



Genetically Modified Organisms in food and feed

Annual Report 2010 of the Dutch National Reference Laboratory

RIKILT Report 2011.006

I.M.J. Scholtens-Toma, B. Molenaar, S. Zaaijer, M.M. Voorhuijzen-Harink, T.W. Prins, E.J. Kok



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Summary

This is the annual report of the Dutch National Reference Laboratory (NRL) for Genetically Modified Food and Feed (RIKILT – Institute of Food Safety). The report gives an overview of the NRL activities carried out in 2010.

In 2010 RIKILT participated in one ring trial for inter laboratory validation of an event-specific GMO detection method organised by the European Union Reference Laboratory for Genetically Modified Food and Feed (EURL-GMFF). Both RIKILT and the Routine Field Laboratory of the new Dutch Food and Consumer Product Safety Authority (nVWA) participated with good results in several proficiency tests.

Also RIKILT participated in EURL/NRL workshops and the Working Group on Method Verification, the Task Force 'New Techniques' NTTF and the Working Group Unauthorised GMOs.

In 2010 four times confirmatory analysis were carried out for the nVWA on rice samples potentially containing Kefeng rice and/or Bt63 rice. All nVWA sample results were confirmed by RIKILT.

RIKILT has a flexible scope accreditation for real time PCR GMO analysis in raw materials, food and feed.

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1 Introduction

The Dutch Ministry of Health, Welfare and Sports and the Ministry of Economic Affairs, Agriculture and Innovation are responsible for the maintenance of EU regulations in the area of GMOs, i.e. 'European Regulation (EC) 1829/2003 and 'European Regulation (EC) 1830/2003. Both legislations 1829/2003 and 1830/2003 came into force in 2004.

Regulation (EC) 1829/2003 'European Regulation (EC) on genetically modified (GM) food and feed' states that food or feed products containing GMOs must be labelled as such. There is a 0.9% labelling threshold for the unintentional presence of GMOs that are authorised in the EU in non-GMO batches. The producer of a GMO to be authorised in the EU must supply reference material and an event-specific quantitative detection method to the EURL-GMFF. These methods are evaluated by the EURL-GMFF and subsequently validated in interlaboratory ring trials organised by the EURL-GMFF in cooperation with the European Network of GMO Laboratories (ENGL). RIKILT is a member of the ENGL.

Regulation 882/2004 stipulates which institutes within the EU member states are NRL for GMO analysis tasks. In the Netherlands RIKILT is NRL for GM Food and Feed.

This report describes all NRL tasks and activities in the area of GM feed and food, as stipulated in national and EU GMO regulations and as far as they are not yet part of other national projects (e.g. in the WOT Theme 4 project on the validation and accreditation of methods for GMO analysis).

2 NRL tasks

The NRL tasks are laid down in Directive 882/2004. There are two enforcement laboratories (Routine Field Laboratories) in the Netherlands. For the analysis of food samples this is the new Food and Consumer Product Safety Authority (nVWA) and the analysis of feed samples is done by RIKILT.

The following NRL tasks have been carried out:

- Assist the EURL in ring trials for GMO detection methods and exchange of information through e.g. EURL questionnaires
- Participate in EURL/NRL meetings and workshops
- Participate in proficiency tests
- Perform confirmative analysis on samples of other enforcement laboratories, if requested
- Provide relevant information and advice to Routine Field Laboratories
- Check proficiency test results of Routine Field laboratories

3 NRL activities 2010

3.1 Annual EURL/NRL workshops 2010

In 2010 two EUGL/NRL meetings were attended.

The 3rd NRL workshop was integrated into the 13th ENGL Plenary Meeting. The minutes of this meeting are available in Annex I, section 5. SESSION 882/2004 (3rd NRL Workshop).

The 4th NRL Workshop was integrated into the 14th ENGL Plenary Meeting. The minutes of this meeting are available in Annex II, section 5. SESSION 882/2004 (4th NRL Workshop).

3.2 EURL Working Groups

RIKILT participated in the NRL/ENGL 'Working Group on Method Verification' for which the 4th Working Group on method Verification was attended on 07th-08th October 2010 in Ispra. In November 2010 a final draft version of the document was presented at the 14th ENGL meeting (Verification of real time PCR methods for GMO testing when implementing interlaboratory validated methods, Guidance document from the European Network of GMO laboratories (ENGL), prepared by the ENGL working group on 'Method Verification').

RIKILT also participated in the ENGL Task Force 'New Techniques' NTTF for which several conference calls were attended and for which a document was prepared (New Plant Breeding Techniques and Challenges for Detection and Identification, Report from the, "New Techniques Task Force", (NTTF).

RIKILT also participated in the ENGL Working Group Unauthorised GMOs for which one meeting was attended in May 2010 in Ispra. A final document was presented at the 14th ENGL Plenary Meeting in November 2010 (Overview on the Detection, Interpretation and Reporting on the Presence of Unauthorised Genetically Modified Materials, Prepared by the ENGL ad hoc working group on 'unauthorised GMOs').

All three guidance documents will become available to the ENGL laboratories after finalisation.

3.3 EURL Questionnaires

In 2010 RIKILT filled out a survey for the Food Chain Evaluation Consortium (FCEC - Civic Consulting, Agra CEAS Consulting, Van Dijk Management Consultants and Arcadia International), on behalf of the Directorate General for Health and Consumers of the European Commission (DG SANCO). The objective of this survey was to collect the NRL's views on the quality and relevance of the assistance provided by the EURL and on the functioning of the EURL-NRLs network. The information collected through this questionnaire will be used for determining if and how the assistance provided by the EURL might be improved in the future.

3.4 Proficiency tests (GeMMA and ILC-EURL-GMFF-CT)

In 2010 RIKILT participated in the GeMMA proficiency tests for DNA, food and feed matrices listed in Table 3.4.1. Satisfactory qualitative results and Z-scores were obtained in all tests.

Table 3.4.1. RIKILT participation in GEMMA proficiency tests.

