen et al. 2003). The methods are therefore only described briefly below.

Sampling and testing for the presence of GM material may be carried out at several points "from farm to fork". For instance, samples can be taken of the seed before sowing, of the plants in the field, of the crop products after harvest and at various points during further processing. Further, samples of feed and manure can be tested. For the purpose of this report only sampling of seed and feed is described.

A decision on how to take samples and test for GM content will be a balance between which analysis will be most relevant and the costs of these analyses, as most analyses are still very costly.

A “European Commission recommendation on technical guidance for sampling and detection of genetically modified organisms and material produced from genetically modified organisms as or in products in the context of Regulation (EC) No 1830/2003” (on traceability and labelling) is expected to be adopted in 2004. A draft of the recommendation contains guidance on protocols for sampling genetically modified seed lots, for sampling of bulk agricultural commodities (grain), and for sampling lots of food and feed products. In addition, the draft contains guidance on analytical test protocols.

6.1 Sampling

For all sampling methods the challenge is to take a sample that is representative of the original lot. Thus, the results of analysis can be totally dependent on the original sample and subsequent sub-samples being representative of the original lot (see below under “Special analytic problems”). There is also a relation between the size of the sample and the threshold value to be complied with. The lower the threshold value, the larger the sample has to be. The relationship between threshold values and corresponding sample sizes is illustrated in table 5.

Threshold value	Laboratory sample size (number of particles)	
	Homogeneous distribution of GM particles	Inhomogeneous distribution of GM particles
0.1 %	35,000	100,000
0.5 %	7,000	20,000
1 %	3,500	10,000
2 %	1,750	5,000
5 %	700	2,000
10 %	350	1,000

Table 5. Sizes of laboratory samples in case of a homogeneous and an inhomogeneous distribution of GM particles (20 % overall sampling error) (elaborated from Hübner et al. 2001).

6.1.1 Seeds

For the seed testing, one may use the rules on sampling from ISTA (International Seed Testing Association). A working group under the EU's Standing Committee on Seeds and Plant Propagation Material recommends that these rules should be used for checking conventional seed for its GM content. Furthermore, the group recommends a laboratory sample size of 3,000 seeds to detect threshold values of 0.3-0.7 %.

In the context of the introduction of provisions on thresholds for labelling of the GMO content of seeds in the EU seed trade directives a regulation on a protocol for sampling and testing of seed lots of non-genetically modified varieties for the presence of genetically modified seed is expected to be adopted in 2004.

The European Commission Joint Research Centre has issued a review on different existing sampling approaches for grain lots described in documents from ISTA, USDA/GIPSA, CEN, ISO, WHO/FAO, and in a specific EU directive (98/53) (Kay 2002). The laboratory sample size in the sampling approaches specifically related to testing for GMO content ranges between 2,400 and 10,000 grains.

Seed weights vary depending on the plant species concerned. The sample sizes mentioned in Hübner et al. (2001) when testing for compliance with a 1 % labelling threshold give rise to the laboratory sample weights shown in table 6 for different cereals and oilseeds.

Species	Seed weight (mg)	Laboratory sample size (g)	
		Homogeneous distribution 3,500 particles	Heterogeneous distribution 10,000 particles
Barley	37	140	370
Corn	285	1,000	2,850
Oat	32	112	320
Oilseed rape	4	14	40
Rice	27	95	270
Rye	30	105	300
Soybean	200	700	2,000
Wheat	37	140	370

Table 6. Laboratory sample sizes of different cereals and oilseeds needed when testing for compliance with a 1 % labelling threshold (from Hübner et al. 2001).

6.1.2 Feedstuffs

As regards animal feeds there is a general EU directive that states methods for sampling different types of feedstuffs and for the official testing of feedstuffs (First Commission Directive 76/371/EEC of 1 March 1976 establishing Community methods of sampling for the official control of feedingstuffs).

However, for the specific detection of GMOs in feed the expected ISO/DIS standard on sampling methods for the detection of GMOs in food would also seem to be applicable.

6.2 Analysis

The current GMO analytical methods can roughly be divided into protein-based and DNA-based methods.

6.2.1 Protein based methods

The protein-based methods are the fastest, cheapest and the most simple to perform. The methods are based on the development of antibodies that are specific against new proteins that are produced in the GM plants. The currently commercially available methods for analysis for GM plants have been developed for B.t. toxins, which result in insect resistance, and for herbicide tolerance. As some of the proteins are common in different GM plants, the methods can only be used for detection of the GM characteristic but not for identification of the individual GMOs.

The most sensitive protein-based method is the so-called ELISA method (Enzyme-Linked Immuno Sorbent Assay) which is a laboratory-based method. The method is

suitable for both detection and quantification, but since the protein content may vary considerably, the quantitative determination is not considered to be reliable.

The lateral flow strip test is an analysis that can be carried out in just 10-20 minutes. The method does not require a laboratory. Tests can be carried out "in the field", for example on seed lots. The test can only be used for detection (but not the quantification) of the mentioned GM types and is a useful provisional screen for GM content.

6.2.2 DNA based methods

The most often used DNA-based methods are the so-called PCR (Polymerase Chain Reaction) methods, which can be used for both qualitative (detection and identification) as well as quantitative analyses.

The PCR tests must be carried out in a laboratory, and require more time and are more expensive than the protein-based methods. On the other hand, they are far more sensitive and specific than protein-based methods. PCR analyses are considered to be 10 and 100 times more sensitive, respectively, than ELISA and lateral flow strip tests. This method is used when one has to determine unambiguously which GM genes may be present in a given product.

With a PCR test the presence of the inserted gene itself is studied. If one examines the transition between the inserted gene and the plant's own DNA, the individual GM plant ("transformation event") can unambiguously be identified. A PCR-test is performed with the use of pairs of primers which are small pieces of DNA specific for the DNA to be analysed.

In the PCR methods, the logical sequence is first to carry out a qualitative analysis to detect the GM genes. This is followed by quantification of GM content if the first analysis is positive. The limit of reliable quantification of GM content is generally considered to be 0.1 %.

6.2.2.1 Qualitative methods

Qualitative methods are used for detection and identification of GMOs. Detection of whether GMOs are present or not is typically the first step in an analysis for GMO content. For this purpose the use of screening techniques are often used, i.e. screening for common genetic elements found in a range of GM plants (e.g., the 35S promoter).

Once the presence of GMOs has been detected it is relevant to analyse which specific GMO is present. This is to determine whether the GMO (s) in question is (are) approved for commercialisation in a given country or area. For this purpose primers which are specific for the event (s) in question is (are) used in the PCR-test.

6.2.2.2 Quantitative methods

The two principal tests used for quantification of GMO content are competitive PCR and real-time PCR.

In competitive PCR tests the target sequence to be analysed is amplified together with an additional target sequence of known concentration. The two targets then compete for available nucleotides, primers and DNA polymerases in the reaction. The amount of end products is measured when the PCR reaction is completed. Because the relative quantity

of end products, that are present when the reaction is finished, is assumed to correspond to the relative quantity of the two targets at the beginning of the PCR reaction, it is possible to calculate the original GMO content of the sample.

With real-time PCR tests the amplification of target sequences is measured directly during the reaction by measuring a fluorescence signal which develops in the course of the reaction. The advantage of this method compared to competitive PCR is that it is faster to perform and involves fewer steps that might cause cross contamination. As a result, real-time PCR is currently the preferred method of the two.

An additional requirement when performing a quantitative PCR-test is the amplification of a reference gene which typically is a species-specific single copy gene. This is because the GM content is measured relatively to the genome copies of the species in question.

It is not necessarily simple, however, to find suitable species-specific genes which only occur as a single copy. As described by Hübner et al. (2001) many cultivated crop plants contain a high number of gene duplication as a result of the breeding processes. As an example, several varieties of maize should be tested to verify that the copy number of a given target sequence does not vary within this species.

6.2.2.3 Biochips

The relatively recently developed "micro-array" technique is suitable for screening and identifying many GM plants in a single test. The test is performed on a small glass plate on which a specific piece of DNA from each GM plant that is to be analysed is fixed. In the analysis, DNA from the inserted genes in the GM plants that may be present in the tested sample is fixed to the corresponding DNA on the glass plate. The analysed DNA is labelled beforehand so that it can subsequently be visually detected on the glass plate.

In this way, it will be possible to test for the presence of all GM plants that are approved in the EU at the same time. Depending on getting access to specific DNA sequences from the GM plants that, e.g., is approved in the USA but not in the EU, it will be possible to include these in the tests as well.

Currently, there is an EU project running with a view to developing these methods for testing the GMO content in foods (www.gmochips.org).

