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## **New real-time PCR methods available for routine GMO detection labs-applicability and performance**

Theo R. Allnutt, Stefan Baeumler, Christoph Bahrtdt, Gilbert Berben, Yves Bertheau, Mark van den Bulcke, Adrian Härri, Boris Kuznetsov, Valerie Laval, Ralf Seyfarth, et al.

### ► **To cite this version:**

Theo R. Allnutt, Stefan Baeumler, Christoph Bahrtdt, Gilbert Berben, Yves Bertheau, et al.. New real-time PCR methods available for routine GMO detection labs-applicability and performance. Co-Extra International Conference, Jun 2009, Paris, France. 150 p. hal-02755750

**HAL Id: hal-02755750**

**<https://hal.inrae.fr/hal-02755750>**

Submitted on 3 Jun 2020

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# INTERNATIONAL CONFERENCE



2 - 5 June 2009  
Paris, France

CO-EXISTENCE & TRACEABILITY



## Programme and Abstracts

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**GM AND NON-GM SUPPLY CHAINS: THEIR CO-EXISTENCE AND TRACEABILITY**

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[www.coextra.eu](http://www.coextra.eu)

**CoExtra Conference**  
June 2 - 4, 2009  
AgroParisTech  
16 rue Claude Bernard  
Paris 75 005, France

**Stakeholder Workshop**  
June 5, 2009  
Palais du Luxembourg  
15 rue de Vaugirard  
Paris 75 006, France



## Content

<b>Programme.....</b>	<b>5</b>
<b>Abstracts of Oral Presentations.....</b>	<b>9</b>
<b>Session 1: Introductory Presentations.....</b>	<b>9</b>
1.1.    Report on the coexistence of genetically modified crops with conventional and organic farming .....	9
1.2.    SIGMEA results on coexistence at the farm level.....	11
1.3.    TransContainer: Overview and Progress.....	13
1.4.    Co-Extra introduction .....	15
<b>Session A1: Methods for Managing Gene Flow.....</b>	<b>17</b>
A1.1.    Biological measures for gene flow mitigation.....	17
A1.2.    Bio-containment of maize by cytoplasmic male sterility and xenia.....	18
A1.3.    Pollen containment by cleistogamy in oilseed rape.....	20
A1.4.    Chloroplast transformation and transgene containment .....	22
A1.5.    Mesoscale dispersal of maize pollen and implications for gene flow.....	24
<b>Session A2: Coexistence and Traceability in Agriculture and Food Production.....</b>	<b>25</b>
A2.1.    Empirical analysis of coexistence in commodity supply chains .....	25
A2.2.    Modelling coexistence between GM and non-GM within supply chains.....	28
A2.3.    Costs and benefits of segregation and traceability between GM and non-GM supply chains of final food products .....	30
A2.4.    Consumers' attitudes to the EU traceability and labelling regulation. ....	33
<b>Session B1: Technologies for Managing the Supply Chain.....</b>	<b>35</b>
B1.1.    GMO sampling strategies in the food and feed chain.....	35
B1.2.    Rationalization of GMO testing by appropriate sub sampling and control plans .....	38
B1.3.    Modular Approach Implemented: Pros, Cons and Future Perspectives.....	41
B1.4.    Validation of novel methods and technologies.....	44
B1.5.    Reference materials and reference PCR assays for GMO quantification.....	46
<b>Session B2: Detection of GM ingredients in foods and Feeds.....</b>	<b>49</b>
B2.1.    New real-time PCR methods available for routine GMO detection labs - applicability and performance .....	49
B2.2.    Reliability and costs of GMO detection.....	51
B2.3.    Non-PCR based Alternative Analytical Methods.....	53
B2.4.    Detecting unauthorised and unknown GMOs.....	55
B2.5.    New multiplexing tools for reliable analysis of GMOs .....	57

<b>Session 3: Legal, Liability &amp; Redress Issues .....</b>	<b>59</b>
3.1. Legal, Liability & Redress Issues .....	59
3.2. Scientific expertise and the judges.....	60
3.3. Juridical cost-benefit analysis of coexistence: uneasy this task!.....	61
<b>Session 4: Stakeholder Views in EU .....</b>	<b>62</b>
4.1. Stakeholder views and interactions .....	62
<b>Session 5: Decision Support Systems.....</b>	<b>63</b>
5.1. The Co-Extra Decision Support System: A Model-Based Integration of Project Results.....	63
5.2. Analytical DSS module – how to support decisions in the analytical lab.....	65
5.3. DSS modules on transportation (TM module) and on unapproved GMOs (UGM module).....	67
<b>Session 6: Experiences from third countries .....</b>	<b>69</b>
6.1. Benefit-Cost Analysis, Food Safety, and Traceability .....	69
6.2. Segregation Measures for (Non-) GM crops and their Implications for Supply Chains in Japan .....	71
6.3. Co-Existence and traceability: Costs and benefits in food and feed supply chains .....	73
6.4. A Company Perspective.....	74
6.5. Protecting European quality agriculture: Non-GM feed supply and production.....	75
7. Integration of Co-Extra results in EU tools for coexistence & traceability.....	78
8. Summary of main Co-Extra deliverables & results, perspectives, information dissemination & application.....	79
<b>Poster Abstracts .....</b>	<b>96</b>
P1. A cost-effective P35S/Tnos multiplex screening assay with internal positive control.....	96
P2. The problem of when to label in presence of low amounts of transgenic material: the case of botanical impurities.....	97
P3. NIR imaging and chemometrics in support to the detection at the single kernel level of GMO .....	99
P4. Performance of TaqMan®, LNA, Cycling Probe Technology, Lux and Plexor real-time PCR chemistries in quantitative GMO detection .....	100
P5. GMO analysis: towards assuring confidence in a result.....	101
P6. Detection of <i>Bacillus thuringiensis</i> by real time PCR.....	103
P7. Development of a new probe for qualitative identification and quantification of Bt 11 maize .....	105
P8. Development of construct-specific TaqMan real-time PCR for detection and quantification of transgenic Bt11 maize ( <i>Zea mays</i> ).....	106
P9. State of the art on sample preparation and assessing the validity of procedures deriving test portion from laboratory samples.....	108
P10. Designing the PCR markers <i>Agrobacterium tumefaciens</i> gall-forming strains. ....	110

P11.	A rapid & simple point-of-use diagnostic for GMO detection in plants.....	112
P12.	Development of an integrated platform for the detection of materials derived from genetically modified crops in food and feed products.....	113
P13.	Use of pJANUS™-02-001 as Calibrator Plasmid for GTS 40-3-2 (Roundup Ready Soybean) Detection: An Inter-Laboratory Trial Assessment .....	114
P14.	Testing the “Modular Approach”: an example with Round-Up Ready Soybean .....	115
P15.	NAIMA: a fast quantitative method for high-throughput GMO diagnostics in food and feedstuffs .....	116
P16.	GMO <i>versus</i> mycotoxins sampling plan: a pragmatic approach .....	117
P17.	Approaches to monitor the adventitious presence of transgenes in <i>ex situ</i> collections of national gene banks.....	119
P18.	Monitoring the adventitious presence of transgenes in <i>ex situ</i> cotton collections of the National Gene Bank.....	120
P19.	Molecular diagnosis of commercialized or unapproved Bt crops of India using qualitative and quantitative PCR assays .....	121
P20.	Multiplexing of SIMQUANT .....	122
P21.	Use of computational subtraction to search for unknown genetic modifications .....	124
P22.	Effect of different storage conditions on PCR amplificability of genomic DNA extracted from pellets containing maize MON 810 maize.....	125
P23.	Multiplex DNA Detection System For Identification Of Genetically Modified Organisms (GMOs) In Food And Feed Chains; Co-Extra WP6 results .....	127
P24.	The Co-Extra website, a key tool in the Co-Extra external communication strategy.....	128
P25.	Influence of the (non-GM) soybean price on compound feed price.....	130
P26.	The cost-effectiveness of the coexistence of GMHT oilseed rape in Ireland: an analysis of crop management strategies.....	131
P27.	Modelling coexistence between GM and non-GM supply chains.....	133
P28.	Supply chain description and analysis for maize, potatoes and fresh tomatoes in Slovenia .....	135
P29.	Preference heterogeneity among German consumers regarding GM rapeseed-oil .....	137
P30.	Costs of co-existence and traceability systems in the food industry in Germany and Denmark .....	138
P31.	Analysis of the extra-costs generated on French “Label Rouge” chicken supply chain by non-GM feed policy.....	139
P32.	Towards an optimal management regime to facilitate the coexistence of GM and non-GM oilseed rape in Ireland .....	141
P33.	Brazilian GMO Free Areas Experiment and the Release of RR Soybeans.....	143
P34.	A bibliometrics approach on Soybean Research in Brazil .....	144
P35.	The Agroindustrial Chain of Soybean in Brazil: Brief Notes on the Contract of Sale .....	145
P36.	Time Requirements and Financial Expenditures for Coexistence Measures and Their Impact to Profitability of Genetically Modified Plants in Switzerland .....	146

TUESDAY, JUNE 2			
8:00 to 18:00	<b>Setting up : Exhibition and Posters</b>	AgroParisTech (APT)	
14:00 to 18:00	<b>Conference registration (also required for attending the reception on Tuesday evening)</b>	APT– main entrance (16 rue Claude Bernard, 75 005 Paris)	
15:00 to 16:00	<b>Press Conference (access reserved for press)</b>  Chair: <i>François Houllier (DSPPV, Scientific director of Plants and derived products, France)</i>  <i>Yves Bertheau (INRA, France), Frédérique Angevin (INRA, France), Co-Extra Executive Committee Members and Bernhard Koch, Prof. of Tort Law (Innsbruck Uni.)</i>	APT - salle des conseils	
19:00 to 20:30	<b>Reception:</b> Welcome cocktail & Welcome address	City Hall - Mairie de Paris Vème 21 Place du Panthéon 75005 Paris V Métro : Luxembourg (15 minutes walking distance)	
WEDNESDAY, JUNE 3			
SESSION 1: EC Research			
8 :00 - 10:00	<b>Registration</b>	APT – main entrance	
9:00 - 9:10	<b>Welcome address</b> <i>Remi Tousain (Director of AgroParisTech, France) and Yves Bertheau (INRA, France)</i>	APT - Amphi Tisserand & Risler	
9:10 - 9:50	<b>Chair: Yves Bertheau (INRA, France)</b>  <b>1.1 Report on the coexistence of genetically modified crops with conventional and organic farming</b> <i>Sigrid Weiland (DG Agriculture and Rural Development, EC), Alice Stengal (DG Environment, EC), Ciaran Mangan (DG Research, EC)</i>		
9:50 - 10:20	<b>1.2 SIGMEA results on coexistence at the farm level</b> <i>Jeremy Sweet (NIAB, UK)</i>		
10:20 - 10:50	<b>1.3 TransContainer: Overview and Progress</b> <i>Ruud A. de Maagd and Kim Boutilier (Plant Research International B.V., The Netherlands)</i>		
10:50 - 11:30	<b>BREAK AND VISIT OF CONFERENCE EXHIBITION &amp; POSTERS</b>		
11:30 - 12:00	<b>1.4 Co-Extra introduction</b> <i>Yves Bertheau (INRA, France)</i>		
12:00 - 13:30	<b>MIDDAY BREAK</b>		
SESSION 2: Parallel sessions (detailed programme on page 6)			
13:30 - 15:00	<b>Parallel session A.1</b>  <b>Methods for Managing Gene Flow</b>  Chair : <i>Joachim Schiemann (JKI, Germany)</i>	<b>Parallel session B.1</b>  <b>Technologies for Managing the Supply Chain and Detection of GM ingredients in foods and Feeds</b>  Chairs : <i>Kristina Gruden (NIB, Slovenia) &amp; Roberta Onori (ISS, Italy)</i>	Session A: Tisserand  Session B: Risler
	15:00 to 18:00	<b>Parallel session A.2</b>  <b>Coexistence and Traceability in Agriculture and Food/Feed Production</b> Chairs: <i>Frédérique Angevin (INRA, France &amp; Morten Gylling (FOI, Denmark)</i>	
19:00	<b>Conference Dinner on River Seine boat</b>		