Test	Event	Matrix
GeMSU35	35S promoter	100% maize flour
GeMSU35	NOS terminator	100% maize flour
GeMSU35	MON88017 maize	100% maize flour
GeMMU13	GA21 maize	Contamination in wheat
GeMD26	LL62 rice	DNA
GeMSU35	35S promoter	100% maize flour
GeMSU35	NOS terminator	100% maize flour
GeMD28	A5547-12 soy	DNA

RIKILT also participated in two proficiency tests organised by the EURL (ILC-EURL-GMFF-CT 01/10 and ILC-EURL-GMFF-CT 02/10). These tests are mandatory for NRLs. In these tests DNA had to be isolated from two samples containing different levels of the indicated GMO and these levels had to be quantified.

Table 3.4.2. RIKILT participation in EURL proficiency tests.

Test	Event	Matrix
ILC-EURL-GMFF-CT 01/10	NK603 maize	Maize meal
ILC-EURL-GMFF-CT 02/10	MON810 maize	Maize meal

Satisfactory results were obtained in the NK603 test. The report of the second test (MON810) will be issued in 2011.

As part of the NRL tasks the new Food and Consumer Product Safety Authority was asked to report their proficiency test results to the RIKILT. The nVWA participated in the GeMMA proficiency tests for DNA, food and feed matrices listed in Table 3.4.3.

Table 3.4.3. VWA participation in GEMMA proficiency tests.

Test	Event	Matrix
GeM SU32	MIR604 maize	100% maize flour
GeMSU34	MON863 maize	100% maize flour
GeMSU36	MON810 maize	100% maize flour
GeMSU37	Roundup Ready soya	100% soya flour
GeM MP07	MON810 maize	Baked product e.g. biscuit
GeMSU42	MON863 maize	100% maize flour

Satisfactory qualitative results and Z-scores were obtained in all tests, except for the GeMMP07 MON810 test where the z-score was 2.1. Statistically there is a 1:20 chance for a z-score >2 or <-2, even if there is no technical problem. After repeated analysis by the nVWA satisfactory results were obtained. In addition, at the beginning of 2011 the RIKILT was asked for assistance with this sample. The RIKILT could confirm that this sample was difficult to quantify, because of low MON810 copy numbers in the DNA of the sample.

Also the nVWA participated in the same two EURL proficiency tests as in Table 3.4.3. Satisfactory results were obtained in the NK603 test. The report for the second test will be issued in 2011.

3.5 Participation in EURL-GMFF organised ring trials

In 2010 RIKILT participated, as a member of the ENGL, in the MON87769 ring trial. This ring trial was carried out for interlaboratory validation of the MON87769 soya event (Roundup RReady2Yield™) specific test delivered by the manufacturer. The method and report of this test will become available at <http://gmo-crl.jrc.ec.europa.eu/statusofdoss.htm>.

3.6 Assistance to other laboratories

In 2010 GMO rice construct tests were carried out 4 times (a total of 7 food samples) to confirm nVWA test results for the unauthorised KeFeng6 rice. The results obtained by the nVWA were confirmed in all cases. The tests were developed by Reiting et al. (2010) and consist of three rice GMO construct tests (Pubi-Cry, 35S-hpt and cpti-NOS) if all three are positive they are an indication for the presence of KeFeng6 rice.

In 2010 a maize seed sample was analysed for the presence of maize GMO events for NAK (Nederlandse Algemene Keuringsdienst voor zaaizaad en pootgoed van landbouwgewassen; Dutch General Inspection Service for agricultural seeds and seed potatoes), on behalf of the VROM Inspectie Zuid-West (Inspection South-West of the Dutch Ministry of Housing, Spatial Planning and the Environment, nowadays the new Ministry of Infrastructure and the Environment in October 2010).

3.7 Other aspects

In 2011 the VWA will remain the Dutch representative in the ENGL Steering Committee and RIKILT will be the official back-up representative. RIKILT and nVWA will both continue to attend the ENGL Plenary Meetings. The division of these tasks may be changed in 2012.

4 Conclusions

In 2010 RIKILT participated in several proficiency tests with good results and participated in one ENGL ring trial. Also confirmatory tests were carried out for the nVWA (unauthorised Kefen6 rice and/or Bt63 rice).

RIKILT attended two NRL Workshops in Italy, participated in two ENGL Working Groups for Method Verification and Unauthorised GMOs and the Task Force 'New Techniques' NTTF.

References

Inter laboratory validated GMO methods: <http://gmo-crl.jrc.ec.europa.eu/statusofdoss.htm>.

Definition of minimum performance requirements for analytical methods of GMO testing:
<http://gmo-crl.jrc.ec.europa.eu/guidancedocs.htm>.

Reiting R., Grohmann L., Mäde D., 2010, A testing cascade for the detection of genetically modified rice by real-time PCR in food and its application for detection of an unauthorised rice line similar to KeFeng6, Journal für Verbraucherschutz und Lebensmittelsicherheit, DOI 10.1007/s00003-010-0573-3.

Annex I

13th ENGL PLENARY MEETING REPORT

(Ispra, 19-20 May 2010)



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection
Molecular Biology and Genomics



Ispra, 15 June 2010

13th ENGL PLENARY (Ispra, 19-20 May 2010)

MEETING REPORT

1. Approval of the Agenda + Introductory remarks

The Chairman welcomed the participants to the 13th ENGL Plenary, attended by a record of more than 70 participants including:

- ENGL/NRL members (all 27 Member States represented, incl. Malta)
- Observers from EFSA and Turkey
- External guests from India, Japan, USA, Canada (apologies from China) - this important international participation was linked to the ad hoc sampling session on the second day of the Plenary meeting and to the meeting of the Global Conference Organizing Committee, which was to be held after the ENGL Plenary.

The Chairman added that the next ENGL Plenary meeting of November 2010 may be attended by representatives from Latin America.