It is not yet possible to carry out reliable quantitative analyses using micro-arrays. At present the method can be used for the initial detection and identification of GM plants, after which the quantity should be determined by quantitative PCR.

6.2.3 Alternative methods

A number of alternative methods of GM testing have been developed. Several of the methods relate specifically to the characters expressed by the relevant GM plants. Some of these methods are described briefly below.

6.2.3.1 Germination tests (bioassays)

These tests are suitable for screening for the presence of herbicide tolerant GM crops by letting seed germinate on a herbicide-containing medium. Such tests are relatively inexpensive but will typically last between 7 and 10 days. Examples are glyphosate tolerant

maize (Goggi and Stahr 1997), glufosinate-ammonium tolerant maize (Payne 1998) and glufosinate-ammonium tolerant oilseed rape (Pfeilstetter et al. 2000).

At present commercial germination tests have been developed for Roundup Ready (RR) soybean, RR and Liberty Link (LL) maize, RR and LL oilseed rape, RR cotton and LL sugar beet (see, e.g., www.mwseed.com/gmo-testing.htm).

6.2.3.2 Tetrazolium test

Another method for screening for presence of herbicide tolerant seeds is the tetrazolium test in which sliced seeds are first imbibed in a herbicide solution and then soaked in a tetrazolium solution (Ayala et al. 2002). In this test the embryos of herbicide tolerant seeds are coloured red whereas embryos of herbicide susceptible seeds in comparison have a more whitish colour. Compared to the germination test this test is quicker to perform, lasting only 24 hours.

6.2.3.3 Insect resistance bioassay

A bioassay method for identification of insect resistance in maize (corn borer resistance) has been developed by the French institute Institut National de la Recherche Agronomique (INRA). Within the context of the International Union for the Protection of New Varieties of Plants (UPOV) such a method is being discussed in their Technical Working Party for Agricultural Crops.

6.2.3.4 Chromatography

For GM plants with altered composition in, e.g., fatty acids as a result of genetic modification, it is possible to use chromatographic methods to detect differences in the chemical profiles between GM and conventional plants (Bonfini et al. 2002).

Such methods are, however, only suited for qualitative detection because of natural variations in the contents of such compounds.

6.2.3.5 Near infrared spectroscopy (NIR)

NIR-analysis can be used to detect altered fibre structures in plants resulting from genetic modification. In Bonfini et al. (2002) an example with detection of Roundup Ready soybean is described.

6.2.3.6 Microfabricated devices and nanoscale GMO analysis

A brief technical description of potential future methods for analysing very small samples and/or for rapid analysis for GMO content can be found in Bonfini et al. (2002). Several of the methods could be applicable for analysis on-site, i.e. outside the laboratory. Some of these methods are based on the PCR technology whereas others are not.

6.3 Future developments

The increasing number of GM plants that are expected to appear on the market in the future makes it increasingly difficult to test for their presence in any given material to

be analysed. However, in 2003 a couple of new developments appeared which could make it easier to identify GMOs in the future.

In February 2003 the National Institute of Agricultural Botany (NIAB) in the UK was granted a patent on a so-called bar-coding technique (www.niab.com). The patent exploits the nature of DNA as a molecule which holds information. By using the bases that naturally make up DNA molecules to encode information that is not "genetic", it is possible to insert several kinds of information into the genetically modified plants. The patent comprises four different systems for encoding information.

One simple application is to insert information common to all GM plants. In this way the detection of presence of GM material in, e.g. seed or feed would be simple because the laboratory would only have to look for the presence of one particular sequence.

For the purpose of identification a particular GM plant, additional information about company name, species, the year of commercialization and the composition of the gene construct could be inserted.

In addition, in the March 2003 issue of Nature Biotechnology, Marillonnet et al. describes a somewhat similar system for encoding information on company name, date of production and a database reference number into the DNA of GM organisms.

7 Validation of methods of analysis

For GMO labelling thresholds to be enforced there is a requirement to use validated methods so that one can be sure that the results of a GMO analysis is the same independent of the laboratory having performed the analysis. In addition, the laboratory should also be accredited according to international standards.

For the testing of foods the European Commission has issued a recommendation of 25 January 2002 concerning a coordinated programme for the official control of foodstuffs for 2002. The recommendation contains a list of validated methods to be used to test certain foodstuffs for compliance with the food labelling regulations. Some of the methods would also be applicable for the testing of feed.

On the homepage of the "Biotechnology and GMOs Unit" of the European Commission Joint Research Centre (http://biotech.jrc.it/) there is a link to a page on validated methods with a further link to a database of validated methods as well as links concerning criteria to be met prior to method validation. The "Database of validated methods"-page contains links to the database as such as well as links to reports on PCR, PCRELISA and ELISA methods.

In addition, in the context of the EC Regulation 1829/2003 on genetically modified food and feed implementation rules are expected to be issued in 2004. A draft of the implementing rules contains guidelines on validation of GMO detection methods in an annex to the rules. In the annex there is reference to a document entitled "Definition of minimum performance requirements for analytical methods of GMO testing". The document proposes a set of method acceptance criteria as well as method performance requirements.

"Method acceptance criteria" are criteria, which should be fulfilled prior to the initiation of method validation by the future Community Reference Laboratory (CRL). The "method performance requirements" define the minimum performance criteria that the method should demonstrate upon completion of a validation study carried out by the CRL. The aim of setting these criteria is to ensure that the methods validated are fit for the purpose of enforcement of the regulation on genetically modified food and feed.

The process of validation and subsequent standardisation of analysis methods can be illustrated as shown in figure 3.

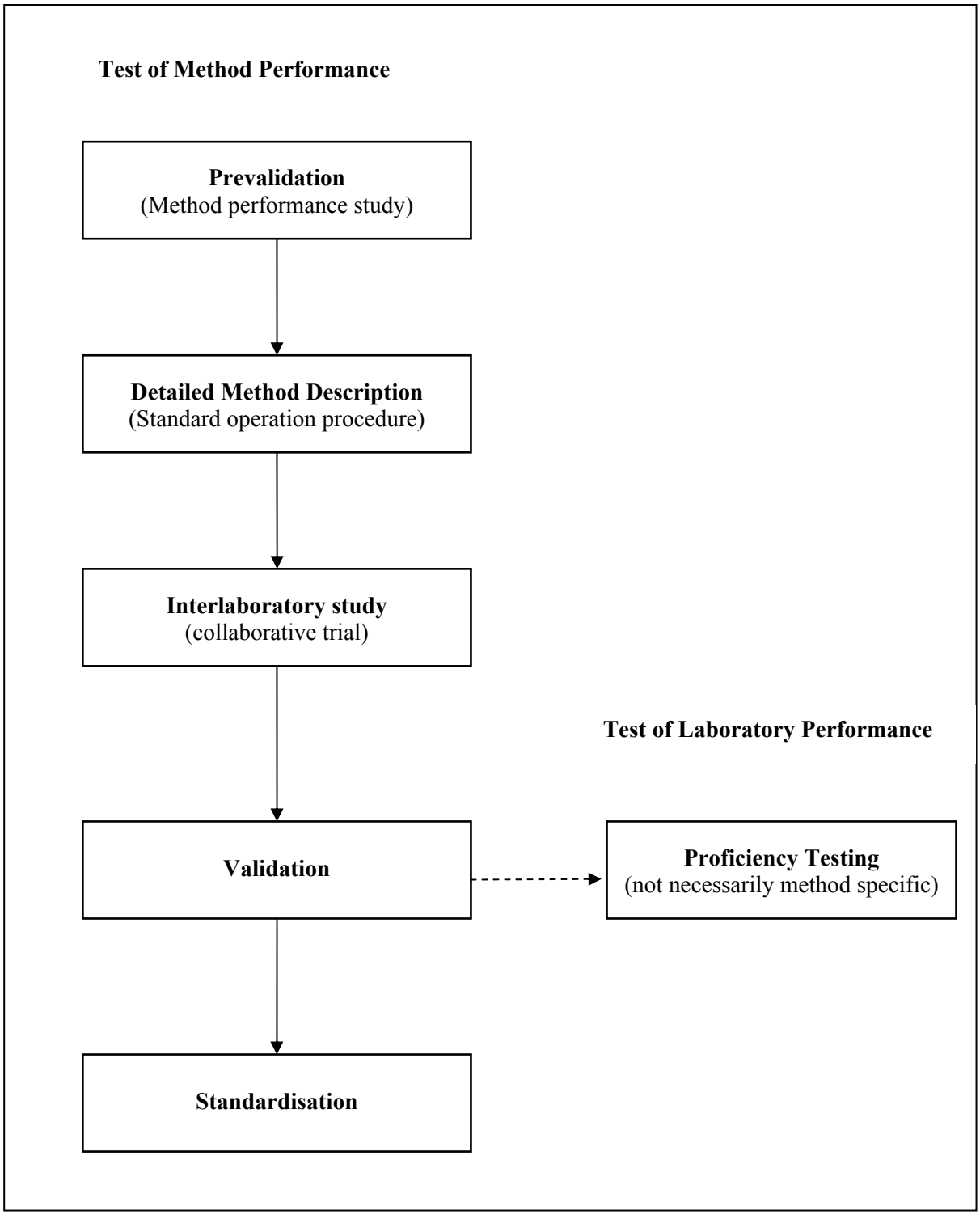


Figure 3. Process of validation of GMO analysis methods.