Sessions A and B - Details		
WEDNESDAY, JUNE 3, AgroParisTech, France, Paris		
	<b>Session A1 Methods for Managing Gene Flow</b>	Chair: <i>Joachim Schiemann (JKI, Germany)</i>
13:30 - 13:50	A1.1. Biological measures for gene flow mitigation	<i>Alexandra Hüsken (JKI, Germany)</i>
13:50 - 14:10	A1.2. Bio-containment of maize by cytoplasmic male sterility and xenia	<i>Maria Munsch (ETH, Switzerland)</i>
14:10 - 14:30	A1.3. Pollen containment by Cleistogamy in oilseed rape	<i>Xavier Pinochet (Cetiom, France)</i>
14:30 - 14:50	A1.4. Chloroplast transformation and transgene containment	<i>Ralf Bock (MPI, Germany)</i>
14:50 - 15:10	A1.5. Mesoscale dispersal of maize pollen and implications for gene flow	<i>S. Dupont and Y. Brunet (INRA, France)</i>
	<b>Session A.2. Coexistence and Traceability in Agriculture and Food Production</b>	Chairs: <i>Frédérique Angevin (INRA, France) &amp; Morten Gylling (FOI, Denmark)</i>
15:10 - 15:40	A2.1. Empirical analysis of coexistence in commodity supply chains	<i>James Copeland (FERA, UK) and Nicolas Gryson (University College of Ghent, Belgium)</i>
15:40 - 16:10	<b>BREAK</b>	
16:10 - 16:30	A2.2. Modelling coexistence between GM and non-GM within Supply Chains	<i>Louis-Georges Soler (INRA, France)</i>
16:30 - 16:50	A2.3. Costs and benefits of segregation and traceability between GM and non-GM supply chains of final food products	<i>Klaus Menrad, Andreas Gabriel (WZS, Germany)</i>
16:50 - 17:10	A2.4. Consumers attitudes to the EU traceability and labelling regulation	<i>José M. Gil &amp; Montserrat Costa Font (CREDA-UPC-IRTA, Spain)</i>
17:10 - 18:00	Questions and discussion	
	<b>Session B.1. Technologies for managing the Supply Chain</b>	Chairs : <i>Kristina Gruden (NIB, Slovenia) &amp; Roberta Onori (ISS, Italy)</i>
13:30 - 13:50	B1.1. GMO sampling strategies in the food and feed chain	<i>Marina Miraglia (ISS, Italy)</i>
13:50 - 14:10	B1.2. Rationalization of GMO testing by appropriate sub sampling and control plans	<i>Yves Bertheau (INRA, France) and Roy MacArthur (FERA, UK)</i>
14:10 - 14:30	B1.3. The modular approach implemented, pros, cons and future perspectives	<i>Mark van den Bulcke (IPH, Belgium)</i>
14:30 - 14:50	B1.4. Validation of novel methods and technologies	<i>Marco Mazzara (JRC-IHCP, Italy)</i>
14:50 - 15:10	B1.5. Reference materials and reference PCR assays for GMO quantification	<i>Isabel Taverniers (ILVO, Belgium)</i>
15:10 - 15:30	Questions and discussion	
15:30 - 16:00	<b>BREAK</b>	
	<b>Session B.2. Detection of GM ingredients in foods</b>	Chair: <i>Arne Holst-Jensen (NVI, Norway)</i>
16:00 - 16:20	B2.1. New real-time PCR methods available for routine GMO detection labs - applicability and performance	<i>Doerte Wulf (Genescan, Germany)</i>
16:20 - 16:40	B2.2. Reliability and costs of GMO detection	<i>Kristina Gruden (NIB, Slovenia)</i>
16:40 - 17:00	B2.3. Non-PCR based alternative analytical methods	<i>Guy Kiddle (Lumora, UK)</i>
17:00 - 17:20	B2.4. Detecting unauthorised and unknown GMOs	<i>Arne Holst-Jensen (NVI, Norway)</i>
17:20 - 17:40	B2.5. New multiplexing tools for reliable analysis of GMOs	<i>Maria Pla (CSIC, Spain)</i>
17:40 - 18:00	Questions and discussion	



THURSDAY, JUNE 4		
<b>SESSION 3</b>		
9:00 - 9:20	<b>Legal, liability &amp; redress issues</b> Chair: <i>Bernhard Koch (ECTIL, Austria)</i>	APT - Amphi Tisserand
9:20 - 9:40	<b>3.1. Legal, liability &amp; redress issues</b> <i>Bernhard Koch (ECTIL, Austria) and M. A. Hermitte (CNRS, France)</i>	
9:40 -10:00	<b>3.2. Scientific expertise and the judges</b> <i>C. Noiville (CNRS, France)</i>	
	<b>3.3. Juridical cost-benefit analysis of coexistence: uneasy this task!</b> <i>M-A. Hermitte, G. Canselier (CNRS, France) &amp; Y. Bertheau (INRA, France)</i>	
<b>SESSION 4</b>		
10:00 - 10:30	<b>Stakeholder views in EU:</b> Chair: <i>Kristina Sinemus (Genius, Germany)</i>	APT - Amphi Tisserand
	<b>4.1. Stakeholder opinions and attitudes on co-existence of GMOs with conventional and organic supply chains</b> <i>George Sekallaris ( NHRF, Greece) and Renè Custers (VIB, Belgium)</i>	
10:30 - 11:00	<b>BREAK AND VISIT OF CONFERENCE EXHIBITION &amp; POSTERS</b>	
<b>SESSION 5</b>		
11:00 - 11:20	<b>Data integration &amp; Decision Support Systems</b> Chair: <i>Nevena Alexandrova (ABI, Bulgaria)</i>	APT - Amphi Tisserand
11:20 - 11:40	<b>5.1. The Co-Extra Decision Support System: A model-based integration of project results</b> <i>Marko Bohanec (JSI, Slovenia)</i>	
11:40 - 12:00	<b>5.2. Analytical DSS module – how to support decisions in the analytical lab</b> <i>Kristina Gruden (NIB, Slovenia)</i>	
12:00 - 13:30	<b>5.3. DSS modules on transportation (TM module) and on unapproved GMOs (UGM module)</b> <i>Esther Kok (RIKILT, The Netherlands)</i>	
12:00 - 13:30	<b>MIDDAY BREAK</b>	
<b>SESSION 6</b>		
13:30 - 14:00	<b>Experiences from third countries</b> Chair: <i>Morten Gylling (FOI, Denmark)</i>	APT - Amphi Tisserand
14:00 - 14:30	<b>6.1. Benefit-cost analysis, food safety, and traceability</b> <i>James Hammitt (Harvard University Centre for Risk Analysis, Boston, USA)</i>	
14:30 - 15:00	<b>6.2. Segregation measures for (non-) GM crops and their implications for supply chains in Japan</b> <i>Masashi Tachikawa (Ibaraki University, Japan)</i>	
15:00 - 15:30	<b>6.3. Co-Existence and traceability: costs and benefits in food and feed supply chains</b> <i>Bill Wilson (North Dakota University, USA)</i>	
15:30 - 16:00	<b>6.4. Company Perspective</b> <i>Randal Giroux (Cargill, USA)</i>	
15:30 - 16:00	<b>BREAK</b>	
16:00 - 16:30	<b>6.5. Protecting European quality agriculture : non-GM feed supply and production</b> <i>Renaud Layadi, (Region Bretagne, France)</i>	APT - Amphi Tisserand
16 :30 - 17:00	<b>7. Integration of Co-Extra results in EU tools for coexistence &amp; traceability</b> <i>Guy van den Eede &amp; Emilio Rodriguez-Cerezo (European Commission/JRC)</i>	
17:00 - 17:30	<b>8. Summary of main Co-Extra deliverables &amp; results, perspectives</b> Information dissemination & application <i>Yves Bertheau (INRA, France)</i>	
17.30 - 18:00	<b>9. Concluding Comments</b> <i>from Co-Extra, INRA, EC representatives</i>	
19:00	<b>Free evening</b>	





Stakeholder workshop Palais du Luxembourg, Paris		
Friday, June 5		
8:00 - 8:30	<b>Registration</b>	Entrance
	<p><b>Chair (whole day):</b> <i>Yves Bertheau (INRA, France)</i></p> <p><b>Stakeholder panel:</b> <i>Garlich von Essen (European Seed Association, ESA), Arnaud Petit (Committee of Professional Agricultural Organisations, General Committee for Agricultural Cooperation in the European Union, COPA-COGECA), Agnès Davi (Confederation of Food and Drink Industries of the EU, CIAA), Olivier Andrault (UFC Que Choisir, Federal Union of Consumers), Mireille Ferri (Vice-présidente Région Ile de France), Maaïke Raaijmakers ("Platform Biologica")</i></p> <p><b>Moderator:</b> <i>Olivier de Lagarde (journalist)</i></p>	Salle Médicis
8:30 - 9:00	<p><b>Introductory talk</b> <i>Marion Guillou (CEO INRA, France)</i></p> <p><b>Introduction to Co-Extra</b> <i>Yves Bertheau (INRA, France)</i></p>	
9:00 - 9.20	<p><b>From seeds to silo: agricultural coexistence and traceability issues</b> <i>Frédérique Angevin (INRA, France)</i></p>	
9:20 - 10:30	<p><b>Round table</b> Panel questions, then audience questions</p>	
10.30 - 10:50	<p><b>Legal issues</b> <i>Bernhard Koch (ECTIL, Austria)</i></p>	
10:50 - 12:00	<p><b>Round table</b> Panel questions, then audience questions</p>	
12:00 - 14:00	<b>LUNCH BREAK</b>	Salle René Coty
14:00 - 14:30	<p><b>Supply chain management and economic issues</b> <i>Morten Gylling (FOI, Denmark)</i></p>	Salle Médicis
14:30 - 15:30	<p><b>Round table</b> Panel questions, then audience questions</p>	
15:30 - 16.10	<p><b>Stakeholder opinions and attitudes</b></p> <p><b>Some lessons from stakeholder interactions for the future of co-existence</b> <i>Renè Custers (VIB, Belgium)</i></p> <p><b>Statement</b> <i>Pascale Hebel (CREDOC, France)</i></p>	
16.10 - 17:10	<p><b>Round table</b> Panel questions, then audience questions</p>	
17:10 - 17:40	<b>Final comments</b>	
17.40 - 17.55	<p><b>Co-Existence of GM and non-GM supply chains: the point of view of the Commissioner in charge of Agriculture.</b> <i>Julien Mousnier (Member of the cabinet of Ms Fischer Boel, EC, Brussels)</i></p>	
17:55 - 18.10	<b>Conclusions by Jean-Louis Borloo (French Minister of Environment)</b>	

## Abstracts of Oral Presentations

### Session 1: Introductory Presentations

#### 1.1. Report on the coexistence of genetically modified crops with conventional and organic farming

Sigrid Weiland and Alice Stengal,

European Commission, Brussels

Coexistence refers to the choice of consumers and farmers between conventional, organic and GM crop production in compliance with the legal obligations regarding the labelling of GMOs. GMOs as well as food and feed containing, consisting of, or produced from GMOs have to be labelled in order to guarantee an informed choice. As this potentially implies economic losses, e.g. where GMOs appear in conventional or organic products, suitable technical measures have to be taken to segregate GM from non-GM production. Whilst environmental and health aspects of GM crop cultivation must be exhaustively addressed already during the authorisation procedure, they are not to be considered in the context of coexistence. Coexistence measures have their focus on the economic impact.

Member States may take appropriate national measures on coexistence in order to avoid the unintended presence of GMOs in other products. The Commission Recommendation on guidelines for the development of national strategies and best practices on coexistence is intended to help Member States develop national legislative or other strategies for coexistence.

The Commission published recently its second report on coexistence providing an update of the state of national coexistence measures based on information provided by the Member States. The report also gives an overview of the activities undertaken in response to the mandate provided by the conclusions of the Agricultural Council of May 2006.

With 15 Member States having adopted legislation on coexistence, compared to four in 2006, there has been significant progress in the development of coexistence legislation. The approaches applied in Member States differ with respect to administrative procedures and the technical specifications of segregation measures. These differences reflect the regional variation of agronomic, climatic and other factors determining the likelihood of GMO admixture to non-GM crops. A study launched by the Commission shows that all national jurisdictions foresee a minimum protection in cases of economic damages resulting from GMO-admixture in non-GM crops under regular conditions of tort law which differs between Member States. The majority of them has not adjusted the conditions of general tort law to the specific case of GMO admixture.

In parallel to the development of national coexistence regulation, there has been a moderate expansion of the cultivation of GM crops. However, commercial experience necessary for the assessment of the best way forward to address coexistence is still limited.

Research activities concerning various aspects of coexistence are still ongoing in many Member States, illustrating the need for further developing the knowledge base. In view of further assessing and enhancing the efficiency of national coexistence measures, the European Coexistence Bureau (ECoB), created by the Commission, is developing, in collaboration with the Member States, crop specific Best Practice Documents.

From the present report the Commission concludes that there is no need to deviate from the subsidiarity-based approach towards coexistence. The Commission will continue to foster the exchange of information with Member States regarding coexistence and support further coexistence related research based on clearly identified needs.

## 1.2. SIGMEA results on coexistence at the farm level

Antoine Messéan<sup>1</sup> & Jeremy Sweet<sup>2</sup>

<sup>1</sup> Eco-Innov, INRA, BP1, 78850 Thiverval-Grignon, France

<sup>2</sup> The Green, Willingham, Cambridge CB24 5JA, United Kingdom

In 2003, the European Commission established the principle of coexistence which refers to “the ability of farmers to make a practical choice between conventional, organic and GM-crop production, in compliance with the legal obligations for labelling and/or purity standards” and laid down guidelines defining the context of this coexistence<sup>1</sup>.

What needs to be accounted for if we are to introduce in a sustainable manner GM crops throughout Europe so that coexistence is feasible? The cross-disciplinary European SIGMEA Research Project was set up to provide to decision-makers science-based information about the appropriate coexistence and traceability measures that would be needed.

To this end, SIGMEA brought together the principal teams and thereby the principal programmes studying gene flow in a large number of countries across Europe, representing a wide range of agricultural systems including organic farming.

Within the last 5 years, SIGMEA has (i) collated and analysed European data on gene flow and the environmental impacts of the major crop species which are likely to be transgenic in the future (maize, rapeseed, sugar beet, rice, and wheat), (ii) designed predictive models of gene flow at the landscape level, (iii) analysed the technical feasibility and economic impacts of coexistence in the principal farming regions of Europe, (iv) developed novel GMO detection methods, (v) addressed legal issues related to coexistence, and (vi) proposed public and farm scale decision-making tools, as well as guidelines regarding management and governance.