The Chairman explained that the latest agenda version includes a few amendments compared to the one circulated two weeks ago:

- The presentation on "IT-based strategy supporting GMO detection" will be made by Belgium rather than Slovenia (item 4.2)
- One additional presentation on control plasmids will be made by Germany (item 4.4)
- The presentation from the NL during the ad hoc sampling session is cancelled because of the unavailability of the invited Dutch expert.

The Chairman highlighted the important ad hoc session on "sampling for GMO testing" to be held on 20 May morning, where active input from ENGL members will be highly welcome to identify future ENGL actions needed on sampling.

The agenda was then approved with no additional item to be discussed under "miscellaneous".

The Chairman also made reference to:

- Distribution of an ENGL feed-back form to be filled in by participants, in order to further improve future ENGL plenary organisation (this was done for the first time in this 13th ENGL Plenary, based on an ENGL Steering Committee decision in February. It will now be done at each ENGL Plenary)
- Recent change of name and logo for the CRL, from CRL to EURL (European Union Reference Laboratory for GM Food Feed), which is linked to the implementation of the Lisbon Treaty on the European Union
- The recent ABC publication on GMO analysis, which includes many papers from ENGL members. Further copies of this booklet can be obtained through IRMM.

Finally, the Chairman confirmed the dates of the next ENGL meetings:

- 7/8 October: 19th ENGL Steering Committee
- 9/10 November: 14th ENGL Plenary

2. Debriefing of the Chairman

2.1 Administrative items

The Chairman debriefed the participants on the outcome of the 18th ENGL SC meeting of February 2010, including:

- The ENGL membership list, which is now publicly available on the MBG Unit website (see <http://mbg.jrc.ec.europa.eu/>)
- The upgrade of the ENGL website planned by end 2010 (in the meantime the outdated ENGL website has been closed down)
- The new concept of an ENGL dynamic action list – this was introduced for the first time, and welcomed, at the ENGL SC meeting of Feb. 2010 and will also now be used at ENGL Plenary. The ENGL Secretariat presented shortly the concept (i.e. action list with responsibilities and deadlines, plus a colour coding system: green for actions completed, yellow for actions pending but still within the deadline and red for actions pending beyond the deadline). This should allow a more rapid and systematic follow-up of ENGL meeting decisions.

2.2 EURL/ENGL "compendium of reference methods"

The chairman explained that the upcoming publication of a "compendium of reference methods" will come under the EURL 882/2004 mandate and that following the "e-voting" procedure launched early April this will be a joint publication EURL/ENGL.

JRC presented the structure and contents of this "compendium of reference methods" which will contain a list of approx. 110 methods, all appropriately validated according to international standards. Each method will be described in a 2-page summary.

The compendium should be published in summer 2010. At a later stage the compendium will be the basis for a complete upgrade of the existing on-line GMO detection database (end 2010).

It was asked whether the compendium, in addition to detection methods, could also include DNA-extraction methods. This may be added in an update of the compendium in 2011.

The Chairman added that as soon as available (i.e. in summer 2010) copies of the compendium will be circulated to ENGL members, together with copies of two other upcoming JRC publications – an overview booklet on EU legislation on GMOs and an updated CD-rom on EU activities on GMO detection.

2.3 International Activities

JRC provided a short update on:

- Global capacity-building programme (recent training in Turkey, upcoming workshops/trainings in Singapore, Croatia, South Africa)
- 2nd Global Conference on GMO analysis – the Scientific Programme of 2nd Global Conference on GMO analysis is available on ENGLNet. Hard copies were also distributed in the meeting room. Further input from ENGL on speakers/topics is welcome.
- ESOF2010 – ESOF is the EuroScience Open Forum that will be held in Torino on 2-7 July 2010 (see <http://www.esof2010.org/>). The Scientific Programme will include on 6 July afternoon a panel session on "GMO testing - a global and scientific challenge" that will include

A. Holst-Jensen (ENGL), R. Mac Arthur (ENGL), C. Paoletti (EFSA), E. Rodriguez (JRC) as panel speakers and G. Van den Eede (JRC) as panel moderator.

The session on "International Activities" was concluded with a presentation from Dr. Gurinder Jit Randhawa (India) on an example of "harmonisation of GMO analysis" outside the EU.

Dr Gurinder gave background information on GMOs in India (cotton is the commercial GM crop in India - 87% of cotton grown in India is GM, with a total of 6 different events – 10 other crops are in field trials involving approx. 100 events). GMO detection is therefore becoming more and more important in India for legislation enforcement. In this frame, Dr Gurinder welcomed the scientific support received from JRC and ENGL (on-line guidelines and protocols, participation to meetings, trainings and proficiency testing). Future developments could include further guidance on sampling, a global database of GMO labs, alternative methods to PCR.

Further to a question on Bt-eggplant, Dr Gurinder confirmed that a positive risk assessment was given on the safety of the product but the Ministry of Environment has expressed some concerns which have delayed the commercial release in India.

3. ENGL COMMON SESSION

3.1 ENGL Working Groups

- Working Group on Unauthorised GMOs – WG-UGM

In the absence of the chairman (A. Holst-Jensen), JRC debriefed on the outcome of the latest WG-UGM meeting of 18 May 2010 – a final document will be presented to the ENGL Steering Committee in October for decisions on next steps.

It was asked whether the WG-UGM was working on validation of UGM detection methods, which would be useful for control authorities in case of legal challenges about UGM detection. However it was recognised that unavailability of reference materials and control samples, plus urgency required in case of UGM detection, make validation of UGM detection methods difficult. Working under quality accreditation (flexible scope) may be the best way to confirm the reliability of results obtained on UGM detection.

- Working Group on Method Verification - WG-MV

The chairwoman (L. Hougs) updated the ENGL and confirmed that a final doc from the WG-MV is expected by end 2010.