Prior to a real validation study the method in question should undergo a pre-validation study in order to make initial assessments of method performance. This is because larger-scale validation studies require considerable efforts and resources. A pre-validation study typically involves the participation of 2-4 laboratories.

The output of the pre-validation study is a detailed method description (the Standard Operation Procedure; SOP) which is to be followed by the laboratories participating in the validation study. The validation study is performed with the participation of at least 8 laboratories.

If the method performs satisfactorily in the interlaboratory study it can be validated. A range of parameters to be assessed during the validation process have been defined (Anklam et al. 2002b). These include, e.g., specificity, accuracy, precision, detection limit, quantification limit and robustness of the method.

Once validated the method may be accepted as an international standard by the European (CEN) or international (ISO) standardisation bodies. The European standards organisation CEN is currently in the process of developing standards for sampling and analysis of GMOs.

GMO analysis laboratories may participate in proficiency testing programs. Such tests are not test of methods but rather tests of the ability of the laboratories to reach a correct result. The tests are carried out by distributing test materials which contain specific amounts of GM material unknown to the participating laboratories. The choice of the method to be used is left to the laboratories.

8 Special analytic problems

8.1 The sampling process

There is a whole range of potential problems associated with taking samples and preparing them for analysis. In addition, at each step in this process an error is introduced. The challenge is to minimize the unavoidable sampling error.

A sample can be either homogeneous or heterogeneous for the character to be analyzed (GM/non-GM; see tables 5 and 6). If the sample consists of a mixture of GM and non-GM particles it is defined as heterogeneous. A lot or sample containing GM mixed with non-GM seed is thus to be regarded as heterogeneous because it is made up of two kinds of particles. Feed consisting of such a mixture would also be regarded as heterogeneous.

In a heterogeneous sample the distribution of the GM particles can be either random or non-random. Furthermore, in a sample with a non-random distribution the GM particles can be evenly distributed or aggregated in clumps.

The typical sampling and preparatory steps when taking seed samples are illustrated in figure 4.

The first sampling stage in this process is normally regarded as the most critical one as the actual distribution of the genetically modified material in the lot is not known beforehand. For seed it is reasonable to assume that the distribution of heterogeneity in the lot in most cases will be non-random, whereas in feed, which is often a mixture of different components, the distribution of GM particles in the lot would tend to be more random.

The effect of heterogeneity at this sampling step (lot to bulk sample), when sampling kernel lots, has been investigated in a recent publication (Paoletti et al. 2003). The results of simulations showed that the current procedures used to produce bulk samples, as laid down in international sampling guidelines, are not very suitable if the distribution of GM-kernels in the lot is non-uniform. In cases of heterogeneous distribution of GM kernels, bulk samples thus have a high probability of not correctly representing the lot. In addition, there is a high probability that the bulk sample will not contain any GM kernels and thus give rise to a false-negative result.

In the subsequent sampling stages, where the bulk sample is reduced in size, the distribution of genetically modified material often can be regarded as random provided that thorough mixing of the sample is done. However, through the processes of seed grinding and DNA extraction changes in the proportions of GM/non-GM units may be introduced.

As can be seen from the figure, at the grinding and DNA extraction steps there is a change in the type of units sampled (from kernels to particles, and from particles to molecules).

Depending on the plant family there may be different contributions of GM DNA from the seed coat, endosperm and embryo as can be seen in figure 5. Grinding may thus introduce a change in proportions of GM and non-GM units in the sample because of different parental contributions and different ploidy levels of the tissues.

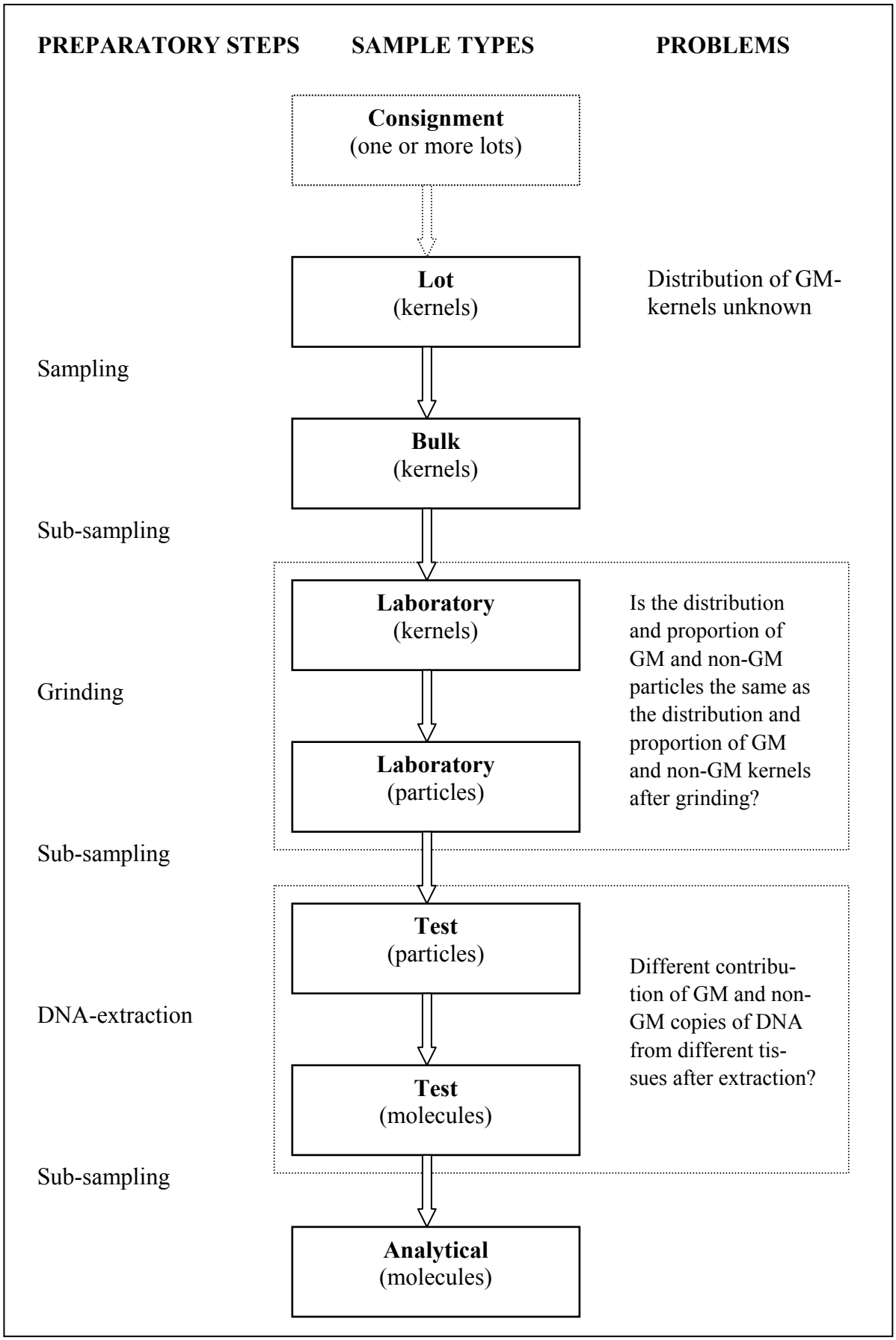
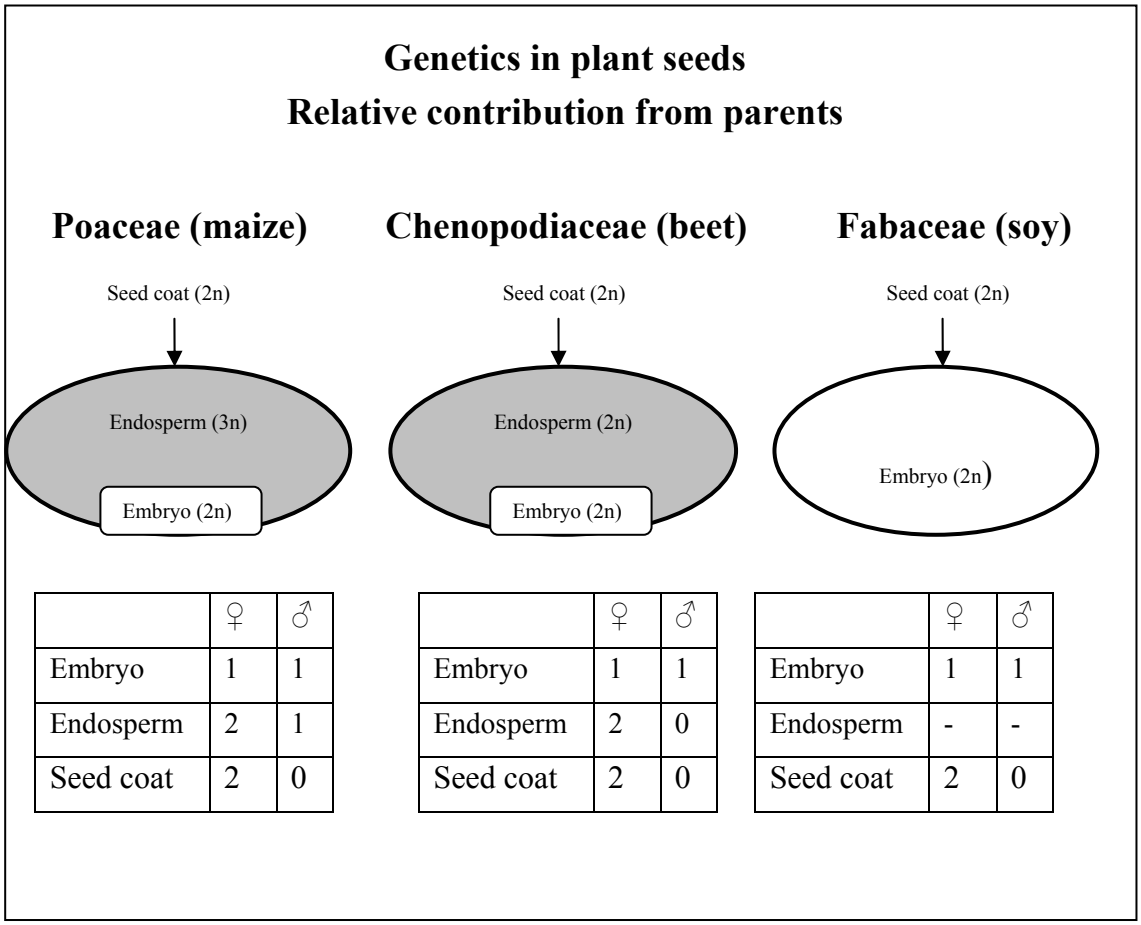