SIGMEA has produced a practical toolbox for addressing GM impacts in agriculture:

1. A unique database including more than 100 data sets on geneflow and ecological impacts which may inform decision-makers on factors driving gene flow at the landscape level and on the variability of such processes across Europe, help regulators to set up coexistence measures at National levels as well as help scientists to identify further research priorities in that area.
2. LandFACTS is a user-friendly windows-based software to simulate crop allocation to fields by integrating typical crop rotations and crop spatio-temporal arrangements within agricultural landscapes and could be used for a practical implementation of coexistence measures
3. The generic gene flow platform LandFlow-Gene, including validated rapeseed and maize modules, is now available as a prototype. It has been used to assess the feasibility of coexistence at the landscape level under various contexts (climate, landscape, cropping systems, adoption rate) and test the effect of coexistence measures. This platform could be extended to other crops to provide a general framework for informing coexistence in all cropping systems of Europe.
4. Structural and organisational factors affecting coexistence in practice have been identified and strategies for managing coexistence at the regional level have been proposed;

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<sup>1</sup> Commission recommendation of 23 July 2003

([http://ec.europa.eu/agriculture/publi/reports/coexistence2/guide\\_en.pdf](http://ec.europa.eu/agriculture/publi/reports/coexistence2/guide_en.pdf))

5. A user-friendly decision-support system (SMAC-Advisor) to assess maize coexistence feasibility at the field level was designed.
6. A comprehensive overview of monitoring and legal issues has been provided and general recommendations have been made.

Altogether, these tools and outcomes can be combined to assess coexistence at various spatial scales (field, farm or region) and various decision-making levels (farmers, elevators, member states, EU).

SIGMEA findings make it possible to address issues such as "what will happen, in terms of gene flow, if a particular GM organism is introduced into a particular European region?" and "how can crops be deployed at the landscape level so as to maintain the adventitious presence of GMOs in conventional crops within the legal thresholds, or any specific market-driven requirements?".

The outcome of both field and modelling studies carried out in SIGMEA is that best practices for coexistence are highly variable and depend on local characteristics, crop practices, environments as well as farmer strategies and preferences, and that the feasibility of coexistence directly depends on the targeted threshold.

For maize, coexistence (defined as complying with the official threshold) for hybrid varieties should be achievable through the use of high purity seed, the management of cross pollination by using varieties that flower at different times and/or spatially separating fields, or the installation of buffer zones or the practice of discarding where fields are in close proximity. For low thresholds (0.1%) or in regions with high density of maize, requested measures such as isolation distances may be impossible to implement and a geographical separation between GM and conventional crops is a reasonable solution. For supply chains, such as organic farming – which requires a total absence of GMOs in their crops – coexistence at a local scale is technically impossible.

Based on regional case studies findings, contrasting global coexistence scenarios may be defined by considering different regulation approaches:

- A "bottom-up" approach, which would let the private actors (collectors, farmers) free to choose the best way to achieve coexistence guidelines and to meet regulatory or market-based threshold requirements;
- A "top-down" approach, based on the strong intervention of public authorities with the implementation of compulsory uniform measures (e.g., isolation distances);
- and a "third way" approach, which provides a focused response of authorities to lift some constraints on private actors.

It has been stressed that a coexistence regime based on "uniform isolation distances", as implemented so far in several member states, is not optimal, not proportional and may lead to unnecessary additional costs or render coexistence impossible in practice.

SIGMEA thus recommends that coexistence measures should be as flexible as possible and depend on local climatic, agronomic and environmental factors. This approach would lead to more cost-efficient measures. However the current regulatory framework to support such an approach is still to be developed.

SIGMEA has developed tools to support the definition and implementation of flexible measures. Predictive gene flow models are now available (currently only for maize and oilseed rape but easily extendable to other crops). These can help decision-makers assess the feasibility of coexistence at the field, farm and silo level for the various targeted thresholds under various environmental and agronomic conditions. In addition simple decision-support tools, like SMAC Advisor can be used by farmers or advisors who would like to quickly assess coexistence feasibility using limited amounts of information at a local field level.

### 1.3. TransContainer: Overview and Progress

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#### Background

The spread of transgenes from genetically modified crops to conventional and organic crops or to wild relatives remains a source of public and scientific concern in Europe. While movement of transgenes from genetically modified crops approved for cultivation to conventional or organic crops is strictly speaking not a biosafety issue, the EU policy for GMO crops is one of co-existence and traceability, i.e. the concurrent existence of all three systems (GMO, conventional, organic) should be facilitated (1). This has led to the development of country-specific “co-existence measures” regulating the growing, processing, and tracing procedures for GM crops (2). Containment measures may be classified as physical, temporal or biological. Current co-existence measures use physical containment, namely minimal isolation distances and pollen barriers, between GMO and conventional or organic crop fields, as well as measures to prevent adventitious mixing during harvesting and processing.

Co-existence of GM and non-GM crops may be promoted by the implementation of biological transgene containment strategies, involving modification of the GMO crop in such a way as to minimize the spread of transgenes through pollen, seed or both. The containment mechanism used for a particular crop needs to be carefully chosen for the mode of transgene spread that is most relevant for that crop, and be compatible with the harvested product (vegetative parts, fruits, or seeds). While not the focus of the above-mentioned EU policy, biological containment may also be beneficial when the spread of transgenes may be undesirable because of human health risks (pharmaceuticals or raw industrial products) or where outcrossing to wild relatives is a considerable risk. Depending on the particular application, biological containment strategies need to be proven failsafe to varying degrees.

#### TransContainer

The EU FW6 project TransContainer, which is coordinated by the authors, comprises 13 partners from universities, research and government institutes, SMEs and one industrial partner. The project is investigating and developing a number of strategies for biological containment:

- Plastid transformation as a means to prevent transgene spread;
- Prevention of flowering as biological containment strategy;
- Controlling transgene transmission through pollen and seed

Where necessary, we aim to complement these strategies with tightly controllable switches to restore fertility. The crops used are European crops grown for their seeds (oilseed rape), fruits (tomato and eggplant), or vegetative parts (sugar beet, rye grass, red fescue, poplar and birch). For some of these crops, several strategies are being developed. Besides developing biological containment strategies, the project also:

- Investigates the impact of the implementation of these strategies on environmental and food safety and on the possible improvement of co-existence rules,
- Assesses the agro-economic effects for European agriculture and compares different scenarios for co-existence,

- Invokes stakeholder dialogue on socio-economic and environmental issues by holding interviews and workshops for stakeholders and the public,
- Communicates co-existence issues and results of the project to stakeholders and the general public through workshops, the project's website, and production of a DVD.

The first results on the biological containment technologies of the Transcontainer project are beginning to emerge, and will be discussed (3, 4). Stakeholder involvement has proven to be a difficult task, as a large part of the public and the users (farmers) are only just coming to terms with the introduction of GM crops and the associated co-existence measures. As a result, many of the stakeholders are not aware of the different biological containment options or have not had time to consider them. When opinions on this technology have been given, they usually follow the lines of the extremely polarized camps in Europe: proponents welcome the option or think that they are unnecessary, while opponents at best denounce release of all GMOs, and at worst see a plot to get GMO-crops accepted or even an excuse to develop GURTs, the infamous "Terminator" technology.

This work was supported by the EU Framework 6 Programme (Contract nr.023018).

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## 1.4. Co-Extra introduction

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Co-Extra is an FP6 (contract 007158) research program of the priority 5 (Food safety and quality) of the European Commission which started in April 2005 and finishes in September 2009.

Its main aim is to provide practical tools to implement coexistence and traceability for the coexistence of supply chains using either GMO, conventional products or organic agriculture derived products. This integrated project completes the two complementary STREPS: SIGMEA working mostly on field coexistence and Transcontainer focusing on biocontainment methods.

The coexistence is understood as the ability to farmers to produce the agricultural products they wish, while still enabling the freedom of choice of consumers. The documentary and analytical traceability studied in Co-Extra are two tools necessary for both managing the coexistence of supply chains and controlling the results of this management. The products to be managed originate either from the European agriculture or from imports from third countries. In several aspects this management of supply chains does not differ from systems already in place, such as waxy maize, or seeds productions. The segregation of such specialities is quite well known and controlled in the EU and several third countries, and does not impact too much European supply chains costs. The main issue in segregating GM and non-GM products lies thus in a rather low labelling threshold of 0.9% and the use of the DNA unit to measure this, as recommended by the EC.

Co-Extra first attempted to address coexistence from the farm to the retailer by starting empirical studies and modelling in fields, and studying their outcomes management in the upper parts of the supply chains. Gene flow studies on long distance of pollen dispersion on fragmented landscape were undertaken and statistical models were validated for e.g. maize. Biocontainment methods, designed to minimize gene flow, were also studied. The effects of seeds admixtures, as well as those of stacked genes, on fields outcomes on current pollen flow models and seeds purity were assessed.

Costs-benefits analyses of coexistence and traceability were undertaken while looking for the most cost-effective detection methods to reduce their impact on the final costs. The practices of traders and third countries farmers were analyzed in order to determine trends that may predict the future of European supply chains.

As a consequence of the 178/02 European regulation, documentary traceability is a well known and implemented practice in European companies. GMO traceability differs from this general request of traceability by adding a longer period of documents preservation. Studies of documentary traceability, particularly in third countries, were undertaken for its positive impact on cost-effectiveness on final prices and its current use in the EU. While the European policy opened the door to analytical controls, documentary traceability is a underestimated way to trace products at the lowest costs in supply chains provided the critical points of supply chains are clearly identified and mastered after initial analytical controls.

As it was exemplified in a previous European study (Kelda / Keste) sampling large batches such as shipments of several thousand tons is not an easy task. The same apply to sampling in fields. As sampling is also carried out for several other purposes such as mycotoxins, pathogens, allergens, a survey of sampling plans was carried out and the interest of combining different sampling plans tested.

Thanks to the 1829/03 and 1830/03 regulations, detection methods (currently Quantitative Real-Time PCR) of EU approved GMOs are all validated through collaborative trials by the CRL (Community Reference Laboratory of the Joint Research Centre at Ispra). However, the



implementation of such methods validated by using a particular chemistry and generally a particular kind of apparatus may be costly and thus induce inappropriate analytical costs. Co-Extra thus decided to compare chemistries and apparatuses to provide an enlarged freedom to laboratories applying these techniques. Alternative detection methods to PCR were also studied as well as fit-for-purpose apparatus to be used in fields. More generally speaking, several ways to improve the cost-effectiveness of current analytical methods were assessed.

As the GMO production is increasing worldwide, numerous incidents of involuntary release of GMO occurred over the last years. GMO approved earlier in third country (e.g. asynchronous approvals between e.g. USA and the EU) have appeared on the European markets. More worrying, newcomers in GMO production, such as some emerging countries, have developed unapproved GMO which have now reached the European markets. In response to this arrival of several EU unapproved GMO, Co-Extra launched studies for developing detection methods for detecting EU unapproved GMOs. The same applied to GMO with stacked genes; some being unapproved though their isolated counterpart may be approved, and to determine accurately the kernels contents of samples having GMO mixtures of stacked and non-stacked genes.

In order to retrieve information from stakeholders and share results with stakeholders, a dialogue was initiated through the web site ([www.coextra.eu](http://www.coextra.eu)), newsletters, focus groups, and a Stakeholder Advisory Board. In addition, the interviews carried out for the supply chains management and economic studies. This dialogue was also improved during a large study of consumers' attitudes and opinions in several European countries. From some attitudes observed in the focus groups Co-Extra started studies on how to solve the issue of 'low botanical presence', where, for example a non GMO cargo may be admixed with very low levels of a different GMO cultivar.

The coexistence and the impact of traceability are both legal issues, thus several studies were launched on the current status of coexistence and traceability legal frame, liability and redress mechanisms. As the scientific expertise per se is also prone to legal contests, a study was launched on this, as well a cost-benefit analysis from a legal point of view on a supply chain case study.

All the results to be issued from Co-Extra are difficult to synthesize in a way that makes them easily made available and mastered, particularly by all stakeholders such as SMEs. This is also true for the laboratories analysts who in routinely face several issues difficult to solve (as for instance the detection of unapproved GMO). Co-Extra thus launched a set of modules of a DSS (Decision Support System), integrating economic parts, management of supply chains with decision rules, laboratory analytical parts including careful assessment of the need for detecting unapproved GMOs in a sample.

All together, the 4 years research of Co-Extra has been performed by more than 200 scientists, with their teams and has been attempting to provide insights of current practices and solutions to issues as well as providing solutions for unpredictable situations. For the first time, a EU research program has been addressing the whole supply chains, from seeds to retailers shelves, their practices, their requirements for taking into account both their current solutions and providing new ones. The needs of the supply chains and their impact on production of crops provided new questions on coexistence and traceability, including cost- and time-effectiveness of analytical methods.

The practical implementation of the several observations and solutions developed by Co-Extra will have important technical, scientific, economic and legal impacts.

## Session A1: Methods for Managing Gene Flow

### A1.1. Biological measures for gene flow mitigation

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WP1 (“Biological measures for gene flow mitigation) of Co-Extra is aimed at assessing and developing biological tools and methods to allow producers to grow the kinds of crops they choose with minimal levels of admixture between GM, conventional and organic products. Therefore, the general objective of this WP is to analyse, further develop and validate methods for restricting gene flow during cultivation by removing or reducing the fertility of pollen or seeds as well as to identify the major drivers of pollen flow over fragmented landscapes. It focus on crops for which GM varieties are already approved or close to authorisation (maize and rapeseed), and on crops whose authorisation is expected during the next 5 years (sunflower, tobacco). The main aim of WP1 is to test the stability and reliability of biological containment tools like cytoplasmic male sterility in maize and sunflower, cleistogamy in oilseed rape and plastid transformation in tobacco. Therefore, parameters of gene flow of CMS-maize and cleistogamous oilseed rape has been studied under field conditions located at different sites in Europe. Moreover, data mining was performed to gain information about the suitability of chloroplast transformation as a containment strategy.