- Task-Force on Plant Breeding New Techniques – NTTF

JRC made a presentation on the activities of a JRC Task-Force (incl. various ENGL members) on "detection challenges raised by Plant Breeding New Techniques" (a list of seven specific new techniques is looked at one by one). A technical report from the NTTF will be provided to DG Sanco by end 2010. It will then be up to DG Sanco and the Member States to take, if need be, any decision on the regulatory status (GM or not) of Plant Breeding New Techniques. Further to a question, it was confirmed that industry will be consulted in due time (autumn 2010) on the work of the NTTF.

- Working group on Method Performance Requirements WG-MPR

This WG is not yet active but a revised mandate was adopted by the ENGL SC in February. A call of interest for members/chairman will be circulated soon.

In this frame, the JRC made a presentation on a recent "bioinformatics survey" from the EURL on sequences referred to in various GMO detection methods. In a few cases it showed some similarities between different detection methods for different events. The topic is being discussed with industry. It is too early to say whether this may have an impact on the acceptance of future methods for validation. If need be, some issues on "sequence similarities" could be indicated in the method validation report.

- Working Group on Sample Preparation Procedure – WG-SPP

This WG is not yet active but some discussions on its future mandate already took place at the ENGL SC meeting of February 2010. On this basis, Italy made a presentation on the future activities of this WG, who plans to provide a document in June 2011.

Following various questions on the scope of the WG-SPP activities and in relation to the upcoming sampling session of 20 May, it was decided that the mandate of the WG-SPP will be reviewed and confirmed at the ENGL SC meeting of October 2010.

JRC also referred to ISO/TC 34 document N1424 "Update on IWA, International Workshop Agreement on Bulk commodity grain sampling", which is presently under discussions and for which comments (incl. statistical input) have been requested. It was noted that there are already some standards existing on sampling for GMO testing (incl. a CEN Technical Standard) so it would be important to confirm the status (binding or not) of this new ISO document. JRC will now clarify with ISO the procedure for adoption of this document (incl. possibility to circulate the document, timing for comments) and its future status (binding or not). If need be, ENGL members will be asked to comment on it (possibly through national standardisation bodies).

3.2 On-going EU regulatory discussions

The representative from DG SANCO first confirmed that DG SANCO is now managing all GMO-related topics within the EU Commission (re-organisation from DG ENV). She provided an update on on-going discussions on EU GMO legislation:

- A proposal on a "new approach" allowing decisions from Member States on GMO cultivation will be presented before the summer.
- A proposal on "LLP" is still in internal inter-services consultation. No timelines can be provided for a proposal to be presented but the general approach would be a 0.1% tolerance threshold for GMO events with a valid EFSA application and a validated detection method. It was noted that collaboration with EURL will be essential since analytical uncertainty is high at such low level of presence.
- Previous draft proposals related to the RASFF Annex on GMO notifications or on botanical impurities still need to be further discussed with the Member States.
- Emergencies: on-going contacts with China on GM rice and with Canada on GM flaxseed

The representative from EFSA made a presentation on "New Guidelines for GMO Risk Assessment".

- An updated guidance from EFSA on GM FF Risk Assessment is going to be incorporated in a formal (EC) regulation.
- A new guidance from EFSA on Environmental Risk Assessment has been developed and is being discussed with stakeholders.
- An EFSA working group on "comparators" has been established, which outcome may be of relevance to ENGL.

3.3 Rapid Alert System Food Feed (RASFF)

Linseed/Triffid case

A representative from the Canadian Grain Commission made a presentation on flaxseed and the FP967 Triffid case and highlighted the various steps taken in Canada to handle the issue. He confirmed that a 1st protocol for GM testing of flaxseed was agreed between Canada and the EU in October 2009 and a 2nd protocol in March 2010. Some ENGL members confirmed that testing on EU linseed has been done in the EU since the Canadian case and that all results were so far negative.

Rice/Kefeng case

Germany made a presentation on the "testing cascade" approach that is followed in Germany for detection of GM rice. This approach is based on a literature review on the various GM rice events existing (not necessarily all well characterised), on the construction of a plasmid to replace control material (not necessarily available) and on testing for 35S and NOS.

JRC made a presentation on preliminary testing done at the EURL on the so-called "Kefeng 6" event (based on the German approach and on market samples) but so far no conclusion can be drawn in the absence of control samples. In this frame the Chairman informed ENGL that some samples of various Chinese rice events have been sent to EURL from China. Once analysed and confirmed as suitable, these samples could be circulated to ENGL members as control samples for various Chinese GM rice events.

3.4 Global harmonisation (ISO/CEN and Codex)

JRC made a presentation on the latest discussions on GMO testing at 3 recent meetings:

- CEN (Germany, Dec. 2009) and ISO (Japan, Feb. 2010)
- Codex (Hungary, March 2010)

Discussions on standards relevant for GMO testing take place in CEN TC275/WG11 and ISO SC16 and need to be closely followed since CEN and ISO standards are referred to in regulation (EC) No 882/2004. JRC is therefore attending the relevant CEN/ISO meetings. Some follow-up from ENGL members is also welcome through national standardisation bodies (for instance in the on-going expert discussions on sampling within CEN TC275/WG11).

JRC added that EURL is considering submitting EU validated methods as ISO standards but discussions are needed with the technology providers.

At Codex level, an agreement on a text was found at the latest CCMAS (Codex Committee on Method of Analysis and Sampling) and the final text should now be adopted by the CAC (Codex Alimentarius Commission) in July 2010. The scope of the document has been broadened (the new title is "Proposed Draft Guidelines on Performance Criteria and Validation of Methods for Detection, Identification and Quantification of Specific DNA Sequences and Specific Proteins in Foods") but still refers to foods derived from modern biotech and requirements are (partly) based on ENGL criteria.

A question was raised about which endogenous genes should be used as reference genes. It was acknowledged that existing EU validated methods include various reference genes and that a reduction of the number of reference genes would be welcome. Discussions are on-going with industry about this.

Referring to the broader scope of the latest Codex document, the Chairman added that the expertise of the EURL GMFF could be useful to other EURLs active in other fields. ENGL members were invited to suggest any area in which the expertise of the EURL GMFF could be transferred.