PREPARATORY STEPS
SAMPLE TYPES
PROBLEMS
Consignment
(one or more lots)
Lot
(kernels)
Distribution of GM-kernels unknown
Sampling
Bulk
(kernels)
Sub-sampling
Laboratory
(kernels)
Is the distribution and proportion of GM and non-GM particles the same as the distribution and proportion of GM and non-GM kernels after grinding?
Grinding
Laboratory
(particles)
Sub-sampling
Test
(particles)
Different contribution of GM and non-GM copies of DNA from different tissues after extraction?
DNA-extraction
Test
(molecules)
Sub-sampling
Analytical
(molecules)

Figure 4. Typical sampling and preparatory steps for seed analyses.



	♀	♂
Embryo	1	1
Endosperm	2	1
Seed coat	2	0

	♀	♂
Embryo	1	1
Endosperm	2	0
Seed coat	2	0

	♀	♂
Embryo	1	1
Endosperm	-	-
Seed coat	2	0

Figure 5. Examples of different relative contributions of DNA from seed tissues depending on plant family (adapted from presentation by Arne Holst-Jensen, The Veterinary Institute, Norway, at the 3rd meeting of the project group, 23-24 April 2002).

This effect may be even more profound during the extraction step. The contribution of GM copies and non-GM copies of DNA depends on the ploidy of the tissues. In addition, there may be differences in the quantity and quality of DNA that can be extracted from reserve tissues in comparison to embryonic tissues. Furthermore, in some tissues cell nuclei may have undergone endoreduplication.

8.2 The analysis process

An example of a potential error in the analysis process is the result of differences in DNA primer design between the target DNA and the DNA used as a reference.

During the process of amplification of DNA in a PCR analysis the reference DNA and target DNA may be amplified with different efficiency due to such differences. If, for example, the rate of amplification per PCR cycle is 1.9 for the reference DNA and 1.8 for the target DNA, then after 30 cycles the reference DNA will have been amplified five times more efficiently than the target DNA ($(1.9/1.8)^{30}$).

8.3 The expression of test results

There is concern that the expression of GMO content can lead to different conclusions depending on expression as % seeds or % DNA content. As mentioned in the maize example in chapter 5, there are examples where, e.g., seed would have to be labelled for GMO content whereas the flour resulting from grinding the same seed would not. As long as no consensus has been reached on this issue, this contributes to confusion regarding how to interpret GMO analysis results.

8.4 Variation between laboratories in analytic results

Several proficiency testing programs have shown a certain variation between laboratories in their ability to obtain correct results after testing samples with specific GMO contents. Examples of this are reported in the description above of the proficiency tests arranged by ISTA. As illustrated in the described examples, it is important that GMO analysis laboratories participate in such programmes in order to improve their ability to report correct results. It would be a precarious situation if the test result is too much dependent on which GMO laboratory is chosen to carry out a GMO analysis. An example of this problem is illustrated in the next section.

9 Ring analysis experiment

As a way to illustrate the possible variation in results when a given sample is sent to different laboratories for analysis of GMO content a simple ring test experiment was carried out among the members of the project group. This was to show the analysis situation at a moment when there was no standards existing yet as to how to perform the analysis. In other words, each laboratory used its own methods, only the samples were presumed to be identical.

The material to be analysed in the ring test was maize meal imported to Iceland from USA, soy meal imported to Denmark from USA, and oilseed rape seed made up as mixtures of conventional seed and GM seed in Sweden. It was known a priori that the maize meal contained the transformation events MON810, Bt11 and Bt176, that the soy meal contained Roundup Ready (RR) soy, and that the two oilseed rape seed samples was made up of 10 RR seeds in 10,000 conventional seeds (0.1 %; sample "A") and 50 RR seeds in 10,000 seeds (0.5 %; sample "B"), respectively.

Contrary to the oilseed rape samples, the true GMO concentrations in the maize and soy samples were not known beforehand. Accordingly, for these samples only the variation between laboratories can be compared. The results of the ring test are shown in table 7.

Species	**Norway** Veterinary Institute	**Denmark** GeneScan	**Iceland** GeneticID	**Finland** Customs Laboratory	**Sweden**		
					Livsmedel-verket	Scan-Gene	Agrogene
Maize							p35S: 13 %
Total				13.7 %	22 %	24.6	tNOS: 22 %
- MON810	36 %	21.5 %	42 %	5.2 %	6.7 %	%	
- Bt11	9 %	8 %		4.4 %	10.2 %		
- Bt176	17 %	9 %		4.1 %	5.1 %		
	10 %	4.5 %					
Soy							
- RR	< 2 % (0.5 %)	4 %	1.3 %	3.4 %	2.5 %	1.6 %	1.3 %
Oilseed rape							
- RR (A)	Detected	< 0.1 %				0.1 %	0.2 %
- RR (B)	Detected	0.5 %				1.7 %	> 50 %

Table 7. Results of GMO ring test.

As can be seen from the table, some of the tests were carried out by national laboratories, and others by commercial laboratories.

For the maize sample the results varied between 13.7 and 42 % total GM maize content which is a fairly large variation. However, the GMO content is in any case way above any existing labelling threshold. But the variation serves to illustrate how different the results of a GMO analysis could be at that time depending on which GMO laboratory carried out the test.

The analysis of the soy sample showed comparably much less variation than the maize analysis. Again the true concentration was not known, but the result varied between 1.3 and 4 %, which in any case is above the EU labelling threshold. However, the Norwegian result is below the Norwegian labelling threshold, so in this case the soy meal would have to be labelled in the EU countries but not in Norway.

Only four of the participating laboratories were able to test for GM content in oilseed rape. Apart from the result which showed a GM content above 50 % (which is probably an artefact) the results were pretty close to the true value. However, the results concerning the “B” sample illustrate a situation were the seed would have to be labelled in the EU in one case and not in the other (0.5 and 1.7 %, respectively).

The possible sources of variation of especially the maize results could be uneven distribution of GM material in the sample, variation caused by different methods of sample preparation and variation caused by using different methods of analysis.

10 Overview of international activities on GMO analysis

Below a non-exhaustive description of GMO analysis related activities in diverse international forums is presented.