Tools modelling velocity and pollen concentrations over heterogeneous fields were also developed to assess the cross-pollination rates between GM and conventional maize over large distances and fragmented landscapes. Based on gathered data a model of fluid mechanics was successfully validated. Field experiments were carried out to gain information about the major drivers of maize pollen flow over fragmented landscapes. Various factors involved in maize pollen emission and pollen flow were analysed through existing data analysis and due to field experiments. Seed lots are starting points in an ever increasing supply food chain; therefore field experiments of maize seed admixture (1% GM seeds) have been conducted to evaluate the effect of seed thresholds on the final outcrossing rate in the harvest product.

In this presentation, certain results will be presented, which have been obtained in the work package.

## A1.2. Bio-containment of maize by cytoplasmic male sterility and xenia

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While the genetically modified (GM) cultivations are spreading all over the world, the question of coexistence between the different farming systems is a main concern in Europe. For GM maize cultivation, the main issue is the release of GM pollen in the environment and the potential fertilization of conventional and/or organic neighboring fields. Beside studies on isolation distances between the fields, another approach for gene flow mitigation consists of the biological containment of the transgene in cytoplasmic male-sterile (CMS) plants. Cytoplasmic male sterility in maize (*Zea mays* L.) is a natural trait due to a dysfunction in the mitochondrial DNA affecting sporogenesis. CMS plants do not produce and release functional pollen. Three major types of male-sterile cytoplasm (T-, C- and S-type) has been defined in maize according to the specific nuclear restorer genes (*rf* genes) that are able to countermand the male sterility and restore fertility. Breeders used this maternally inherited trait since the 1950s to minimize the costs in hybrid seed production. The Plus-hybrid system, i.e. growing suitable mixtures of GM cytoplasmic male-sterile plants (80%) and unrelated non-GM male-fertile plants (20%), the latter acting as pollen donors, is an interesting way for controlling the release of pollen from genetically modified maize. The Plus-hybrid system relies on the fact that the female fertility of CMS plants is not affected, and seeds can be set if vital pollen is provided. One prerequisite is however essential; the male-sterile trait must be reliable under various environmental conditions.

### European CMS hybrids are reliable bio-containment tools <sup>[1]</sup>

Our hypothesis in this study was that one or more environmental factors may influence the expression of the male sterility. Therefore, field investigations were carried out in 2005 and 2006 in the frame of the European project Co-Extra. Twenty modern CMS hybrids from different European breeding companies representing all three cytoplasm types were tested in 17 environments in Switzerland, Bulgaria, Germany and in France. Stable and unstable male-sterility occurred in all three CMS types. The reversion to fertility was due to an interaction between genetic (presence of minor *rf* genes) and climatic (air temperature, photoperiod and water vapor) factors. CMS-T was identified as the most stable type of male-sterile cytoplasm; nevertheless, due to its susceptibility to the fungi *Bipolaris maydis*, its use may be limited to the growth of small-scaled transgenic fields, e.g. molecular farming. While CMS-S was often subject to restoration of fertility, the C type of male sterility was similar to the T type with regard to maintaining the male sterility and could be applied in a larger scale for the growth of e.g. *Bt*-maize (in mixture with non-transgenic male-fertile plants). Even in situations, where the male-fertile component of the Plus-Hybrid needs to be genetically modified too (e.g. herbicide tolerant trait), such a cultivation system can reduce the release of transgenic pollen by 80% compared to a regular GM maize stand, where 100% of the hybrids release transgenic pollen.

### Maize Plus-Hybrids increase grain yield<sup>[2, 3]</sup>

Beside their potential as a bio-containment tool, maize Plus-Hybrids combine benefits of male sterility (CMS effect) and allo-pollination (xenia effect) regarding the grain yield. They often outperform the corresponding male-fertile sib-pollinated hybrids. The potential gain in yield afforded by modern European Plus-Hybrid was investigated in a preliminary field trial in 2005 (3 locations in Switzerland) and in a European ring trial in 2006 and 2007 (12 locations in Switzerland, Bulgaria, Germany and in France). Many Plus-Hybrids increased grain yield, on average, by 10% or more and by up to 20% in specific environments. The Plus-Hybrid effect affected both yield components, CMS leading mainly to a higher number of kernels and the xenia effect mainly to an increase in the thousand kernel weight. While the CMS effect depended strongly on the environment, the xenia was consistent in all environments but its extent varied.

Cytoplasmic male sterility is an elegant way to minimize or even eliminate the problem of GM pollen flow of adjacent conventional or organic fields if stable T- and C-cytoplasm is used. The Plus-Hybrid system would be a useful tool to achieve an agricultural bio-containment system. For this system, a high level of male sterility must be maintained, as shown by this study. Furthermore, appropriate combinations of CMS hybrids and fertile pollinators can lead to a significant gain in yield that would definitely boost the acceptance of a bio-containment system with cytoplasmic male sterility.

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### A1.3. Pollen containment by cleistogamy in oilseed rape

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The diversification of farming production systems with the apparition of transgenic crops, as well as the specialization of crops cultivars for different markets, require measures to prevent adventitious presence in productions at the field, storage and refinement level. For instance, in oilseed rape crops, such means are necessary to allow the coexistence of productions requiring different fatty acid composition. In fields, adventitious presence in adjacent fields is mainly due to pollen flow, which have to be reduced to make possible the co-existence of different crops. Pollen flow between adjacent fields may be reduced by physical ways: by putting separation distances between fields grown with the same crops or by surrounding the crop of which the pollen is considered as contaminant with a buffer crop strips. Biological ways of containment, such as male sterility or cleistogamy, may also be used depending on the species. One simple way to prevent pollen flow between oilseed rape is to ensure that their flowers do not open. Cleistogamous plants do not exist naturally among the genetic resources of the oilseed rape species, but different lines of cleistogamous oilseed rape were obtained by chemical induced mutagenesis at INRA Rennes (Patent FR 97 15768). The cleistogamous trait is controlled by one gene (Renard and Tanguy 1997) and would be a good way of securing biocontainment, on condition that this trait is stable during the flowering period and under various environmental and agricultural conditions. One aim of our study was to test the stability of the cleistogamous trait in the field under several environmental conditions. In this goal, the flower opening level was observed at different dates during the flowering period, on two cleistogamous genotypes tested in three locations, during two successive years and under two treatments (with or without the application of a growth regulator at the vegetative restarting at the end of winter). The second aim was estimate the rate of adventitious presence of cleistogamous lines by allo-pollen under several environmental conditions. In this goal, the allo-pollination in seed sets collected on Clg1 plants was tested in three locations using erucic acid as a marker during two successive years.

#### Material and Methods

The stability of the cleistogamous trait was assessed for two rapeseed cleistogamous lines, Clg1 and Clg2, corresponding to the lines 17046 and 16960, respectively, provided by INRA-Rennes (Patent FR 97 15768). Control cultivars were used in each site. In each site, a split-plot field design, using a randomized block design, in four replications, was carried out, with elementary plot having areas between 22.5m<sup>2</sup> and 47,5 m<sup>2</sup>. The development of the crop was characterized by notations of the dates when key development stages were reached and the plant height at maturity. During the flowering period, the stability of the cleistogamous trait was assessed visually by scoring of opening level on mature flowers of the inflorescence with a three-level scale: the first class corresponded to the full opened flowers, the second class of the totally closed flowers that appeared like a big yellow bud, and the last class of the partially opened flowers. Ten plants were scored per plot, with notation of at least five flowers on the main stem and on one secondary stem.

The allo-pollination was assessed for one rapeseed cleistogamous line (Clg1, corresponding to the lines 17046 provided by INRA-Rennes (Patent FR 97 15768)). As a pollinator cultivar a high erucic

acid rapeseed line (Markant) was used in each site. The trial was isolated by at least 500m from other rapeseed fields. The trial was composed of 2 neighbouring plots :The first plot was sown with a mixture of 99% of Marcant (erucic line) seeds and 1% of Clg1 (cleistogamous line) seeds.The second plot was sown with the cleistogamous line Cleisto1. Each plot was 50m long and 50m large and the sowing rows had the same direction as the limit between the two plots, and as the dominant wind. Correlations between rates of seeds derived from crosses with the erucic line and the erucic acid content in seed sets were established in each site according to the erucic acid content of seeds produced by manual crosses between Clg1 and the erucic line.

## Results

The first experiment showed that flowers of cleistogamous lines are mostly totally closed, but a variable proportion of flowers were observed as partially open. The average percentage of totally closed flowers (Clg1 and Clg2) reached 72.03% at location 1 (2007), 80.91% at location 2 (2007), 85.05% at location 3 (2007), 86.96% at location 2 (2006), 88.91% in at location 1 and 89.69% at location 3 (2006), with standard deviations of 26.6, 24.3, 19.3, 9.54, 7.9 and 6.6, respectively in each site x year. Global analyses of all the data from the six site x year combinations revealed that the environment (site x year) had an effect on the stability of the cleistogamous trait, as differences among sites and years were observed. The main effect of genotype (Clg1 or Clg2) explained 33% of the variability of the percentage of totally closed flowers. This statistical result reflects the difference of mean and of variance showed by the two genotypes: in each environment, Clg1 showed a high stability level for the cleistogamous trait, whereas Clg2 showed a higher and more variable rate of partially open flowers. Finally, a low but significant difference was also observed between the notations done on the primary or on secondary stems, and the application of growth regulator had no significant effect.

The second multi-site experiment showed that the environment (site x year) had an effect on the allo-pollination, as differences among sites and years were observed. Allogamy rates of Clg1 under a high pressure of allopollen (Clg1 sampled in erucic block) vary in three locations between 4.4% and 16.2%. The samples collected on open pollinated cleistogamous plants (Clg1 sampled in Clg1 block) at different distances from the erucic plots showed that the percentage of allogamy rates rapidly dropped over the initial meters around the pollen source and decreased as the distance from the pollen source increased. In samples (location 2) collected on plants at 0m from the erucic plot, the erucic acid content reached at mean 1.64%, but at 6m, we observed only 0.26% of erucic acid. However, erucic acid was also detected in samples collected at 48m from the erucic plot, showing that adventitious presence, at low rates (less than 0.2%) may occur at large distances.

## Conclusions

The main result from our various studies is that cleistogamy has a major potential for limiting crosspollination due to the strong reduction of the pollen cloud. We suggest that isolation distances implemented for oilseed rape could be dramatically reduced when using cleistogamic oilseed rape as a containment strategy.

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## A1.4. Chloroplast transformation and transgene containment

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Plants with transgenic plastid genomes (referred to as „transplastomic“ plants) provide an attractive alternative to conventional transgenic plants (Ruf et al., 2001; Bock and Khan, 2004) and are increasingly used in metabolic engineering, resistance engineering and molecular farming (Bock 2007a). The plastid transformation technology offers several technical attractions, such as, high-level transgene expression (reaching foreign protein accumulation levels of up to >70% of the plant's total soluble protein; Oey et al., 2009), convenient transgene stacking in operons, absence of epigenetic transgene instability (no gene silencing and position effects) and precise transgene integration by homologous recombination (Bock, 2001; Bock 2007a). Furthermore, the increased biosafety provided by transplastomic plants is of particular relevance to future applications of genetic engineering in agriculture and biotechnology. Plastids (chloroplasts) are maternally inherited in most crops. Maternal inheritance excludes plastid genes and transgenes from pollen transmission (Bock 2007b). Therefore, plastid transformation is considered to provide a superb tool to ensure transgene containment and improve the biosafety of transgenic plants. In a large-scale study, we have recently assessed how strict maternal inheritance is and how much increase in transgene confinement plastid transformation technology confers. We have developed an experimental system facilitating stringent selection for occasional paternal plastid transmission (Ruf et al., 2007). In a large genetic screen, we detected low-level paternal inheritance of transgenic plastids in tobacco (*Nicotiana tabacum*), one of the currently most preferred species in molecular farming (i. e., the high-yield production of pharmaceuticals in plants). While the frequency of transmission into the cotyledons of F1 seedlings was approximately  $1.58 \times 10^{-5}$  (upon 100% cross-fertilization), transmission into the shoot apical meristem was significantly lower ( $2.86 \times 10^{-6}$ ). As these experiments address the worst-case scenario (100% cross-fertilization, strong selection for the transgenic plastids), our data demonstrate that plastid transformation provides a highly effective tool to increase the biosafety of transgenic plants (Ruf et al., 2007). However, in cases where pollen transmission must be prevented altogether, stacking with other containment methods will be necessary to eliminate the residual outcrossing risk.

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## A1.5. Mesoscale dispersal of maize pollen and implications for gene flow

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The growing introduction of genetically modified (GM) crops has generated a host of research efforts aimed at investigating the possibilities for coexistence between GM, conventional and organic farming systems. Published experimental and modelling studies aimed at characterizing pollen dispersal have shown that most pollen emitted by a source field deposits within a short distance from the latter, but also that the observed dispersal functions have long fat tails, making it possible for pollen to contaminate plants at rather long distances.

Such possibility has been recently confirmed from (i) a series of airborne measurements of maize and oilseed rape pollen concentration and viability in the atmospheric boundary layer, (ii) chamber measurements of pollen viability in a large range of temperature and humidity conditions and (iii) observations of fecundations in isolated plots of white-kernel maize, at several km from any maize field.