3.5 Prospects for FP7 projects

The Chairman simply commented that no positive feed-back was received from DG RTD and therefore prospects for new EU research projects were limited. No further news were available from ENGL members

3.6 Miscellaneous

None

4. SESSION 1829/2003

4.1 EURL-GMFF robustness study

JRC made a presentation on "Transferability of validated qPCR methods across real-time PCR platforms". A robustness study was recently run with 3 different EU-validated methods on 6 different RT-PCR platforms. All in all, the results confirm "robustness" of the EU-validated methods i.e. their transferability across different PCR platforms. The results are of course not intended to promote one or the other brand of PCR machine.

4.2 IT-based strategy supporting GMO detection

Belgium made a presentation on an on-going joint project with Slovenia. The project aims at developing an IT-tool which would allow to collect and to structure web-based information in order to reach conclusions on presence or absence of a GMO (through an IT-tool rather than through actual GMO analyses). Further work is still needed.

4.3 Availability of Reference Materials

The representative of IRMM made an update on the reference materials available from IRMM. Reference materials are also available from AOCS but under different payment conditions. There may also be an issue with stock availability from AOCS since they tend to produce their reference materials in limited quantities. The Chairman invited ENGL members to inform about any issue regarding availability of reference materials (since EU legislation requires reference materials to be available for any GMO approved in the EU throughout the whole lifecycle of the product).

4.4 Control Plasmids

Germany made a presentation on the pros and cons of using plasmids as control samples. The main benefits would be fast, cheap and large quantity availability of control material when plasmids are used. However performance criteria would need to be defined (ideally at EU level) before using such control plasmids. Reference was also made to "GMO screening methods" and the idea of EU-validation of "GMO screening methods" was supported.

4.5 Sequencing

JRC made a presentation on the sequencing activities of the EURL-GMFF. So far 6 events have been sequenced and have shown no difference between the insert sequence included in the application and the insert sequence checked by EURL-GMFF.

4.6 Digital PCR

The representative of IRMM made a presentation on Digital PCR. Different tests were done on MON810 material and appeared satisfactory. Digital PCR looks to be a technology which allows to obtain easily a quantitative result (in DNA copy numbers), with no need for a calibrant. However the costs are still so far very high (around 200-300 EUR/sample).

4.7 EURL-GMFF ExpertGroup on Fuzzy Logics

A first meeting of experts on fuzzy-logics is planned in June. Further steps regarding use of fuzzy logics will be decided later on.

4.8 Miscellaneous

None

5. SESSION 882/2004 (3rd NRL Workshop)

5.1 Comparative testing schemes (proficiency testing)

The JRC made a presentation on the 1st round of proficiency testing organised in Spring 2010. A total of 93 laboratories participated (mainly from ENGL but also from third countries).

Results are going to be based on Z-scores. Preliminary results show all in all a very good performance from the participating laboratories (Z-scores lower than 2). A final report will be published and individual results communicated to participating laboratories by December 2010.

A 2nd round of proficiency testing is planned for autumn 2010.

Some comments were made on the reporting to be done by participating laboratories, including on the possibility to provide comments in addition to the raw results. This will be taken into account in the reporting form for the 2nd round.

Italy also made a presentation on collection of test materials from official controls, explaining the usefulness of collecting positive test materials. A system is now in place in Italy and around 50 samples have been collected from the Italian network.

The Secretariat will now consult ENGL members about interest to have a similar initiative at ENGL level. If interest is confirmed, the ENGL Steering Committee will define the rules concerning the collection of these test materials.

5.2 EU harmonisation/validation of GMO screening methods

The Chairman explained that the upcoming "compendium of reference methods" (see item 2.3) will include some screening methods, which could be used for validation by the EURL. He recalled also that Member States have been calling for EU validation of screening methods.

He will therefore propose to the ENGL Steering Committee to organise an ad hoc session on "screening methods" at the next ENGL Plenary in order to identify next actions on the topic.

5.3 High-through put detection systems

JRC made a short presentation on on-going activities regarding "Assessment of the use of ready-to-use GMO detection plates on processed foods and in at low levels in soy and maize grains". A publication on the topic is under preparation. Different set-ups of "ready-to-use" plates can be used for different purposes (e.g. screening to better focus further testing or LLP detection). All in all "ready-to-use" plates perform very well.

5.4 Task Force on "Accreditation for GM FF analysis"

The IRMM representative made a short presentation on the outcome of a meeting on "accreditation for GM FF analysis" held in IRMM in April 2010.

The main objective was to stress the importance of "flexible scope accreditation", in particular to the representative of the European Accreditation body. Further to this meeting, a technical document on "flexible scope" will be sent to the European Accreditation body in summer 2010.

5.5 ENGL knowledge transfer to other EURLs

This topic was addressed after the discussions on Codex (item 3.4)

5.6 Miscellaneous

None

AD HOC SESSION "SAMPLING FOR GMO TESTING"

In introduction, JRC made a short presentation on the sampling training workshop that was held at Ispra on 4-5 February 2010.

Portugal then made a detailed presentation to illustrate an example of "Adaptation of Lab Sampling Processes" following this sampling training workshop. The whole sub-sampling strategy has been adapted and resulted in improved testing results (less variation).

This introduction was then followed by presentations from:

G. Gandini - Border Inspection - Italy

Made a presentation on how sampling for GMO testing was done in Italian harbours. For GMO testing, the focus in Italy is on sugarbeet, cotton and soya crops. Sampling, both dynamic and static, is based on ISO standards. Because of the huge volumes involved (25000 tonnes shipments), sampling may raise practical difficulties.