10.1 The European Union

a) The European Commission has issued a recommendation of 25 January 2002 concerning a coordinated programme for the official control of foodstuff for 2002. The recommendation aims to check the compliance with community law regarding labelling of foods and food ingredients, which may contain or consist of or may be produced from GM soy and maize. It states that Member States should carry out GMO analysis on foodstuffs containing GM soy and/or maize using recommended validated methods. The results of member state inspections and controls should be reported to the Commission on a record sheet provided in the recommendation.

In the recommendation there is a list of methods that at the date of issue were being used in a large number of proficiency tests and either had been annexed to the CEN standards on GMO testing that were under development (elaborated by the CEN working group CEN/TC275/WG11) or would be submitted before long.

b) The European Commission Joint Research Centre (JRC) provides scientific support for the development and implementation of the biotechnology regulations in the EU. The JRC is involved in developing methods for GMO detection, identification and quantification. Furthermore the JRC is responsible for validating these methods, and strengthen the harmonisation of qualitative and quantitative GMO analysis. In connection with the validation process, ring-trials are being conducted and organised. Once validated the method protocols are submitted to the user community and international standardisation bodies.

The GMO unit of JRC is mainly involved in the development and the validation of methods for GMO detection and quantification as well as other issues such as sampling problems. Current projects involve research in the stability of plant transgenes, identification and promotion of protein and DNA based analytical methods and identification and promotion of appropriate sampling strategies. In relation to these activities the unit has set up various relevant databases such as:

- A database which provides an overview of analytical methods for DNA and protein detection and quantification.
- A molecular register to host all DNA sequences of authorised GMOs as well as the tools for analysis.

In addition, JRC has set up the European Network of GMO Laboratories (ENGL) which provides a platform for experts to discuss technical issues such as those related to GMO analysis (http://engl.jrc.it). In addition, ENGL participates in the technical evaluation

and validation of quantitative detection methods for GM food and feed. ENGL currently consists of more than 70 national control laboratories.

Of special interest to the subject of the present report is the "KeLDA" (Kernel Lot Distribution Assessment) project which aims to investigate the distribution of GM admixture in soybean grain lots imported in the EU from different countries outside the EU. This project is the first study which will provide data on the real situation concerning such admixtures. The results of this study will give insight into the way GM material is distributed in raw bulk materials. It is expected that the outcome of the study will be the elaboration of sampling strategies that will allow effective sampling of such lots. Until the end of 2003 12 soybean lots had been sampled and analyzed for the presence of GM material. A preliminary analysis of the results has indicated that distribution patterns do vary among lots and do show heterogeneity.

c) According to the EC Regulation 1829/2003 on genetically modified food and feed a Community Reference Laboratory (CRL) on GMO testing has to be established. The reference laboratory has now been established by the JRC. A new web-site of the CRL – gmo-crl.jrc.it – was launched in April 2004 at the time of application of Regulation 1823/2003.

d) As part of the 5th framework programme the European Commission has subsidised the research programme ENTRANSFOOD (the European Network on Safety Assessment of Genetically Modified Food Crops; http://www.entransfood.com/). This programme package consisted of four working groups: 1) Safety Testing of Transgenic Foods, 2) Detection of Unintended Effects, 3) Gene Transfer, and 4) Traceability and Quality Assurance. The results of the research programme were presented at a concluding conference held in May 29-30, 2003.

A part of the work on traceability and quality assurance has been the QPCRGMOFOOD project (period February 2000 to July 2003) which aimed at developing reliable and transformation event-specific tests for qualitative and quantitative detection of genetic modifications in food and for determination of the diversity of genetic modifications in food (http://www.vetinst.no/Qpcrgmofood/Qpcrgmofood.htm).

As a continuation of the QPCRGMOFOOD project a new project on the development of biochips to detect Genetically Modified Organisms (GMOs) in food has been initiated in 2001 (www.gmochips.com, duration 2001-2004).

e) Within the European Union there has been set up a network for inspectors from regional and national GM inspectorates involved in sampling, testing and monitoring of GMOs. The network which is called the European Enforcement Group on Deliberate Release of GMOs (EEG-DR) was initiated in 1999 by the Ministry of the Environment, Nature and Forestry, Schleswig-Holstein Land, Germany. On their webpage (http://eep-mon.iitb.fhg.de/) it is possible to find, e.g., Standard Operating Procedures (SOPs) on sampling methods and documentation.

10.2 OECD

The Organisation for Economic Co-operation and Development (OECD) has been working in biotechnology related topics for almost 20 years. The internal Co-coordination group for Biotechnology facilitates co-operation between the various OECD activities and there are various working groups associated with activities in the genetic

genetic engineering field. These working groups include; Working group on Harmonisation of Regulatory Oversight in Biotechnology and Task Force on the Safety of Novel Foods and Feed which in the 2003-2005 period will be involved with risk and safety assessments, outreach activities such as maintaining and developing the BioTrack Database of Field Trials and the development of consensus documents which provides technical information.

OECD has in February 2002 published guidelines for the designation of a unique identifier for transgenic plants. The unique identifier is an alphanumeric code designed for each biotechnology product approved for commercial use. It has been designed to ensure lack of duplication between different products. The purpose of the unique identifier is for it to be used as a 'key' to access information in the OECD product database and interoperable systems (see below for further details).

Other relevant work that has been undertaken by the Harmonisation of Regulatory Oversight in Biotechnology Working Group includes a questionnaire which was circulated to OECD member countries. The aim of this questionnaire was to be an information gathering exercise on the topics of detection, monitoring and identification of products/organisms derived from modern biotechnology (OECD 2003). The report concluded that most of the 20 respondents, including non EU member states, had some sort of framework and labelling requirements in place.

The GMO issue is also discussed in the context of the OECD Schemes for the Varietal Certification of Seed Moving in International Trade (see Chapter 5).

10.3 ISTA

The International Seed Testing Association (ISTA) has established a working group called the GMO Task Force. The aim of the task force is 1) to establish an ISTA Rules Chapter for the detection, identification and quantification of GMO in conventional seed lots, 2) to organise proficiency tests on GMO testing in conventional seed, and 3) to set up a platform for the exchange of information between laboratories.

Regarding the rules chapter, it will not contain specific methods, but will define a level of reproducibility required to report test results on an ISTA International Seed Lot Certificate.

The task force is structured into four working groups: 1) a Strategy Working Group, 2) a Rules Chapter Working Group, 3) a Proficiency Test Working Group, and 4) an Exchange of Information Working Group.

The Proficiency Test Working Group organized an international proficiency test on GMO testing in May 2002. 43 laboratories across the world took part, including both private and governmental laboratories. The aim of the test was to check the ability of the participating laboratories to detect the presence of GM seeds (originating from the transformation events ('MON810' and 'T25') in samples of conventional seed of *Zea mays*. The result of the 1st proficiency test showed that approximately 70 % of the participants were able to identify all 30 maize samples correctly with the remaining 30 % showing problems with GMO testing to a varying degree (ISTA 2002).

The 2nd proficiency test started in February 2003 with the aim of checking the participant's ability to detect and quantify the presence of GM maize seeds originating from

the transformation event 'MON810'. 47 laboratories took part in the qualitative test out of which 85 % reported correct results for all samples and the remaining 15 % reported false results for all or some of the samples. 13 laboratories took part in the semi-quantitative test where the sample had to be classified above or below 1% GMO content. One laboratory classified all samples correctly and 11 laboratories categorised between one and five samples falsely. 19 laboratories reported quantitative test results. The quantitative test was performed either as a test to estimate the percentage value of the GMO content or a test to determine whether the GMO contents were above or below 1 %. A range of different results were reported on these tests. Only 4 laboratories classified the GMO content correctly (ISTA 2003).

A 3rd proficiency test started in December 2003. The results of this test have not been published at the time of writing the present report.

In addition to these activities, the ISTA GMO Task Force arranges workshops on statistical aspects of GMO detection.

10.4 Codex Alimentarius

The Codex Alimentarius Commission (http://www.codexalimentarius.net/) established the ad hoc intergovernmental Task Force on Foods Derived from Biotechnology in 1999. The purpose of the Task Force was to develop standards, guidelines or recommendations on foods derived form biotechnology. The scientific basis for the work of the Task Force was provided by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organisation (WHO) through a series of scientific expert consultations. The Task Force established two Ad Hoc Working Groups at its first session in Japan in March 2000. The first group, chaired by Japan, was to develop texts on; the principles for the risk analysis of foods derived from modern biotechnology and guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants. The second group, chaired by Germany, was to compile a list of analytical methods including those for the detection or identification of food ingredients from biotechnology. The Task Force has completed the work and a full report has been submitted to the Commission. Further relevant activities includes that of the Codex Committee on Methods of Analysis and Sampling which on the 26th session in June-July 2003 agreed to consider criteria for methods of analysis for foods derived from biotechnology for the next session in the spring of 2004.