In order to better understand long-range dispersal of maize pollen an approach has been developed to simulate the trajectories and dehydration of pollen grains in the atmosphere at regional scale. To this purpose the non-hydrostatic mesoscale Meso-NH model has been modified so as to introduce source terms for pollen emission, conservation equations for pollen concentration and moisture, and a deposition velocity. Simulations have been performed over South-West France on several days during the maize pollination period. MesoNH is run in a two-way nested configuration including three nested computational domains down to a 2-km horizontal resolution. GIS-based landuse maps are used for the surface conditions, featuring all the maize fields of the region, as previously identified from satellite data. Considering several days during which airborne measurements were performed, observed and simulated concentration profiles are found to agree well throughout the atmospheric boundary layer. The simulations allow the pollen plume to be characterized through each day and deposition maps of viable pollen to be produced. The calculated deposition rates at remote distances from the maize fields are in the same range as those observed in situ. The results provide evidence that background fortitious contamination is unavoidable at regional scale. Additional test simulations will be performed using specific landuse patterns in order to quantify the impact of landscape structure on regional pollen deposition.

## Session A2: Coexistence and Traceability in Agriculture and Food Production

### A2.1. Empirical analysis of coexistence in commodity supply chains

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#### Introduction

Coexistence refers to the ability of farmers and consumers to make a practical choice between conventional, organic, and genetically modified (GM) products, based on compliance with the legal obligation for labelling and/or purity standards. Adventitious mixing of GM material with a non-GM product can occur at various stages along the product supply chain, from the field where the crop is grown to the handling and processing plant. In the framework of Co-Extra, the organization of different supply chains were analysed and sensitive points and processes were identified with respect to GM and non-GM admixture and traceability. Seven commodity supply chains were investigated in various countries: soybean, maize, sugar beet, rapeseed, wheat, fresh tomato and potatoes.

#### Methodology used

This empirical analysis of coexistence was based upon supply chain analysis and stakeholders' interviews. Interviews focused upon a general description of companies and processes, and on the solutions currently adopted to deal with coexistence between GM and non-GM products. Supply chains have not been faced to the coexistence issue with the same degree, especially due to the fact that only a few GM varieties have been authorized in Europe. Thus, questionnaires also included questions about existing specialties supply chain (such as waxy maize, upper standard rapeseed, erucic rapeseed, etc) to gain an insight into how some stakeholders cope with the coexistence between different types of conventional products.

## Results and discussion

The study of different commodity supply chains enabled the identification of critical points from seed production to retail. Furthermore, different strategies stakeholders may choose were identified, as well as the prerequisites, strengths and weaknesses of different strategies.

### *What are the critical points within the supply chains?*

One of the most crucial points in the supply chain is crop production. Admixture at this stage of the supply chain may be spread over many different end-product batches and should be thoroughly managed. Admixtures at crop production level may be due to seed impurities, volunteers, cross-pollination between GM and non-GM crops, and insufficient cleaning of sowing and harvesting machinery in case a non-GM crop is sown and/or harvested after a GM crop. The level of risk associated with volunteers, seed impurities, and cross-pollination is highly dependent upon the crop biology.

Elevators are identified as one of the main sources of unintended admixture, as in the wheat, soybean, rapeseed, and maize supply chains. There are also risks of admixture the processing level when crushing rapeseed, wet-milling maize and processing potatoes. The risks of admixture increase with the number of operators in the supply chain and product flows. Within the processes of storage, processing and trading various critical points were identified. Therefore, the ex ante perception of coexistence feasibility differs from one commodity to another, and from one stakeholder to another. On the one hand, some stakeholders consider that GMO and non-GMO are just different commodity qualities and can be processed like any other quality as far as an adapted quality assurance system is undertaken. On the other hand, several stakeholders find it difficult to cope with coexistence and consider that ensuring coexistence between GM and non-GM commodities requires restructuring of their process and additional investments.

### *What kind of strategies can be adopted to handle issues arising at these critical points?*

At the moment, there is little experience on coexistence between GM and non-GM products (mainly soybean and to a lesser extent, maize). Interviews showed that downstream stakeholders require conventional (non-GM) products to be compliant with a lower threshold (0.1% or 0.01%) than the 0.9% regulatory threshold. In situations where GM and non-GM coexist, strategies adopted to handle coexistence are different between food and feed supply chains. In fact, no labelling rules apply for products derived from animals fed with (non)-GM feed. On demand of the retailers, food processors have replaced GM soy ingredients with alternative ingredients derived from non-GM-critical crops such as sunflowers. For the soybean feed supply chain, systems of identity preservation of non-GM products have been introduced in order to guarantee a purity threshold of 0.1%. As a result, an increased level of contract detail and some vertical integration of activities have been observed in the soybean chain. Furthermore, several stakeholders have introduced books of charge, which describe the conditions of production and delivery of specific products, in order to ensure the segregation of GM and non-GM flows. Next to production requirements, these books include requirements with respect to sampling plans, GMO detection, registration of activities and management. All activities are inspected by independent third parties. However, as animal product labelling is not possible, the feed industry has trouble in assigning a value to the efforts made by the manufacturers.

At the elevator and processing level, several scenarios for coexistence were identified: (i) spatial isolation (dedicated plants), (ii) line isolation (use of separated production lines), and (iii) temporal specialisation with alternation of products. The dedication of companies or plants to either GM or non-GM production offers the lowest risk of admixture. In the line isolation strategy, dedicated

production lines are used in the same plant, which increases the risk for admixture and decreases the overall flexibility in the company. Both strategies however may suffer from under-capacity use because of changing demands. The temporal specialization of process lines is more flexible but requires regular cleaning of equipments or downgrading of non-GM batches. Due to differences in size and structure, the choice for a specific strategy should be taken on a case-by-case basis and is likely to be driven by market demand.

## A2.2. Modelling coexistence between GM and non-GM within supply chains

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### Introduction

Coexistence is an approach allowing farmers to choose between conventional, organic and genetically modified (GM) crops and allowing consumers to choose between different food products subject to obligations regarding labelling and purity. Coexistence between GM and non-GM supply chains is a complex issue, because adventitious mixing of GM material with non-GM product can occur at any one of the stages of production and anywhere along the supply chain, from the field where the crop is grown to its handling and processing. Another major facet of GM and non-GM coexistence is the fact that the GM content of a product is not a visible attribute. Means to bridge the gap in information do exist (product testing, using model), but they are subject to error. In this paper, we present a simulation model of the coexistence between GM and non-GM products along supply chains. More specifically, the framework of the model is inspired by the starch maize supply chain. The aim of this model is to assess the ability of the supply chain to provide final non-GM product compliant with a required threshold (0.9% labelling threshold for example) and to discuss the impact of the means to bridge the information gap on this probability of compliance.

### Material and Methods

The model simulates GM and non-GM flows, and takes into account admixture and dilution functions between GM and non-GM batches along the supply chain. Inspired on the example of the starch maize supply chain, three key stages of the supply chain are considered: grain production at field level, grain collection (including drying), and processing. Firstly, the MAPOD gene-flow model (Angevin *et al.*, 2008) is used to simulate GM adventitious presence in non-GM harvests due to cross-pollination between GM and non-GM maize. Within the downstream supply chain, there is only one dryer and one processing plant. Hence, GM and non-GM material are successively handled in the same equipments. On the contrary, storage capacities are considered non-limiting in the model and admixture due to storage equipments is considered negligible. At the maize collection level, the model simulates on the one hand admixture between several batches blended in a same bin, and on the other hand admixture between succeeding batches during drying process. Finally, the model simulates admixture between succeeding batches at processing. We have adopted a compartmental modelling approach of the process to quantify risks of admixture.

Stakeholders define the frequency at which GM and non-GM flow alternate at drying and processing levels (scheduling parameters). GM and non-GM batches are then randomly ordered according to these variables.

Once sequences of batches have been scheduled, uncertainty remains about the GM content of the batches, all the more that it is not a visible attribute. Three kinds of control system might be set up in the model:

1. Simple traceability: this system allows stakeholders to identify whether the batches comes from either GM or non-GM varieties.
2. Automatic downgrading: the simple traceability system is supplemented by rules on automatic downgrading of non-GM batches dried and/or processed after GM batches.
3. PCR Testing: in addition to the simple traceability system, testing is used to gather information on the non-GM batches. The model takes into account the fact that testing can

be inaccurate (Starbird, 2007). We assumed a proportional error by simulating measurement uncertainty with a lognormal distribution. Testing can be carried out before and/or after processing.

Two contrasted sets of admixture parameters (at drying and processing levels) were taken into account for the simulations, corresponding to low and high level of admixture between succeeding lots. In addition, previous studies have highlighted that the distribution of GM adventitious presence in non-GM harvests is quite variable among regions (Le Bail *et al.*, submitted). Thus, three contrasted distributions of the GM adventitious presence in non-GM harvest were taken into account, in order to assess the effect of the input purity rate on the output purity rate. As far as the scheduling scenarios were concerned, two values of the scheduling parameters were taken into account: 10 and 100.

For scenarios 2 (automatic downgrading) and 3 (PCR testing), the model identifies the strategy that maximises the profit. Profit depends on the number of batches of each type (GM and non-GM), on the testing cost and, on the probability that non-GM batches are compliant with the required threshold, according to clients testing. Client testing is performed several times and the mean value is considered for the profit calculation.

## Results and discussion

Work on the simulation model is still on-going. Nonetheless, first simulation results show that chain organization, from the upstream producers to the downstream stakeholders, plays a crucial role in maintaining or improving the non-GM product compliance with the labelling threshold. In addition, model should allow comparing various strategies.

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## **A2.3. Costs and benefits of segregation and traceability between GM and non-GM supply chains of final food products**

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### **Aims**

The basic intention of this part of the project was to quantify the costs and to identify the benefits of traceability and co-existence systems for GM food (and feed) from the seed to the final product at the retail stage in several countries and supply chains respecting the 0.9 % threshold for labelling of GM food. Thereby the production and processing stages of eligible GM crops like wheat, sugar, rapeseed, soya and maize are analyzed with respect to cost structures originating from efforts to organize co-existence and segregation. The multi-national analysis of several supply chains with partly different end products allows meaningful comparison of economic and technical consequences of co-existence measures on the different stakeholders along the supply chains. As some of the analyzed foodstuffs like soy lecithin, sugar and starch derivatives are used as ingredients for complex food (and feed) products (like e.g. chocolate, frozen pizza, compound feed), the impacts of GM and non-GM co-existence on the value chains of such complex products are analyzed as well. As these complex products are composed of several critical ingredients and this type of product is closer to the food retailer and consumer, the compliance with co-existence regulations and thresholds has to be realized in an even more complex environment. Another target of the project was to detect benefits emerging by the implementation of product differentiation systems and assess their impacts on the different levels of the value chain from the seed producer to the private consumer of foods.

### **Methodology**

For calculating the co-existence and segregation costs, an Excel-based simulation model has been developed which includes potential cost categories on each level of the value chains. The total costs at each level follows the principle to aggregate all incurred costs for cultivating, transportation and processing of the raw material crops on the different levels and to increase the price of the final product at each level. The resulting price for the secured non-GM crop or product represents automatically the non-GM commodity price on the next level of the value chain, while the price of GM commodity is assumed as the current price level without any co-existence and traceability measures. This principle is used at all stages of the supply chain thus aggregating the additional costs for respecting the 0.9 % threshold of adventitious presence on all levels (at the seed level the 0.5% threshold is mainly respected) and setting the price for the non-GM product at the end of the value chain.

This conceptual approach is also perpetuated when identifying the costs in the processing of complex food and feed products like chocolate, frozen pizza and compound soy and wheat feed. The model allows for an isolated view on every single ingredient that carries potential risk of GM contamination and the emerging cost types can be calculated separately.

Subsequently, benefits of introduced co-existence and product differentiation systems (IP, segregation or traceability systems) are analyzed within a literature research and finally the emerging benefits of such systems are confronted with the originating costs.

## Results

The generated cost calculation model was applied on the food and feed value chains of wheat (starch, flour, feed additive), sugar (sugar beet as raw material), rapeseed oil, soy (feed additive) and maize (starch, feed additive) in the participating countries Germany, Denmark, Poland, UK and Switzerland. Basically, the cost structures and the results of the cost calculations between the single countries do not only differ because of national differences in implementing the existing co-existence regulations of the EU, divergent farming or industry structure, but also due to the information given in the conducted interviews and available data e.g. concerning costs of specific activities. While for a Swiss oil mill company the commodity delivering system is quite manageable and the input testing of elevated rapeseed is negligible, the bigger companies in Germany and Poland, with several processing sites cannot manage threats of admixture without monitoring systems at the entry gates. Another example for differing cost structures is the impact of field structures on co-existence schemes in farming in the different countries. Several possible strategies of maintaining isolation distances between GM and non-GM fields can be applied depending on the regional field distribution and national regulation of liability. While for the German farmers it is assumed that the GM rapeseed farmer has to compensate the loss of gross margin by cultivating alternative crops on a certain discard width by the non-GM farmer, the conditions in Polish agriculture determine buffer zones on GM fields as additional effort to maintain co-existence in rapeseed production. Thus, the individual combination of cost types and the particular origin of data have to be respected by the comparison of the country-specific results of the cost calculation.