F. Bekkaoui - National Research Council of Canada Plant Biotechnology Institute (NRC-PBI)

Made a presentation on GM flaxseed testing in Canada. Detailed sampling protocols (different for grains and for certified seeds) have been developed by the Canadian Grains Commission and are available at <http://www.grainscanada.gc.ca/gmflax-lingm/stpfm-mpeevl-eng.htm>

R. Jenkins – United States Department of Agriculture (USDA)

Made a presentation of the GIPSA (Grain Inspection, Packers, Stockyards Administration) activities (incl. proficiency testing). GIPSA works mainly on "rapid testing" i.e. using protein-based test kits. Some questions were raised about the results obtained in the GIPSA proficiency testing, which in general are less satisfactory (Z scores higher than 2) than in other proficiency testing rounds. This may be explained by the unit of measurements used (w/w or copy numbers) and the material used (homozygous or heterozygous).

In conclusion, the Chairman invited all ENGL members to provide further input on sampling in the coming weeks, in order to identify next steps/actions from ENGL on "sampling for GMO testing" (incl. activities of the SPP-WG). Future ENGL activities on sampling will be discussed and decided at the ENGL Steering Committee of October 2010.

Annex II

14th ENGL PLENARY MEETING REPORT

(Ispra, 9-10 November 2010)



EUROPEAN COMMISSION
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection
Molecular Biology and Genomics



Ispra, 29 November 2010

14th ENGL PLENARY MEETING REPORT (Ispra, 9-10 November 2010)

Day 1 – Tuesday 9 November

1. Approval of the Agenda

The Chairman explained that this ENGL Plenary will be a special meeting since it will include on 10 November morning a special session dedicated to the launch of the "Compendium of Reference Methods for GMO Analysis" and the opening of the new EU-RL GMFF laboratory facilities.

The various guests to the 14th ENGL Plenary meeting were introduced: P. Alexander from DG TAXUD (Customs DG in the European Commission), several international partners (from China, India, Japan, Georgia, Moldova, Jordan, Zimbabwe, Latin America) and a biotech industry delegation from Europabio.

The Chairman also explained that DG Sanco will not be present at this meeting but a video conference call will be organized to discuss about Low Level Presence (LLP). He added that these discussions on LLP will be "confidential" and restricted to ENGL members (observers will therefore be invited to stay outside of the meeting room at that time of discussions).

The agenda for the 14th ENGL Plenary was then adopted with the following modifications:

- Item 3.3 (Screening methods) + Item 5.2 (Compendium) moved to 10 November morning
- Additional presentations from Norway (A. Holst-Jensen) on GM aquaculture and on sequencing

2. Debriefing of the Chairman

The Secretariat reviewed the Dynamic Action List (DAL) from the 13th ENGL Plenary meeting, in which most of the actions were completed in time.

The Secretariat also briefly presented two important documents that were adopted by the ENGL Steering Committee (SC) in October 2010: the ENGL 2011 Annual Work Programme (AWP) and the ENGL Internal Rules (both docs are available on ENGLNet).

Regarding the ENGL 2011 AWP, a question was raised about the organisation of the 2nd Global Conference on GMO Analysis. The Chairman confirmed that it was still planned in 2011, but the date and the location could not yet been confirmed. However, decisions and confirmations on the 2nd Global Conference on GMO Analysis are expected by end November.

3. ENGL Common Session

3.1 Update on ENGL Working Groups

3.1.1 Unauthorised GMOs –UGM-WG

The UGM-WG chairman (A. Holst-Jensen) presented the proposed final version of the UGM-WG document (available on ENGLNet). He stressed that the objective was not to introduce a new paradigm for detection of unauthorised GMOs but to follow the same approach as of today. He added that work of the UGM-WG was not fully completed and that a second mandate would be welcome. He finally proposed the following next steps, which were agreed upon:

- Comments from ENGL members to be sent by 20 November 2010
- Endorsement from ENGL SC in December 2010
- Publication of the final UGM-WG document in January 2011

3.1.2 Method Verification – MV-WG

The MV-WG chairman (L. Hougs) presented the proposed final version of the MV-WG document (available on ENGLNet). She added that a second version of the document will need to be prepared in the future. She proposed the same next steps as for the UGM-WG, which were also agreed upon.

The chairman added that finalisation of both documents will be useful and timely in the frame of on-going discussions on LLP. It was also agreed that both documents could already be made available to ENGL international partners, even if not yet formally published.

3.1.3 Method Performance Requirements – MPR-WG

The JRC provided a short briefing on the kick-off meeting of the MPR-WG that was held on 8 November. The priorities for the MPR-WG include first of all qualitative methods, incl. screening methods.

The Chairman recalled that the ENGL SC decided in its meeting of October 2010 that the MPR-WG should also look at the topic of control plasmids (incl. reference genes).

3.1.4 Sample Preparation Procedure – SPP-WG

G. Berben from Belgium presented the mandate and work plan of the SPP-WG as adopted by the ENGL SC in October 2010. Next steps are now nomination of the SPP-WG members and a kick-off meeting planned in February 2011.

3.2 Update on the New (Plant Breeding) Techniques Task Force - NTTF

The JRC presented an update on the work of the NTTF, which is now coming to an end. A final NTTF report is expected by December and will conclude in particular that:

- PCR is the most appropriate method for detection and identification of new plant breeding techniques
- within the list of new plant breeding techniques considered by the NTTF, only a few ones can be differentiated from conventional breeding or natural mutations. The New Techniques which can be identified include Zinc Finger Nuclease 3, Cisgenesis, floral dip.

Next steps will now be integration of the NTTF report in a consolidated JRC report on New Plant Breeding Techniques that will be sent to DG Sanco end 2010.

4.1 Update on on-going EU regulatory discussions

Note: DG SANCO could not attend the ENGL meeting but discussions on EU regulations took place through an ad hoc video conference call included in the agenda at 11.30 am. DG SANCO in Brussels introduced the recent Commission proposal on Low Level Presence (LLP), which will be discussed for the first time with the Member States on 15 November.

DG SANCO explained that in short, the LLP proposal would introduce a tolerance threshold of 0.1% + Measurement Uncertainty (M.U.) in feed for GMOs not yet approved in the EU, as long as a valid EFSA application has been filed and a quantitative detection method has been validated for the relevant GMO.