10.5 CEN

The European Committee for Standardization, CEN (http://www.cenorm.be/cenorm/index.htm), is currently in the process of approving a set of six standards on methods of analysis for the detection of genetically modified organisms and derived products. The standards comprise methods of sampling and methods of protein and DNA analysis:

1) Methods of analysis for the detection of genetically modified organisms and derived products - Sampling (ISO/DIS 21568:2003).
2) Methods of analysis for the detection of genetically modified organisms and derived products - Qualitative nucleic acid based methods (ISO/DIS 21569:2002).

3) Methods of analysis for the detection of genetically modified organisms and derived products - Quantitative nucleic acid based methods (ISO/DIS 21570:2003).
4) Methods of analysis for the detection of genetically modified organisms and derived products - Nucleic acid extraction (ISO/DIS 21571:2002).
5) Methods for the detection of genetically modified organisms and derived products - Protein based methods (ISO/FDIS 21572:2003).
6) Nucleic acid based methods of analysis for the detection of genetically modified organisms and derived products - General requirements and definitions (ISO/DIS 24276:2002).

10.6 USDA/GIPSA

Following the appearance in 2000 of StarLink maize in food products the Grain Inspection, Packers and Stockyards Administration (GIPSA), under the United States Department of Agriculture (USDA), established a special testing program for sampling and detection of StarLink maize. More information on the testing program can be found on this web page: http://www.usda.gov/gipsa/biotech/starlink/starlink.htm.

GIPSA have recently developed a more elaborate biotechnology program to meet the need for product differentiation in the marketplace. In 2001 a biotechnology reference laboratory was set up to ensure the availability of reliable tests. Furthermore GIPSA conducted a Proficiency study to assess the capability of DNA-testing for U.S commercialised biotechnology events in maize. The study showed a significant variation in the capability of the participating laboratories to analyse for biotechnology events. In February 2003, GIPSA began to offer a Proficiency Program, for organisations testing for biotechnology derived grains and oilseeds, in an attempt to improve the reliability of testing. 54 organisations from countries world-wide participated in the program. Approximately half of the participants chose samples for qualitative analysis, and the other half chose samples for quantitative analysis. The samples for qualitative analysis were fortified at 0.1 %, and the samples for quantitative analysis were fortified at levels ranging from 0.1 % to 5.0 %. The variability of the results of the quantitative analysis was relatively high, especially at lower fortification levels. Generally, the average values reported were lower than the target fortification levels, particularly at the higher fortification levels.

10.7 Others

The Convention on Biological Diversity adopted a supplementary agreement to the Convention known as the Cartagena Protocol 29 January 2000 (http://www.biodiv.org/biosafety/default.aspx). The objective of the protocol is to contribute to the protection of biological diversity from the potential risk posed by Living Modified Organisms (LMO's). This is achieved by the establishment of an Advanced Informed Agreement (AIA) procedure. AIA applies to the first intentional transboundary movement of LMO's for introduction into the environment of the party of export. The importing country is supplied with a detailed written description of the LMO, which enables an assessment of the possible risks associated with the import and pro-

vides the means to make an informed decision before agreeing to the import. The Protocol entered into force on 11 September 2003, ninety days after the receipt of the 50th instrument of ratification. The Protocol has also established a Biosafety Clearing-House (BCH) to facilitate the exchange of information on LMO's and to assist countries in the implementation of the protocol. The BCH is established in a phased manner and is currently available in the pilot phase version. The BCH will set up a series of databases containing information on LMOs. One of the databases will provide information on the unique identification of LMOs.

The International Seed Federation (ISF; http://www.worldseed.org/), which is an international organisation of seed companies, has issued several position papers on biotechnology, e.g., on the issue of adventitious presence of GM material in non-GM seeds. Since the entering into force of the Cartagena Protocol on Biosafety on 11 September 2003, the ISF on its website provides information on the documentation requirements for shipments of LMOs for contained use or intentional release into the environment. For example, there is information on the "Advanced Informed Agreement" required according to the Protocol as well as an example of a standard commercial invoice to be used when selling genetically modified seed.

Phytosanitary risks that may be associated with LMOs are not presently integrated into international legislation. The Interim Commission on Phytosanitary Matters (ICPM) is under FAO responsible for the International Plant Protection Convention (IPPC; http://www.ippc.int/), which will provide instruments for risk analysis of certain LMO's that can be defined as: 'any species, strain, or biotype of plant, animal or pathogenic agent injurious to plants or plant products'. ICPM has established an Expert Working Group on Risk Analysis for LMO's, aiming to provide standards on risk analysis procedures as regards the photysanitary risk that may be presented by certain LMO's. A draft supplement to one of the existing International Standards for Phytosanitary measures (ISPMs) – Supplement to ISPM No. 11 (Pest Risk Analysis for quarantine pests) on Pest Risk Analysis for living modified organisms – was released for country consultation in 2003. In order to be able to identify a particular LMO, information on appropriate detection and identification methods and their specificity, sensitivity and reliability is regarded as important.

11 The GMO analysis situation in the Nordic countries

11.1 Norway

11.1.1 Threshold and labelling regulations

Import and marketing of seeds from genetically modified plants, are only permitted if the GMO is allowed in Norway in accordance with the Gene technology Act. Seeds from varieties which have been produced by gene modification shall have a label which says “Genetically modified”.

GM feed products produced in or imported to Norway must be labelled according to a parliamentary decision from 1995. The Norwegian labelling requirements on feed entered into force in 1999. The labelling requirements laid down in Regulation on feedingstuffs are considered to be satisfied if products containing genetically modified ingredients are labelled as such if the genetically modified component constitutes more than two percent of the ingredient. Expressions recommended used in the legislation are ‘genetically modified ….. (name of product)’, or ‘produced of/from genetically modified.... (name of product/ingredient)’. Feed products without DNA and protein do not have to be labelled.

Import and marketing of feeds from genetically modified plants is only permitted if the GMO is allowed in Norway in accordance with the Gene technology Act.

Norway also has a ban against antibiotic resistance genes from GMOs in feed. This ban entered into force in June 2002.

11.1.2 Official GMO analysis activities

The Norwegian Food Safety Authority is responsible for the GMO control in food, feed - for both land animals and fish - and seeds (until 2004 the Norwegian Food Control Authority was responsible for control in food, the Norwegian Agricultural Inspection Service for feed for land animal and seeds and the Directorate of fisheries for feed to fish).

11.1.2.1 Sampling

Sampling of seed is done according to general ISTA sampling methods (sampling for germination and purity tests).

Feed samples are taken according to First Commission Directive 76/371/EEC of 1 March 1976 establishing Community methods of sampling for the official control of feedingstuffs.

11.1.2.2 Analysis

All official GMO analyses are performed by the Norwegian National Veterinary Institute.

Seed is analyzed qualitatively for the presence of transformation events MON810, Bt176 and Bt11 in maize and GT73 in oilseed rape.

Feed is analyzed quantitatively for RR soy and MON810, Bt176, Bt11 and GA21 maize. In addition, feed is analyzed qualitatively for the presence of the antibiotic resistance genes *bla*, *nptII*, *nptIII*, *cat*, *aad*, *tet pBR322*, and *tet pRK290*.

As a result of the EU project QPCRGMOFOOD (see below), qualitative and quantitative analysis of T25 and DBT418 maize and of Topas 19/2 and Ms8xRf3 oilseed rape as well as qualitative analysis of CBH351 (StarLink) maize will be possible, provided that validation of the methods are successful.

11.1.3 Results of GMO controls 2001-2003

Until 1 October 2003 the results of the GMO analyses were stated semi-quantitatively like this:

- Much more than 2 % (>10 %)
- More than 2 % (3-10 %)
- Approximately 2 % (1-3 %)
- Less than 2 % (0.1-1 %)
- Trace amounts (<0.1 %)
- Not detected (0 %)

From 1 October 2003 the Veterinary Institute has begun to state the results more precisely as values.

Despite the rather extensive control activities, GMO admixtures have only detected in a few cases as can be seen in table 8 for feed.

Year	No. of samples	GMO not detected	<0.1 % GMO	0.1-1 % GMO	Appr. 2 % GMO	3-10 % GMO	>10 % GMO
2001	86	66	14	6	0	0	0
2002	92	54	35	2	0	1	0
2003[1)]	71	32	31	7	0	0	1

[1)]: 1 January-15 November

Table 8. Results of GMO analyses of feed samples in the period 2001-2003 in Norway.