The project team faced the most uncertain figures at the producer levels (seed and crop production) of the regarded value chains. Due to the still lacking threshold on GM adventitious presence for most crops in the EU only very few information exist concerning the necessary measures and additional costs of co-existence in certified seed production. Additional costs of co-existence and segregation efforts are calculated with 38 or 86 € per ha respectively for the Danish and German wheat crop production. For rapeseed, a crop with a quite high risk of receptiveness of pollen from other plants and varieties, the total additional costs are stated from 40 € per ha in Denmark up to 74 € per ha in Germany and Poland. At elevator level, within its functions of storage, drying and distribution the risk of admixture is determined as quite high. Depending on the size of the elevator company and its capacities, the additional costs vary from 7 to 16 € per ton wheat (Denmark, Germany), 18 to 64 € per ton rapeseed (Germany, Denmark, Switzerland) and are estimated with around 30 € per ton for the elevating of maize in Germany. The high ranges in the cost figures can be explained with the different possibilities of the company to apply certain segregation strategies. Transferring these additional costs to the final processing level, the mills, crushers and processors, the increased expenses for the used raw materials caused by the co-existence activities in the previous levels of the value chain result in the highest costs for commodities and transport when implementing co-existence and traceability management systems. Over all regarded chains these commodity costs together with costs for required monitoring systems form more than 90% of all costs for implementing co-existence systems. The total additional co-existence costs at the end of the value chain, which had to be added on the general producer price, are calculated with at least 25 € per ton wheat starch in Germany, 11 € per ton wheat flour in Denmark or 22 € per ton rapeseed oil in Poland. The additional co-existence costs in the case of sugar show the lowest exaltations, as beet production and processing imply lower risk and better conditions to avoid admixture and maintain thresholds. The identified additional prize loadings of mono-food products are included in the cost calculation of compound feed and multi-ingredient food products in order to analyze the economic impact of co-existence systems when handling several raw materials in one final consumer product.



## Conclusion

According to the results of the analysed food supply chains, significant additional costs are expected by organising co-existence between genetically modified and non-GM products in the value chain from production of farm crops up to the production/processing levels of the single supply chains and by maintaining mandatory (or voluntary) thresholds and regulations. Depending on factors like crop requirements, farming, storage and elevating systems, processing strategies, monitoring managements etc, the total additional costs of co-existence and product segregation systems can raise up to 13% of the total product turnover at the gates of rapeseed oil mills or starch industry processing wheat and maize. However, as in most value chains the question of co-existence currently is a theoretical one in the EU, the implementation and permanent running of co-existence and segregation systems in the food industry can decrease the additional costs due to savings e.g. in the testing requirements of raw materials or routine procedures during the documentation process.

## A2.4. Consumers' attitudes to the EU traceability and labelling regulation.

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The introduction of new technologies in the food industries have revolutionized the efficiency of food production, but has also exerted important demand side effects that cannot be dismissed. This is because new technologies are associated with scientific uncertainty given that not all the social and individual consequences of their inception are fully known. As Moschini (2008) argued, based on Gaskell et al. (2006), general public opposition or reticence towards genetically modified (GM) agro-food applications responds to: i) human health and environmental concerns, ii) ethical considerations and iii) the role of patents and property rights of multinational corporations. This variety of reasons against GM agro-food production reveals a complex formation process of public opinion towards GM agro-food production and therefore a complex process for understanding consumers' final decision and intentions regarding GM food. The main objective of our study is to investigate consumers' general attitude towards GM food and their willingness to pay (wtp) a premium for conventionally produced non-GM food and organic food.

To do that we have first performed a literature review in order to bring together the published evidence on the behavioural frameworks and evidence on the process leading to the public acceptance of GM food. In doing so, we employ a set of clearly defined search tools and a limited number of comprehensive key words. This review concluded: first, that the population can be segregated in three main groups regarding attitudes toward GM food, namely: (i) anti-GM food or pessimistic, (ii) risk-tolerant or information searchers and finally (iii) GM-accepters or optimistic. Second, that consumer attitudes towards GM food are driven by three main dimensions, i) risks and benefit perceptions associated to GM food; ii) individual values and attributes and finally iii) knowledge and its relation with values.

From the previous review it was also concluded that consumer behaviour towards GM agro-food production has many analogies with other behaviours analysed in the past. This is the case of other risky technologies such as pesticide risk exposure, hormone-treated meat, atomic energy and so forth. For instance previous studies based on the Fishbein Multi-attribute Model (Fishbein, 1963) revealed that an attitude or intention towards a product or behaviour is based on knowledge about the product or behaviour itself (Bredahl, 1998); that is, on the attributes that people associate to the product or behaviour (Frewer et al., 1998). Following this theory, we have considered that the best way to study consumers' final intentions towards GM and non-GM agro-food products entail the application of choice experiments. Within the choice experiment framework individuals are allowed to select among different alternative options, where each option is characterised by a number of attributes with different levels (Burton et al., 2001). Therefore individuals will choose an alternative, among a set of alternatives that generates to them the highest utility.

Following consultation with stakeholders, a number of food commodities for study were to be chosen. On the one hand fresh food, e.g. fresh tomatoes, on the other long-stored processed commodities, e.g. oil seed or cornflakes. The analysis was performed by means of a multi-country survey (Denmark, Germany, Spain, GB and Poland). The main results of the survey can be

summarized as follows. Freshness and flavour can be considered as the most important element for food purchasing. However, in GB, Poland and Spain price is also considered. There is a general negative attitude towards GM food in all countries. University scientists and consumer groups are the more trusted sources of information, and Denmark and Germany responders feel themselves more informed than the rest. Regarding to organic food, only German and Danish consumers do spend on organic food. Moreover there is an agreement among countries regarding positive attitudes towards organic food. The study also revealed that GM technology is not considered by respondents as very risky compared with pesticides, artificial hormones or irradiation. Finally, respondents in all study countries prefer conventional food over GM food. However, Spanish respondents made a slight exception since they were prepared to pay a premium for GM food with health benefits. Moreover, all study country respondents except Polish ones, assigned a higher utility for organic food in relation to conventional counterpart.

## Session B1: Technologies for Managing the Supply Chain

### B1.1. GMO sampling strategies in the food and feed chain

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The sampling plan is the procedure of taking a sample, from a lot, for analysis and is the most crucial step in the “analytical chain” whenever the analyte is not homogeneously distributed in the lot. It is imperative that the sampling step is performed as accurately as possible so that the sample collected is representative of the batch of food or feed under investigation and to get the most accurate “true value”. Without the implementation of a good sampling plan, misclassification of the lot could easily occur, negatively impacting sampling objectives: undesirable economic and legal impacts in trade and inaccurate information being provided to risk assessors/managers. Accordingly, the GMO sampling should be considered with the other sampling issues faced in the domain of food and feed safety and quality.

Among the steps usually employed in the evaluation of the GMO level in a lot (sampling, sample preparation and analysis), the sampling step is the major total error contributor and is dependent on the GMO level. Due to the variance associated with each step of the GMO evaluation, a 100% level of certainty is unachievable; resulting in overestimation and underestimation with inaccurate decisions producing adverse financial or social implications.

Sampling of GMO in food and feed commodities is performed by different stakeholders with a wide spectrum of goals, all of them implying different scenarios and consequently often needing different methodologies.

Commission Recommendation 2004/787/EC of 4 October 2004 provides detailed technical guidance for sampling and detection of GMOs related to Regulation (EC) No. 1830/2003 in bulk and very little and unclear details for packed products. However, among the general principles for GMO sampling, the Recommendation states that the Member States should take into account the point in the supply chain in which testing is being performed and the degree of heterogeneity, therefore indicating that sampling can be modulated depending on the situation. In respect to traceability and internal quality control purposes operators also need an array of sampling procedure throughout the food and feed chain. The Recommendation also underlines the need to use sampling proportionate to the desired specific objectives and the possibility to use sampling strategies other than those indicated in the Recommendation. In other words, it would be appropriate to, in addition to the guidelines suggested by the Recommendation, develop further “fit for purpose” sampling strategies.

Development of sampling methods has been an important goal within the Co-Extra project. In addition, a Modular Decision Support System (DSS) has been developed for producers and official control authorities, to support decisions related to the selection of “fit for purpose” sampling methods. Co-Extra goals include: (i) development of “as simplest as possible” sampling methodologies compatible with reliable results; (ii) meeting the needs of different food and feed operators; (iii) optimization of sampling in the different steps of food and feed chain, of the associated cost and degree of accuracy. This presentation will give an overview of the developments from Co-Extra project relevant to the sampling in different scenarios (field, bulk, processing and retail).

In the Commission Recommendation 2004/787/EC sampling in the field is not specifically addressed while in certain cases of co-existence it is important to determine, before harvesting in the field, the level of adventitious presence of GMOs in a non-GMO field. In the Co-Extra context, field trials were conducted in two successive years aimed at developing a reliable sampling procedure for maize plants in the field (model for fragmented landscape with very small field sizes). Every year 3600 samples were collected to determine out-crossing rate in the field, using data mining techniques. Based on the predictions of spatial variability of out-crossing rate, various possible sampling procedures were tested using the statistical Programme R and different sampling schemes were then developed and validated.

As for the food processing chain there are three types of material which may need to be analyzed for the presence of GMOs: raw materials, primary ingredients and final food products. The soybean processing chain (from grain to lecithin) has been chosen as “case study” with respect to its use in a wide range of foodstuff ingredients and additives. The study also provided a simple framework to assist in the decision making to allocate resources (broadly “sampling” and, “analysis” costs) and to balance the cost of control versus the risk associated with incorrect decisions based on test results. Applying control plans that have been optimised for efficiency between sampling and analysis for the soya bean scenario, fitness for purpose parameters can be attained more easily when sampling for soybean flour is undertaken.

Due to labelling requirements, packaged products are expected to be one of the main targets for control bodies. The problem is multi-faceted depending on many factors including particle type and size of different products. Experimental studies on GM soybean packed products were performed and data were processed via distribution-free statistical procedures supplied by software SISSI (Shortcut In Sample Size), to estimate sampling errors associated with number of incremental samples.

Dedicated software tools to support sampling and sub-sampling plans aimed at GM detection through the food and feed chain were also developed: SISSI a novel approach to estimate the optimal sample size in experimental data collection and OPACSA (OPTimal ACceptance Sampling by Attributes) a new statistical optimisation software including a cost function to find the cheapest and most reliable mode of analysis by sub-sampling.

Finally, consideration of general control plans should be undertaken where several analytes could be sampled, with low-cost sampling methodologies. In this regard, an ongoing experimental study for validating sampling methodologies for mycotoxins (Reg. 401/2006 and following) fit for purpose for GMOs is in progress. The aim of this study is to verify if the current sampling methodologies for mycotoxins (the more heterogeneously distributed analyte in a lot) could fulfill the requisite of a representative sampling also for GMOs and derived products. Initial results of this study are presented.

Commission Recommendation of 4 October 2004 on technical guidance for sampling and detection of genetically modified organisms and materials produced from genetically modified organisms as or in products in the context of Regulation (EC) No 1831/2003. (2004). Official Journal of the European Union L348:18-26.

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## B1.2. Rationalization of GMO testing by appropriate sub sampling and control plans

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Sampling is a general issue for all detection purposes, be it in field, in silos, barges or shipments, with packed or unpacked products, in companies, or on the shelves of retailers. The representativeness of a bulk sample from a lot is an important issue for ensuring the accuracy of measurements made on the lot that is sampled, in particular where the target analytes may be homogeneously or heterogeneously distributed. Sampling uncertainty also plays a major role in the uncertainty of the measurement made on laboratory sample for which generally a small sub-sample, supposed to be representative of the laboratory sample is effectively analyzed.

Numerous CEN, ISO and private sampling plans are available, for general application. In the case of GMOs, the EC released a recommendation on sampling that is not applied by Member States due to its high costs.

It is important to remember that a single lot may be sampled in parallel for to detect several analytes: GMOs, pathogens, allergens and mycotoxins or organisms producing them, and analysed with immunological, physical, chemical or DNA based methods. The first question is thus, are samples taken for one purpose usable for another purpose? Two issues then arise:

1) Does the various different risk statuses that a lot may have for different analytes make a common sampling plan a sensible option?

2) Can we have, in the same location, analytical laboratories working in different fields of analysis in order they can share the samples and define common analytical procedures for sub-sampling and extraction; particularly if the analytes, e.g. proteins and DNA, differ among the analyses?

In the absence of a common sampling plan, the aim of the analysis should be used to define sampling plan: for instance sampling plans for environmental purposes differ from seeds analyses, sampling plans used seeds of commodities differ from those used for costly vegetable seeds, such as salads.

Another issue is how analytical performance impacts on and combines with the uncertainty associated with sampling. For example, the uncertainty associated with a typical quantitative PCR based method might be a factor of 2. Hence, the amount of effort put into sampling for GMOs should be just enough to not increase that level of analytical uncertainty. Once that goal is achieved, adding additional effort towards sampling does not improve the decisions made about lots. Another issue is the need for of more sensitive techniques, be it quantitative or qualitative detection methods. The use of qualitative methods is increasing rapidly in GMO detection for the purpose of detecting unapproved GMOs, for instance with the 'Matrix Approach' using the validated DualChip<sup>®</sup> micro-array developed during the frame of Co-Extra. Such need to use qualitative methods is also amplified by the lack of CRMs<sup>2</sup> below the LOQ<sup>3</sup> of the current quantitative methods for approved GMOs and for unapproved GMOs for which reference materials can be either missing or provided only as DNA extracts. The issue of LLP<sup>4</sup> may also require either more sensitive fully quantitative methods or ways to assess the GMO content of a lot when no quantitative validated methods are

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<sup>2</sup> Certified Reference Material

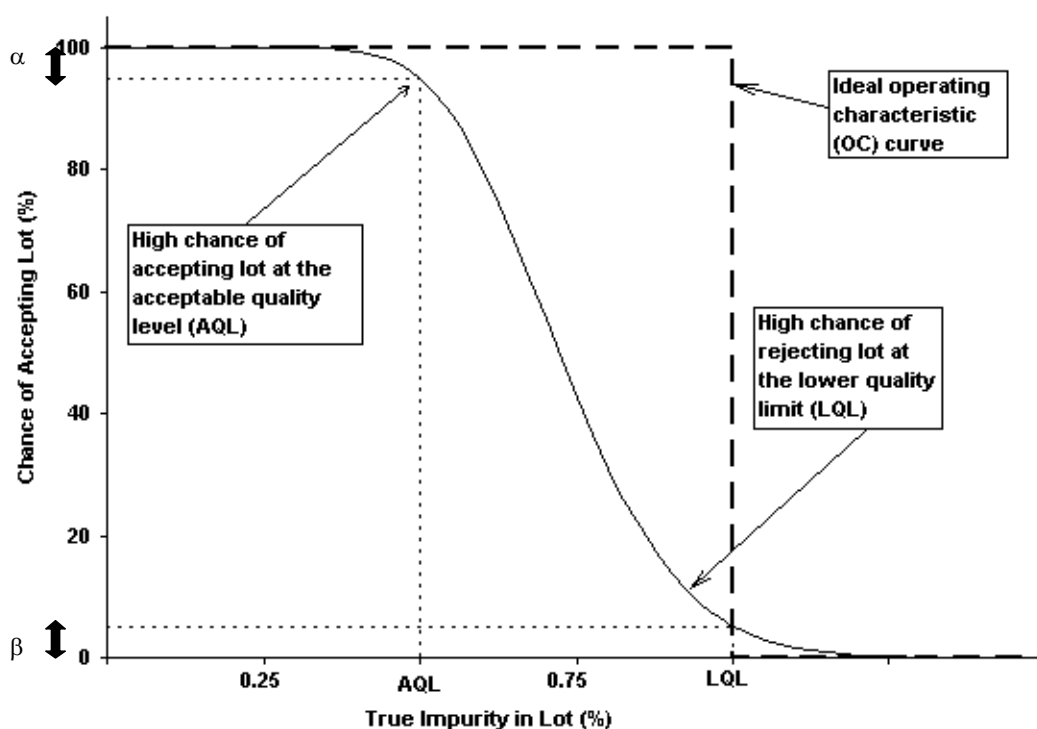
<sup>3</sup> Limit of Quantification

<sup>4</sup> Low Level Presence

available. In that context, the mandate of the CRL<sup>5</sup> should be rapidly extended to the validation of qualitative methods, particularly those used in the ‘Matrix Approach’ and their related controls of donor organisms when needed.