ENGL members asked questions in particular about:

- Determination of the Measurement Uncertainty for different labs
- Reference to mass fraction as unit of measurement of 0.1% (as opposed to Haploid Genome Equivalent in the 2004/787/EC recommendation).

DG SANCO confirmed that "mass fraction" was the unit of measurement to be legally used in any EU food/feed legislation including the LLP proposal.

Regarding determination of Measurement Uncertainty, the LLP proposal makes so far mainly reference to the 2009 IRMM report on the topic but further input from ENGL on this technical issue is still possible.

DG SANCO also invited ENGL members to brief their Competent Authorities ahead of the 15 November meeting since this was a very technical proposal to be discussed.

3.3 GM aquaculture

A. Holst-Jensen from Norway made an introductory presentation on GM aquaculture, in particular on the GM salmon (from the Aqua Bounty company), which is quite advanced in the US regulatory approval process. The introduced trait is "growth enhancement" and comes from a genetic construct based on genes from salmon-related species. Nevertheless identification of the GM salmon should be possible with PCR since the combination of promoter-trait gene used is unique and not present naturally.

D. Zhang from China briefed the ENGL about on-going research on GM carp in China, which is however unlikely to lead to a commercial product in the near future.

It was agreed that ENGL should follow closely any developments related to GM fish.

3.4 Interaction with other EURLs and laboratories

P. Alexander from DG TAXUD (Directorate General on Customs in the European Commission) introduced the activities of the Group of Customs Laboratories (GCL) which was established in 1999 and includes 79 laboratories. Corresponding documentation was also distributed. The structure of the GCL is actually quite similar to the ENGL one and some GCL activities are also related to molecular biology like for instance a recent workshop on "species identification". It was agreed that there was definitely room for collaboration between GCL and ENGL and that dialogue should continue between both networks.

3.5 FP 7 research project calls

In the absence of DG RTD, the Chairman shortly commented that the latest FP7 calls published in July 2010 included very few proposals relevant to the ENGL activities. He added that the JRC-IHCP has joined a consortium who is preparing an application related to "coexistence". However the contribution from JRC-IHCP on detection will be quite limited within the whole consortium activities.

It was agreed to stay in touch with DG RTD ahead of the next round of call publications in July 2011.

4. Session 1829/2003

4.1 Update on on-going EU regulatory discussions

Topic discussed earlier in the agenda in a video conference call with DG SANCO (see before).

4.2 Distribution of control samples

Following discussions in the latest ENGL SC meeting, the Chairman made a communication to clarify the rules regarding "distribution of control samples".

The Chairman confirmed that:

- All ENGL members and associated laboratories have always access to control samples. The only condition to be met is signature of a MTA so that the EU-RL is systematically informed about the various labs receiving control samples.
- Transfer of control samples from one ENGL member to another ENGL member is also possible provided the "receiving" ENGL member has signed a MTA (so that the EU-RL is informed about it).
- For other labs (non-ENGL members) distribution of control samples will be decided on a "case-by-case" by the EU-RL and will anyway also involve the signature of a MTA.

4.3 High-throughput sequencing

P. Hubert from Germany made a presentation on Next Generation Sequencing, which can also be described as massive parallel sequencing. He explained that the latest sequencing techniques do not include anymore PCR-amplification steps and can go to the level of single nucleotide detection. Costs for sequencing are also rapidly decreasing.

At the moment however such high-throughput sequencing cannot (yet) replace PCR in particular since analysis of the huge amount of data generated is still challenging (costs are also still quite high).

A. Holst-Jensen from Norway made a presentation on Transcriptome Sequencing where a mRNA transcriptome strategy and bioinformatic analysis of complementary DNA sequences may be useful to demonstrate GMO presence. However this strategy has some limitations. For instance it implies DNA expression in mRNA (which may not be the case in all tissues) and may not allow identification of a specific GMO event.

4.4 International Harmonisation

Regarding the Cartagena Protocol on Biosafety, the JRC provided an update on the latest COP-MOP5 meeting held in Nagoya, Japan in October 2010 (see <https://www.cbd.int/mop5/>) where the importance of GMO detection was confirmed (for instance in terms of information sharing through the Biosafety Clearing House (BCH) or in terms of capacity building under article 18.3 on LMO identification). In addition to on-going discussions at Codex or ISO/CEN, Biosafety Protocol discussions are therefore also relevant for GMO detection and for ENGL.

Regarding Codex, H. Broll from Germany recalled the various discussions held on GMO detection at Codex level over the last 10 years. Those ended up with the CCMAS conclusions of March 2010, which were formally endorsed by the Codex Alimentarius Commission in July 2010. Subsequently, there is now room for Codex members to submit GMO detection methods to CCMAS. This should ideally be done as EU submission (of all EU-RL validated methods) through DG SANCO.

The Chairman agreed to contact DG SANCO about such a possible EU submission. Regarding any possible similar input from ENGL international partners to Codex, he added that this should not to be misinterpreted as an attempt to "export EU policies".

Regarding JRC capacity-building in GMO detection, the Chairman updated the ENGL on the latest events:

- workshop in Croatia end September 2010
- workshop in South Africa end October 2010

5. Session 882/2004 (4th NRL workshop)

5.1 Comparative testing schemes

The JRC provided an update on the 1st round of comparative testing held in Spring 2010 and the 2nd round held in fall 2010.

In the 1st round, 85 laboratories submitted results and very few obtained Z-scores results outside ± 2 . Final report for this 1st round will be available by end 2010. This 1st round showed in particular a need for harmonisation of reporting.

In the 2nd round, 89 laboratories submitted results. Final report for this 2nd round will be available by mid-2011. A 3rd round will be organised in Spring 2011.

5.3 Update on RASFF cases

The JRC explained that work is still on-going on the Amadea potato and the Kefeng rice.