Comparatively fewer seed samples have been analysed. Until 15 November 2003 no GMOs has been detected in seed.

11.2 Sweden

11.2.1 Threshold and labelling regulations

As an EU member state Sweden respects the EU legislation on GMOs.

11.2.2 Official GMO analysis activities

Inside Sweden the following institutes are responsible for the GMO control:

The Swedish Seed Testing and Certification Institute (SUK) for control in seed, the Swedish Board of Agriculture (SJV) for control in feed, and the Swedish National Food Administration (NFA) for control in food.

In all types of feed and food products the laboratory of NFA is responsible for analyses of adventitious presence of GM material in conventional products.

Seed for sowing of soybean, maize, oil rape, sugar-beet from third countries must be sampled and analysed for adventitious presence of GM seed. Seed lots for export, produced inside Sweden, of oilseed crops and sugar beets are checked in the same way.

The laboratory Agrogene in France has been used to analyse conventional seed lots for adventitious presence of GM seed. In the autumn 2003 the laboratory ScanGene AB in Sweden was checked and approved to make the same type of analysis.

SUK has checked the biosafety/laboratory system at the Hilleshög/Syngenta company used to check the adventitious presence of GM seed in conventional sugar beet seed.

11.2.2.1 Sampling

The ISTA sampling rules are used for sampling of seed. All sampling is done by official sampling officers of SUK.

Sampling of conventional feed is done according to the Commission directive 76/371 ECC. The sampling in raw material and feed products is carried out by inspectors or local authorities.

11.2.2.2 Analysis

Laboratories in France and Sweden, used by Swedish authorities for control of adventitious presence of GM seed in conventional seed lots, are accredited according to ISO 17025. The laboratories are able to detect and quantify all, up to now, known GM seed in conventional maize, soybean , oilseed rape and sugar beet seed.

11.2.3 Results of GMO controls 2001-2003

Species	No. of samples	GM seed not detected	GM seed detected
Winter oilseed rape	35	35	0
Spring oilseed rape	20	20	0
Winter turnip rape	6	6	0
Spring turnip rape	9	9	0
Maize	3	3	0
Total	**73**	**73**	**0**

Table 9. Seed lots checked for adventitious presence of GM seed 2001-2003 in Sweden.

The production of a few lots of organic oilseed rape seed inside Sweden has started. Special care must be done in the future regarding testing for adventitious presence of GM seed in these seed lots.

Species	No. of samples	GM material not detected	GM material detected
Maize	9	5	4
Soybean	15	8	7[1)]
Total	**24**	**13**	**11**

[1)]: All below 1 %.

Table 10. Feed checked for adventitious presence of GM material in 2002 in Sweden.

11.3 Finland

11.3.1 Threshold and labelling regulations

As an EU member state Finland respects the EU legislation on GMOs.

11.3.2 Official GMO analysis activities

The Plant Production Inspection Centre is responsible for the GMO control of seed and feed in Finland. All official GMO analyses are performed by the Finnish Customs Laboratory. The Finnish Customs consists of the National Board of Customs, of five customs districts, and of the Customs Laboratory. The Customs Laboratory primarily carries out laboratory examinations relating to foreign trade and performs services for the Customs, but also to other authorities such as Ministries and central and local au-

thorities. The Customs Laboratory has been given official status for food control under the Food Act (361/95) and a Decision (710/96) by the Ministry of Trade and Industry, with a specified field of competence, which in reality covers the most important inspection methods it applies.

Under the Finnish Food Act (361/95), which conforms to the Council Directive 89/397/EEC, the control of imported food products and transit goods has been assigned to the customs administration, subordinated to the Ministry of Finance. Thus, Finland seems to be the only country in the EU, where the customs authority is not only responsible for the official control of food products as required by the Council regulation (EEC) No 339/93 but is also the competent authority for the import control of food products of non-animal origin excluding foodstuffs covered by specific veterinary legislation. According to the Product Safety Act (914/86) the Customs have to control imported consumer goods.

11.3.2.1 Sampling

One sample has been taken from each targeted lot of seed and feed.

11.3.2.2 Analysis

At routine controls with a view to analysing GMOs, the Customs Laboratory uses the real-time PCR methods (LightCycler) for identifications and quantifications of GMO proportions in different kind of food products.

11.3.3 Results of GMO controls 2001-2003

11.3.3.1 Seed

2001: 5 samples of oilseed rape and 5 samples of turnip rape were analysed and no GMOs was detected.

2002: No seed samples were analysed.

2003: 37 spot samples of 10 different species were analysed and no GMOs was detected.

11.3.3.2 Feed

Year	No. of samples	GMO not detected	< 0.1-1 % GMO in ingredient	> 1% GMO in ingredient
2001	16	4	6	6
2002	4	0	3	1
2003	49	14	30	5

Table 11. Soybean samples tested for GMO content in the period 2001-2003 in Finland.

Year	No. of samples	GMO not detected	< 0.1-1 % GMO in ingredient	> 1% GMO in ingredient
2001	-	-	-	-
2002	1	0	detected	0
2003	12	12	0	0

Table 12. Oilseed rape samples tested for GMO content in the period 2001-2003 in Finland.

11.4 Iceland

11.4.1 Threshold and labelling regulations

Until now no GMO labelling thresholds have existed in Iceland. However, GMO seed is regulated in Icelandic legislation, whereas GMO feed is not.

11.4.1.1 Seed

There is official document control on all imports of seed to Iceland. All seed imported, produced or packed, has to be reported to the Feed, Seed and Fertilizer Inspectorate (Adfangaeftirlit). The agency will in the future also make physical inspections and collect official control samples for analysis. If GMO seed is found it is reported to the Environment and Food Agency by the Adfangaeftirlit and at the same time inspection takes place on the origin and type of GMO involved.

The Environment and Food Agency is responsible for coordination and registration (based on EU approval) of GMO in seeds as stipulated by Icelandic act nr. 18/1996 on GMO and regulation nr. 493/1997 on distribution of GMO into the environment. These are based on Council Directive 90/220/EEC, Commission Directive 94/15/EC, Council Decision 91/596/EEC and Commission Decisions 92/146/EEC, 93/584/EEC and 94/211/EC.

Most plant varieties containing GMOs are not fit for cultivation nor will they be able to produce mature seed in Iceland. The agriculture is mostly based on grassland farming although barley production is increasing. The only possibility of GMO plants would be in greenhouses. Recently an Icelandic company has started research work on GM barley for production of specific proteins for medicinal purpose. This is still in experimental stages. Otherwise no seed of GMO plants is produced in the country.

In 2004 Council Directives 2002/53/EC, 2002/54/EC, 2002/55/EC and 2002/57/EC will be adopted into Icelandic legislation. At the same time proposed EU legislation on GMO seed will most likely also be adopted.

11.4.1.2 Feed

There is official document control on all imports of feedingstuffs to the country. All feed imported, produced or packed, has to be reported to the Feed, Seed and Fertilizer Inspectorate (Adfangaeftirlit). It will in the future also make physical inspections and collect official control samples for analysis.

GMO feedingstuffs from third countries mainly U.S.A. have been freely imported to Iceland if they only contain GMO already approved by the EU. Although not based on analysis for GMO it can be assumed that most compound feeds in Iceland contain GMOs.

In 2004 Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed and Regulation (EC) No 1830/2003 of the European Parliament and of the Council of 22 September 2003 concerning the traceability and labelling of genetically modified organisms and the traceability of food and feed products produced from genetically modified organisms and amending Directive 2001/18/EC will be adopted to Icelandic legislation. After that control of GMOs in feedingstuffs in Iceland will be done by Adfangaeftirlit.

11.4.2 Official GMO analysis activities

There are no official laboratories in Iceland analysing GMOs. No GMO analysis for feed and seed control has been done. However, Regulation (EC) No 1829/2003 and Regulation (EC) No 1830/2003 will be adopted into the EEA agreement and consequently into Icelandic law next year. Similar regulations concerning GMO seeds will be adopted into Icelandic laws. Therefore in the future the Feed, Seed and Fertilizer Inspectorate will be inspecting feed and seed for GMO content, traceability and labelling.

11.4.2.1 Sampling

Random samples for control purpose will be collected in accordance with EU regulations. A limited number of targeted samples can also be collected for specific studies.

The Feed, Seed and Fertiliser Inspectorate will be responsible for the collection and preparation of the samples.

11.4.2.2 Analysis

There are no plans to establish a laboratory for GMO analysis in connection to these control activities, but rather to send the samples abroad for analysis. In the future GMO analysis will probably be performed by LUFA-Nord-West in Germany.

11.4.3 Results of GMO controls 2001-2003

No GMO control samples have been collected regularly. However from the limited number of samples collected it is known that feedingstuffs such as maize and soybean meal from third countries - mainly U.S.A. - have contained GMO already approved by the EU.