Two statistics based methods can then be used to interpret the results of qualitative analytical methods, to give an estimate of the quantity of GMOs present in a lot of a sample taken from a lot. The SIMQUANT method, based on MPN<sup>6</sup> at the level of DNA copies in solution provides a method for measuring the quantity of GMOs at low concentrations using qualitative detection. A sub-sampling strategy, also called control plans by multi-attributes, is another method for measuring the quantity of GMOs using qualitative test results. It provides an estimate with a confidence interval for assessing the GMO content of a sample relative to a threshold. Sub-sampling is a detection strategy particularly used in quality controls (automobiles, manufactured pieces, seeds) which has not, till now, been sufficiently considered in the GMO detection area.

By taking into account a cost function, the OPACSA software also enables the analyst to determine the most cost-effective way to detect any analyte, be it by using single or double stages systems. It also enables the seller and buyer to calculate and negotiate their risks by defining two values, the AQL<sup>7</sup> and the LQL<sup>8</sup> (see figure). The analytical and sampling uncertainty associated with the measurement of GMOs in lots have led stakeholders to apply practical contractual limits of approximately 0.1% estimated GMO content when testing lots against the 0.9% labelling threshold. The practical contractual limits have been freely negotiated by stakeholders based on their assessments of risk and LQL- AQL values. As, the EC recommendation is also based on a sub-sampling strategy, more cost-effective sampling plans could be used by Member States.



<sup>5</sup> Community Reference Laboratory

<sup>6</sup> Most Probable Number

<sup>7</sup> Acceptable Quality Level

<sup>8</sup> Low Quality Level



In conclusion, rationalization of the sampling issue is still a matter of work whose solution depends on the ability of scientists and stakeholders of different analytical methods to work together for developing common and harmonized, more detailed, sampling plans. In the case of GMO, the mycotoxin-sampling plan would be an effective alternative to the EC recommendation while the OPACSA software of Co-Extra could be used for increasing the cost-effectiveness of the detection protocols of European states. Qualitative methods are being more and more used, a new paradigm can be distinguished in the future of analysts training. The experience of ISTA<sup>9</sup> in diffusing such a detection and choice supporting methodology would be very useful for training analysts of the GMO area. The effective cost-effectiveness of such methods should be appreciated more in depth when quantitative methods are available. However, in all cases where qualitative or very sensitive methods have to be used, for instance in case of harmful products, the sub-sampling strategy, with single or double stages, is the method of choice for its ability to both partners of a transaction to negotiate their risks.

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<sup>9</sup> International Seed Testing Association

### B1.3. Modular Approach Implemented: Pros, Cons and Future Perspectives

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The use of Genetically Modified Organisms (GMO) is subjected to legal constraints, either within a deregulating (e.g. USA) or an authorizing (e.g. EC) framework. In either case, compliance with the legal framework is mandatory. Validated methods (and reference materials) represent essential components within the enforcement compliance with the legislation both by the producers and the officials.

Compliance measures invoke investments in quality assurance/quality control (QA/QC) in all enforcement activities of food safety and quality. View the increasing number of GMO, their diversity and their complexity, the high standards set in general for the validation of detection methods at the EC level stresses even stronger the need for an efficient control management in the GMO area. A centralized enforcement organisation at the EC (through Community and National Reference Laboratories) increases transparency for the stakeholders and the consumers. QA/QC processes in production, manufacturing, distribution and sales can in such organisation more easily be streamlined and harmonized. Flexibility and compatibility of QA/QC processes along this chain is an important advantage for increasing transparency and reducing cost.

Within method validation two basic concepts are prevalent: on the one hand, the "global approach", prevalent in the USA and in other detection areas, in which the whole process from the product to the final measurement outcome is to be validated as a whole. On the other hand, within a "modular approach", the different steps in the analytical process of a food or feed matrix ((sub) sampling, homogenization, extraction, etc.) are considered as separate entities and each of these can be validated on its own.

Holst-Jensen and Berdal (2004) have proposed to introduce the concept of 'modularity' for the analytical procedures and validation of methods in GMO analysis. The basic idea is that in general after sampling from bulk lots, GMO analysis consists of a limited set of distinct steps that represent a certain elementary unit with the process, the so-called 'module'. In GMO analysis *stricto sensu*, after sampling, a laboratory analytical sample is subject to the following steps of modular analysis: sub-sampling, sample homogenization, analyte extraction, target detection and finally target quantification.

Within the Co-Extra project, a number of aspects directly concerning the validity of the modular approach have been assessed, especially technology equivalence, the lack of bias-introduction by module interchange and the determination of the measurement uncertainty, if necessary, and the mathematical expression of module interactions and inter-dependencies.

Technology equivalence for various steps in the GMO analysis both in a global as in a modular approach has been assessed: different DNA quantification methods, extraction methods, qualitative and quantitative PCR methods and the commutable use of different types of reference calibrators. While product-type dependency as to the absolute quantities of measured total DNA levels could be demonstrated, the influence at the PCR level was not significant. In the case of DNA extraction, for one method considerable aberrant differences were observed from the other methods at the final % GM measured. A considerable deviation was observed with various PCR methods at different levels, with serious errors at higher GMO % levels. Finally, the exchangeable use of different types of reference calibrators (plasmid and genomic DNA) in quantitative GMO analysis was documented both for maize (commutability study) and soy (inter-laboratory trial).

Bias introduced by varying the methods applied within a particular module (e.g. changing for instance extraction methods within the extraction module) is a second point that was addressed. Here, it was shown that different extraction methods could be used for various products without introducing large variation in the final GM content measurement (except for one particular method, see above). It was also demonstrated that the quality of the DNA analyte could highly influence the GM% measurement due to PCR inhibition. Criteria and methods assessing the PCR inhibition rate have been developed within the project. Finally, the target integrity is another important factor, and some general destabilizing features of target molecules have been identified (esp. neighbouring TT-sequences in the DNA target amplicon).

These results were analyzed by conventional statistical approaches (such as t-test, ANOVA,) to express the inter-dependency between modules and the difference between extraction methods and food and feed products. Within Co-Extra, these statistical approaches were successfully combined with the "vague set" or "fuzzy logic" mathematics, implemented by the software AMPE. In this way, an overall assessment of "fit to purpose" between method-product combinations can be expressed in an alternative mathematical way. As such, any subjectivity or individual preferences about the choice of methods for analysis can be documented. Also, the need for defining transfer criteria between modules was invoked of which analyte purity and integrity were considered as the most important ones.

According to the obtained results within the Co-extra project, the "modular approach" can be considered as a useful approach in GMO analysis. Co-extra documents valid modularity for: DNA content determination, for DNA extraction, for the reliable use of PCR in a wide range of % GM content and for the application of different calibrators in GMO quantification by real time PCR.

As such, the "modular approach" provides a good basis for developing a cost-effective validation process by the stakeholder. Such approach requires however generally accepted performance criteria for the different types of detection methods (e.g. ENGL criteria), accepted statistical evaluation tools (such as AMPE, SeedCalc, etc.) and appropriate reference materials (such as the IRMM CRMs). Further efforts will have to be made to integrate the use of all these elements in the future (e.g. CRMs certified for target copy numbers).

To evaluate whether a certain/new method can be applied within a particular module, requires a reliable reference framework (e.g. a GMO analysis Dbase) supported by a 'Decision Support System' (DSS). Such DSS could then handle also any exceptions to the general applicability of some methods, by applying "fuzzy logics" analysis. An optimal path for GMO analysis (in terms of performance, applicability, cost, etc) can be determined taking into account the experience with food and feed products and the traceability information.

However, nor an accepted "GMO analysis Dbase" or an operational "DSS" are currently available to the stakeholders, leaving the application of the "modular approach" still open for discussion. Establishing a fully operational DSS, that is constantly updated, is thus a major goal for the near future, if the "modular approach" is to be successfully applied by the stakeholders. The development of a DSS for detecting GMO in the broad range of applications, should greatly benefit from the vast experience obtained within the Co-Extra project. In the EC, the ENGL and the EC NRL-GMO networks could play a key role herein.

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## B1.4. Validation of novel methods and technologies

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There is a continuous and increasing need for reliable analytical methods to assess compliance with national and international requirements in all areas of analysis. The reliability of a method is determined by the so-called validation, which is the procedure providing evidence of suitability of an analytical method for its intended purpose. Based on the results of a validation study, a method is considered or not as fit for the intended purposes. In most cases, formal validation requires the assessment of the performance of the proposed method by means of an inter-laboratory study, also known as collaborative study or ring trial.

While well established validation key parameters and data analysis procedures are available for single-target qualitative and quantitative methods (see for example ISO 5725 and [http://gmo-crl.jrc.ec.europa.eu/doc/Min\\_Perf\\_Requir\\_Analyt\\_methods\\_131008.pdf](http://gmo-crl.jrc.ec.europa.eu/doc/Min_Perf_Requir_Analyt_methods_131008.pdf)), the validation of novel methods and technologies developed by the Co-Extra project required the development of novel approaches to summarise the information provided by individual validation indices and tests statistics into comprehensive indicators of method performance.

With the aim of providing easy access to statistical and numerical tools for analytical method validation, the freely available Analytical Method Performance Evaluation (AMPE) software was created. Through AMPE, a variety of validation metrics (indices and test statistics) is provided for comparing measurements from a laboratory analysis and reference values from standard samples. Provisions are also provided for analyses based on blank samples. In its innovative part, AMPE supplies provisions for fuzzy-based aggregation of validation metrics.

Through the application of fuzzy logic, aggregated indicators are proposed as suitable tools for overall evaluation of analytical methods, allowing also objective comparison across different methods. Fuzzy-logic based indicators were developed that allow summarising the information obtained by independent validation statistics into one synthetic index of overall method performance. The possibility of having a comprehensive indicator of method performance has the advantage of permitting direct method comparison, facilitating the evaluation of many individual, possibly contradictory metrics. In its original development, the fuzzy-based expert system was used to validate novel methods developed by the project, the DualChip<sup>®</sup> GMO microarray and the so-called pJANUS plasmids, and to test the “modular approach” to method validation.

The DualChip<sup>®</sup> GMO is a novel multiplex screening method for the detection and identification of GMO, based on the use of multiplex PCR followed by microarray. The technology is based on the “Matrix Approach” i.e. on the identification of quite ubiquitous GMO genetic target elements first amplified by PCR, followed by direct hybridisation of the amplicons on a predefined microarray. The validation was performed within the framework of Co-Extra, in collaboration with twelve laboratories. The method was evaluated with predefined performance criteria with respect to the JRC-IHCP CRL-GMFF method acceptance criteria. Data were processed according to ISO 5725 standard and the overall method performance met the acceptance criteria. However, creating reproducible data with a high level of consistency across array experiments and various platforms is

widely accepted by the scientific community as a major issue. The complex nature of a microarray experiment results in many potential sources of variability, which can affect performance. In response to this challenge, the fuzzy-logic based approach was successfully applied to the analysis and data interpretation of the chip validation exercise.

A second method was developed, based on real-time PCR and the use of novel calibration molecules (plasmid pJANUS) in comparison to genomic DNA calibrant (the classical approach). The method was validated among various laboratories and the evaluation of the inter-laboratory study performed indicated that the dual-target plasmid pJANUS<sup>TM</sup>-02-001 can be used as calibrant in determining the amount of Roundup Ready<sup>®</sup> soybean line GTS-40-3-2. The equivalence of plasmid and genomic DNA templates as calibrants for the quantification of the GM soybean event GTS-40-3-2 was demonstrated: this equivalence was assessed through conventional statistical analysis (ANOVA) and with the application of the fuzzy-logic based approach, that resulted remarkably in line with the expert interpretation of statistical results; this can be seen as a further validation of the suitable application of the novel fuzzy logic analysis in GMO detection methods evaluation.