ENGL members commented that communication on the Kefeng 6 method would be welcome as soon as possible since recent RASFF alerts mainly concern Kefeng 6.

It was agreed that information on the Kefeng method will be communicated to ENGL members as soon as it has been verified by the EU-RL.

5.4 Accreditation

The Chairman explained that in the frame of the 882/2004 mandate, it is planned to organise in 2011 training sessions for ENGL members on implementation of ISO standards in GMO labs.

IRMM commented that discussions are already on-going with the European Accreditation body on the same topic so coordination will be necessary between both initiatives.

5.5 Relativity of Ct values

G. Berben from Belgium made a presentation on a recent study carried out by the EU-RL on animal proteins which concludes that it is useless to compare Ct values between different laboratories.

Different views from ENGL members were expressed on the conclusions from this study but the general feeling was that more than the Ct value itself, what was relevant to compare was the difference between the Ct value from the standard curve and from the analytical sample.

5.6 Collection of test materials from official controls

The Chairman explained that further to the latest ENGL SC meeting of October, ENGL members were invited to express possible interest in the collection at ENGL level of test materials samples coming from official controls. So far very limited interest was expressed. Interested ENGL members should react by end November if they want the initiative to be followed-up.

The Chairman concluded day 1 of the ENGL meeting by inviting all participants to a social dinner in the evening (which will be attended by all guests to the new EU-RL lab opening on day 2).

Day 2 – Wednesday 10 November

The Chairman welcomed all guests to this special session dedicated to the launch of the "Compendium of Reference Methods for GMO Analysis" and the opening of the new EU-RL GMFF laboratory facilities. In addition to the ENGL members, external participants representing various ENGL international partners (from China, India, Japan, Georgia, Moldova, Jordan, Zimbabwe, Latin America) and the biotech industry (Europabio delegation) were present.

- **Presentation from JRC** focused on the history of the EU-RL GMFF from the very first activities on GMO detection at JRC-IHCP in 1998, to the establishment of ENGL in 2002 and the CRL mandate in 2003, the first method validation in 2004 and more recently the Comparative Testing schemes in 2010.

- **Presentation from an ENGL member** (C. Aleixo from Portugal) focused on the added-value for a national laboratory to be member of ENGL

- Presentations from ENGL International Partners

D. Zhang from China focused on the added-value of being an ENGL observer since 2005, which lead to several collaborations like joint publications, researcher exchange or joint databases.

D. Garwe from Zimbabwe introduced the Southern Africa Network of GMO Laboratories (SANGL) in place since 2005 and including 9 countries. The already well established experience of ENGL may be of added value for SANGL. SANGL is still facing some limitations (mainly qualitative methods can be used, not yet quantitative) and therefore future assistance will be welcome.

- **Presentation from ENGL chairman** introduced the "Compendium of Reference Methods for GMO Analysis" which was formally launched today (10 November) and included a summary of 79 GMO detection methods that were validated according to international standards (ISO and/or IUPAC). Copies of the compendium were distributed to all participants. The compendium is also available on the JRC website and will be followed by end 2010 by an on-line database including the same reference methods.

- **Presentation from EuropaBio** (European biotech industry association) was made by the Chairman of the Detection Method WG (DMWG) from EuropaBio who introduced the activities of the DMWG and made in particular the following comments:

- the methods included in the compendium were developed and owned by biotech companies
- the present approach followed by the EU-RL for stack events (i.e. verification process) is satisfactory and should be maintained in the future for multiple stacks.

He also presented a recent bridging study carried out by the DMWG to compare different reference genes in maize, cotton soya and he added that a single reference gene/crop would be welcome.

Finally he introduced an upcoming database of GMO detection methods that will be soon made available at www.detection-methods.com by Crop Life International (CLI the global biotech industry association).

Further to these presentations, successive groups of guests (starting with EuropaBio members) were invited to visit of the new EU-RL GMFF laboratory. In the meantime, ENGL members discussed the last topic on the ENGL agenda:

3.3 GMO screening methods

JRC first made a presentation on various on-going activities including work on GM pollen detection, the compendium of reference methods (incl. some screening methods), the research project GMOSeek.

ENGL members further commented on GMO screening methods that:

- today the screening methods used by labs are mainly in-house validated but "properly" validated screening methods would be welcome (like the present validation by the EU-RL)
- some Member States (like Germany) started working on validation of screening methods but could not agree at national level on criteria for validation for screening methods so input on the topic from an ENGL WG would be welcome (may be the MPR-WG and/or MV-WG and/or ad hoc WG)

Additional comments included:

- the compendium includes methods which were validated some years ago and which may not be in use anymore. Selection criteria for inclusion in the compendium should be fine-tuned ahead of the second updated version of the compendium. It was agreed to consult the ENGL SC about it.
- work on GM pollen detection is already on-going at CEN level and should be taken into account.

In conclusion the Chairman announced that the next ENGL Plenary is planned on 16-17 November 2011 (since the 2nd Global Conference on GMO Analysis is tentatively planned in June 2011).

The Chairman also took this opportunity to stress that this was the last ENGL meeting from W. Moens as ENGL Secretariat and he thanked him warmly for his contribution to the establishment and success of the ENGL over the last years.

RIKILT - Institute of Food Safety is part of the international knowledge organisation Wageningen UR (University & Research centre). RIKILT conducts independent research into the safety and quality of food. The institute is specialised in detecting and identifying substances in food and animal feed and determining the functionality and effect of those substances.

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The research institute in Wageningen is the National Reference Laboratory (NRL) for milk, genetically modified organisms, and nearly all chemical substances, and is also the European Union Reference Laboratory (EU-RL) for substances with hormonal effects.

RIKILT is a member of various national and international expertise centres and networks. Most of our work is commissioned by the Dutch Ministry of Economic Affairs, Agriculture and Innovation and the new Dutch Food and Consumer Product Safety Authority. Other parties commissioning our work include the European Union, the European Food Safety Authority (EFSA), foreign governments, social organisations, and businesses.

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