11.5 Denmark

11.5.1 Threshold and labelling regulations

As an EU member state Denmark respects the EU legislation on GMOs.

11.5.2 Official GMO analysis activities

The Danish Plant Directorate is responsible for the GMO control of seed and feed in Denmark.

Since the incident in 2000 where oilseed rape seed imported from Canada was found to contain small amounts of GMO seed, imports of seed from third countries which are known to grow GM crops of the relevant species have been controlled. Information on seed imports from third countries is received from the Danish customs authorities. In addition, organic seed lots from EU countries where GM crops are grown are controlled.

Until now only organic feed has been controlled for GMO contents. However, when the Regulation on genetically modified food and feed enters into force also conventional feed will be controlled for compliance with the labelling provisions in the regulation.

In order to be prepared to the future control of conventional feed the Plant Directorate tested 102 conventional feed samples collected in 2002 for the presence of GMOs. The results of this survey are presented below.

11.5.2.1 Sampling

For the control of organic seed and feed the procedure has until now been only to take one sample from each lot for analysis. This is because the Danish interpretation of the organic regulations has been that any presence of GMOs was forbidden. Accordingly, there was no need for the sample to be representative. However, after the publication of the Commission Recommendation on coexistence of genetically modified crops with conventional and organic farming this situation is likely to be changed (see below under "Thresholds and labelling regulations").

For the control of conventional seed and feed the general sampling methods apply (ISTA sampling methods for seed, Commission directive 76/371/EEC for feed). It is expected, though, that specific methods for sampling GM seed will appear in the near future accompanying the expected amendments to the EU seed trade directives regarding adventitious presence of GM seed in conventional seed.

11.5.2.2 Analysis

Samples are analyzed qualitatively for presence of RR soy and Bt176 and Bt11 maize in the laboratory of the Plant Directorate. Samples that are tested positive for GMO content are sent to a commercial laboratory for quantification. Since 2003 the Plant Directorate laboratory has also begun to analyze RR soy quantitatively. However, samples are still sent to a commercial laboratory for verification of results.

11.5.3 Results of GMO controls 2001-2003

11.5.3.1 Organic feed

Results of the control for GMO contents in organic feed in the period 2001-2003 are shown in table 13.

Year	No. of samples	GMO not detected	<0.1% GMO or with traces[1)]	0.1-1% GMO in ingredient	> 1% GMO in ingredient
2001	136[2)]	60	26	3	47
2002	210	165	3	34	8
2003	208	188	0	17	3

[1)]: Category used when GMO is found in dust, i.e. in an ingredient that was not declared and must not be found in the mixture.

[2)]: Four seed samples included

Table 13. Results of the control for GMO contents in organic feed in the period 2001-2003 in Denmark.

As can be seen from the table the presence of GMOs in organic feed has generally fallen during the period from 2001 to 2003.

11.5.3.2 Conventional feed

The study was based on 102 feed samples collected in the autumn of 2002 from 40 feed manufacturers in different parts of Denmark followed by DNA analyses (PCR) in order to determine the content of material from 10 GM soy and maize lines.

All the analysed 91 samples of compound feeding-stuffs with a content of soy feed materials (soy-bean cake) contained DNA from Roundup Ready soy (GTS 40-3-2) which is tolerant to the herbicide glyphosate. Quantification of the DNA content in 32 of the samples suggested that the soy-bean cakes usually were derived from crops with a high content of Roundup Ready soy, since the proportion of Roundup Ready soy in the soy constituted 40-100% in 29 of the samples. Analyses of samples from three lots of soy-bean cake for the preparation of compound feed gave similar results with respect to GM soy.

19 samples of compound feeding-stuffs with a content of maize raw materials were analysed for content of GM maize lines with resistance to herbicides or insects. GM maize were detected in five of the samples (MON810, Bt176, T25 or NK603/GA21 maize), but the levels were low and could not be determined. The low occurrence of GM maize materials in compound feed was confirmed by analyses of four maize products used for preparing compound feed.

11.5.3.3 Seed

Until now it has only been necessary to test a few seed lots imported from countries outside the European Union. No GMOs has been found in these samples.

12 Conclusions

A report describing current GMO analysis methods is rapidly outdated because of the continuous development in this area.

In addition, a detailed description of the current GMO analysis methods would only be a reiteration of the currently available scientific reviews and other reports on the same subject. Therefore, the present report only gives a brief overview of the current as well as the expected future analysis methods. Instead it tries to emphasize some of the current problems related to sampling as well as to the applicability of the current methods of analysis for the enforcement of low GMO labelling thresholds.

A general message highlighted by the examples described in Chapter 8 on special analytic problems is that sampling and analysing for GMO contents is not a simple task. Although the analytical methods are very sensitive, the trueness of the analytical result will always depend on whether the sample reflects the actual distribution of GM material in the consignment or lot to be analysed.

As mentioned in the report, labelling thresholds may be viewed as a balance between consumer requests (the lower, the better), company requests (the higher, the better), and technical capabilities (the lower, the larger the error).

Examples in the report illustrates that the practical possibilities of respecting low labelling thresholds – at least with the currently used methods of quantitative analysis - are highly dependent on the species involved. This is a message which is important to be canalised to the political levels where the decisions regarding the setting of labelling threshold levels are made.

In addition, there is a need for international harmonisation of GMO labelling thresholds. Currently, labelling thresholds vary between 0 and 5 %. If GMO labelling thresholds were to be harmonised, the highlighted problems with enforcement of too low thresholds should be taken into account. In this respect the current 0.9 % labelling threshold in the European Union may be considered as being too low. This is, among other things, because it is not applicable for all species (e.g., wheat) given the current methods for GMO quantification.

As illustrated by an example with maize seed, the outcome of an analysis can be very dependent on whether the sample has been grinded or not. This problem highlights the need for standardisation of the way samples are prepared for analysis as well as the way results are expressed.

The expression of GMO contents ought to be percentage of GM DNA in total genomic DNA in order to assure a harmonised approach throughout the supply chain. This recommendation is cited from the latest version (March 2004) of a draft “Commission recommendation on technical guidance for sampling and detection of genetically modified organisms and material produced from genetically modified organisms as or in products in the context of Regulation (EC) No 1830/2003”. In relation to seed, this is a deviation from the traditional way of expressing seed analysis results which is on a percentage of seed basis.

The issue of interlaboratory variability in producing GMO analysis results is highlighted by the results of the small "ring analysis" experiment described in Chapter 9. Even though there were no conditions set as to which methods for sample preparation or analysis should be used, the example does illustrate how results may vary depending on which laboratory performs the analysis. In some cases the result may lie above the labelling threshold and in others below. This is a problem which could be predicted to give rise to difficulties, if controversies regarding the observation of labelling thresholds are to be decided in court.

Finally, Chapter 11 on GMO analysis activities in the Nordic countries illustrates the extent of such activities in the individual countries regarding seed and feed. Apart form Iceland, all Nordic countries analyse seed and feed samples for GMO contents. However, with the adoption of the EU regulations on GM food and feed as well as on traceability and labelling of GMOs into Icelandic law, GMO analysis activities are expected to commence in Iceland too in 2004.

The kinds of laboratories that carry out GMO analyses vary between countries. In Norway and Finland all GMO analyses are performed by national laboratories, in Denmark and Sweden both national and commercial laboratories are used, whereas in Iceland the future GMO analyses will be performed by a commercial laboratory.

As can be seen from the results reported by the Nordic countries the general picture is that no GMOs were found in the seed samples tested whereas feed samples often contain GMOs. Regarding feed this situation probably reflects the lack of labelling thresholds in feed until now. This situation could change with the entering into force of the new EU regulation on GM food and feed.

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Web pages:

Biosafety Clearing House: http://www.biodiv.org/biosafety/default.aspx

CEN: http://www.cenorm.be/cenorm/index.htm

Codex Alimentarius: http://www.codexalimentarius.net/

Community Reference Laboratory: http://gmo-crl.jrc.it

ENTRANSFOOD-project: http://www.entransfood.com/

European Commission Joint research Centre: http://biotech.jrc.it

European Enforcement Group on Deliberate Release of GMOs: http://eep-mon.iitb.fhg.de/

European Network of GMO Laboratories: http://engl.jrc.it

GIPSA: http://www.usda.gov/gipsa/

GMOchips-project: http://www.gmochips.org

International Plant Protection Convention: http://www.ippc.int/

International Seed Federation: http://www.worldseed.org

ISTA: http://www.seedtest.org

Mid-west Seed Services, Inc.: www.mwseed.com

National Institute of Agricultural Botany: http://www.niab.com

OECD: http://www.oecd.org/home/

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