A study was performed to provide a proof-of-concept for the application of the modular approach to analytical methods in the field of GMO testing based on the use of the Real-time PCR. For general information on the modular approach refer to M. Van den Bulcke presentation, "Modular Approach Implemented: Pros, Cons and Future Perspectives".

Modularity implies independency and thus flexibility of combining modules on the one hand, and uniformity and harmonisation on the other hand. If modular validation is to be applied, fit for purpose procedures and general acceptance of minimum requirements for each module are needed in order to evaluate the uncertainties associated with each module. In order to provide scientific evidence on the applicability of this approach, the experiments carried out focused on the interactions between DNA extraction methods and PCR analysis. It was found that for the correct application of the modular approach appropriate performance criteria should be met by DNA extracts (assessment of quality characteristics of DNA) so that they can be fit for the purpose of the following analytical module, independently from the preceding matrix-DNA extraction combination. With the exception of one DNA extraction/matrix combination, the study provided good evidence of independency of the analytical modules tested, suggesting that a modular approach can be correctly employed in method validation and analytical control.

## B1.5. Reference materials and reference PCR assays for GMO quantification

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The EU legislation regulating the approval and environmental release of any new GMO includes several core elements: (1) a safety assessment carried out by the European Food Safety Authority (EFSA), (2) availability of reference materials and event-specific PCR methods for detection, (3) mandatory labelling of GMO-derived or -containing food/feed products above a threshold of 0,9%, and (4) post-market monitoring and traceability (EC 2001, EC 2003a, EC 2003b, EC 2004a, EC 2004b, EC 2004c).

An applicant submitting a dossier for authorization of a new GM event, must provide an event-specific PCR method, which is then evaluated and validated by the Community Reference Laboratory for GMOs (CRL-GMFF). Upon validation and acceptance, the protocol describing the method is published at the CRL website and considered as a reference method for the member states' laboratories in charge of GMO testing for compliance with the EU legislation. Commission recommendation 787/2004 provides technical guidelines on sampling and detection of GMOs and materials containing GMOs in the context of regulation 1830/2003/EC. The GMO impurities must be determined on a single ingredient level, i.e. as a percentage GM-DNA, defined as the relative proportion of the GM-DNA sequence copy number in relation to the plant taxon specific DNA sequence copy number. 'Copy number' refers to the number of 'haploid genome equivalents' (HGE), the EU recommended unit for expressing GMO contents (EC 2004c).

Implementation of the legislation on food and feed labelling and traceability brings up two main requirements: (1) the availability of control materials and calibrants, and (2) the availability of specific and accurate analytical methods, both being crucial aspects for reliable detection and quantification of GMOs in a wide range of agricultural products. This presentation is a summary on both topics.

First, an overview is presented on the different types of (certified) reference materials (RMs, CRMs) and calibrants for GMOs and their specific objectives. Two main types of control materials exist: matrix materials, and calibrant materials. While early materials for GMOs were powders, obtained from raw materials and certified for their mass ratio of GM powder relative to the total species powder, now matrix CRMs and calibrant CRMs exist with certified copy number ratios (haploid

genome equivalents, HGE percentage). An overview is given on these materials and where and how to use them in the analytical procedure for GMO detection and quantification. Main outcomes from the experimental work done within Co-Extra are presented. An interlaboratory comparative study was performed for evaluation of the applicability of different types of DNA calibrants: plasmid DNA (pDNA), extracted genomic DNA (gDNA), multiple strand displacement DNA (mdaDNA). The study also aimed at evaluating the best conditioning of DNA for long-term preservation and stability of DNA over long periods of time. Both gDNA and pDNA proved to be suitable calibrants under the particular conditions of this study. While mdaDNA can only be used for qualitative measurements but not as calibrants for quantification, it was considered as an interesting alternative to produce positive or negative samples when control material is only available in limited amounts. This Co-Extra study contributed to the further development of calibrants suitable for the expression of measurement results in copy number ratios. It is a milestone towards the improved availability of CRMs for calibration purposes.

Second, the importance of reference gene systems of reference assays (RAs) for GMO quantification is extensively discussed. Controlling the adventitious presence of GMOs involves detection of a small proportion of GMO (typically 0-5%) in a background (95-100%) of non-GMO species. For this purpose, an event-specific PCR method is combined with a species- or target taxon-specific PCR assay. The method, submitted by the applicant for international validation and acceptance by the CRL-GMFF, has to fulfill minimum performance requirements which are defined by the European Network of GMO Laboratories (ENGL) and published at the CRL webpage (<http://gmo-crl.jrc.ec.europa.eu>). The new method requirements, which will be applied after 13<sup>th</sup> of April 2009, define several guidelines regarding the target taxon-specific sequence: (1) The absence of allelic and copy number variation has to be demonstrated across a globally representative and diverse species' varieties list; (2) Allelic and/or copy number variation shall be reported by the applicant if known; (3) The specificity of the target sequence shall be *in silico* validated against publicly available sequences databases and experimentally demonstrated by absence of amplification products, when the target sequence specific assay is applied to individual PCRs on pure genomic DNA of a representative sample of the closest relatives to the target taxa, as well as of the most important food crops (ENGL 2008).

Briefly, a reliable reference PCR assay must be specific for the taxon in question i.e. should not give any signal with other closely related taxa on the one hand, and should give a uniform positive signal among different varieties within the taxon on the other hand. Low specificity and uniformity could lead to under- or overestimations of the GM content and thus render the methods unfit for their purpose. Within Co-Extra, existing reference assays for the main GM crops have been extensively evaluated for their copy number, sequence stability, specificity and uniformity and new improved systems have been developed. Several issues will be presented here concerning the formulation of a core collection of species and varieties, to be tested when developing and validating a new reference assay. How should the borders of a GM 'target taxon' be defined? What are the criteria for the selection of species and varieties to be included in such a core collection? What are the factors to be considered in specificity and uniformity testing of any reference target taxon-specific assay?

The purpose of establishing core collections for specificity/uniformity testing is to ensure that these parameters are included in the validation of newly designed reference assays and to assure that the assay will amplify efficiently in all types of plant materials subjected to GMO testing. Ideally, a core collection of species and varieties has to be (1) representative – to cover relevant plant species or other botanical taxa and the existing variation within this/these taxon/taxa; (2) dynamic – to be updated permanently with new varieties/lines; (3) accessible to all parties involved in GMO testing: biotech companies developing PCR detection systems and enforcement labs involved in official control of GMOs.

A reference PCR assay should only be accepted provided that it is target taxon-specific and uniformly amplifies within the whole (market) gene pool. A decision support system is presented for the



selection procedure of botanical taxa and varieties/lines, based on specificity and uniformity. Specificity here concerns the risk for co-occurrence of the non-target species/variety under consideration with the target taxon, and the corresponding risk of co-amplification. Uniformity here concerns the representativity of the species/variety considered relative to the marketed varieties of the species, and what the market share is of the species/variety considered. This is a process which has to take into account taxonomic, phylogenetic, breeding and agronomic data. In this process of selection, reference is made to existing collections of varieties and species, such as the CPVO (Community Plant Variety Office) list of EU-registered plant varieties.

## Session B2: Detection of GM ingredients in foods and Feeds

### B2.1. New real-time PCR methods available for routine GMO detection labs - applicability and performance

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Reliable and cost-effective methods for GMO detection are essential for establishing an efficient system for traceability as well as for monitoring different aspects of GMO coexistence with conventional crops. After several years of primarily gel-based PCR analyses, real-time PCR methods have become state-of-the-art for qualitative detection as well as for quantification of genetically modified components in food and feed. Within the framework of Co-Extra, several new real-time PCR-based methods have been developed in order to enhance efficiency and cost-effectiveness of GMO analysis, further improve reliability of GMO analysis, overcome certain limitations of current real-time PCR methods and finally complement the existing method portfolio with methods for identification and quantification of non-EU approved GMOs formerly not detectable or not quantifiable.

Enhancing efficiency and cost-effectiveness is of major importance as GMO analysis is getting increasingly complex due to the growing number of approved and commercialized GMOs. Whereas in the beginning of GMO analysis a screening for 35S promoter sequences was common practice, the situation has become much more difficult. This is also due to the fact that more and more GMOs are lacking the typical screening elements. Consequently there is an urgent need for multiplex screening and identification methods in order to avoid an increase in cost for traceability to an economically unbearable level. Within Co-Extra several multiplex real-time PCR assays ranging from duplex to pentaplex format have been developed and thoroughly validated providing improved tools for screening for traditional screening elements like 35S promoter and nos terminator as well as tools targeting additional screening targets. Some of the newly developed assays will be presented. Furthermore multiplex-specific requirements for method validation will be addressed.

A second objective of PCR method development within Co-Extra was further improving reliability of GMO analysis. In this context real-time PCR control assays have been developed detecting important donor-organisms of building blocks frequently used in GM plants. In case of positive testing results for screening targets originally derived from *Agrobacterium*, *Bacillus* and figwort mosaic virus the newly developed control assays detecting these donor-organisms can be used in order to confirm that the positive screening results are true indicators of GMOs - and are not due to the presence of bacterial (*Agrobacterium*, *Bacillus*) or viral DNA (FMV) in the food or feed sample. Another means of enhancing reliability of GMO analysis was the development and validation of an improved IPC (Internal Positive Control) which can be used for cost-efficient and sensitive verification of absence of PCR inhibition. Examples of assays including this IPC will be presented.

Another objective of the developmental work within Co-Extra was to overcome limitations of current real-time PCR methods such as to enable GMO quantification in samples with very low DNA content or to make GMO analysis portable for on-site testing. As an example the method for on-site real-time PCR quantification of GT73 *Brassica napus* will be presented.

Finally real-time PCR assays detecting two transgenic potato events which are certified for food consumption in Russia will be highlighted which complement the portfolio of event-specific detection methods available for EU approved GMOs.

## B2.2. Reliability and costs of GMO detection

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The analytical procedure used for GM detection and quantification at the laboratory level is composed of different modules. Each of those modules can impact the accuracy of analytical result qualitatively and/or quantitatively. Within the CoExtra project we have investigated different aspects of the reliability of GMO detection. A system for quantification of GM presence in the samples with low DNA content was established, for example. Additionally, effort was put into improvements related to trade-off between reliability and cost of analysis.

Different DNA-extractions from highly refined materials (lecithin and oil) have been investigated in detail. The sample preparation steps as well as the DNA extraction and purification steps were optimized. Both types of samples benefited from a hexane extraction step followed by DNA extraction and purification. The methods have been optimized for maximum DNA recovery and low hands-on time. Standard operating procedures are available for analytical labs that would like to evaluate the procedure or for further validation studies. The procedure for DNA extraction from lecithin has already been in-house validated and the protocol successfully transferred to different labs. To improve quantification performance, the SIMQUANT approach was developed. The idea is to perform a series of PCR reactions and quantify the target numbers in the sample using the distribution of positive/negative results and most-probable-number statistics. One of the advantages of SIMQUANT is also less sensitivity of qualitative PCR to inhibitors in reactions when compared to quantitative PCR. The SIMQUANT was additionally upgraded to multiplex a version, thus increasing its applicability.

The quality of extracted DNA is known to influence significantly the final result of GM detection and quantification. One approach to control this step is “matrix by matrix” validation of the DNA extraction method. For validation, the quality of DNA solutions should be controlled by testing the presence or not of statistically significant inhibitory effects. This is usually done by adding an exogenous DNA (other taxon genomic DNA than that tested or plasmid, provided they are inhibitor free) containing a specific PCR target into the DNA solutions under study at a concentration close to the limit of quantification or limit of sensitivity (for example, 50-100 copies) and then by amplifying the specific PCR target contained by the exogenous DNA. The most convenient and cheapest way is to use the DNA of other taxon as exogenous DNA. The problem in most routine detection labs is that the matrix is not well defined. Composition of feed and food samples can vary from supplier to

supplier and even from batch to batch, making 'matrix by matrix' DNA extraction validation not feasible. Modular validation can then be performed, providing appropriate controls of PCR inhibition are applied with every sample.

In quantitative analysis, the target number quantification also introduces a bias. Two calculation methods can be used. The  $\Delta\Delta C_t$  method relies on both amplicons having similar efficiencies of amplification for accurate quantification. Therefore the bias of the method would only be acceptable if working with well established matrixes (e.g. with raw materials) if properly validated in combination with DNA extraction method or if the calibration standards are of the same matrix type.

Routine detection of genetically modified (GM) organisms is most often performed on Applied Biosystems machines (ABI7700 and ABI7900), using their prominent chemistry – TaqMan® and their Master-mix. With new developments in this area many different apparatuses and chemistries are available on the market that could potentially outperform the previous systems. Within the project the different apparatuses and performance of alternative chemistries was evaluated, thus extending options in the methodology used in routine practice to recent technological advances. For comparison of apparatuses a small ring trial was organized within which 8 different real-time PCR models were included which were available in the labs of different WP5 partners. Some machines were also replicated in different labs to evaluate interlab variability. Applying CRL validation method acceptance criteria (25% RSD<sub>r</sub>, 50% Bias) results suggest that the type of machine used is not critical in GM quantification, at least for the methods examined here.

Similarly, the comparison of different available chemistries was organized to test those most widely used (MGB®, SYBR® Green and Molecular Beacons) in different laboratories and targeting different genes, while the more recently introduced were tested less extensively (Plexor, LNA, lux). The conclusion was that TaqMan®, MGB®, LNA are equal in performance characteristics and they can be used whenever they are better suited for the particular application, e.g. if there is special needs regarding specificity or target regions are problematic for design of longer TaqMan® probes. Molecular Beacons systems were more difficult to design to achieve a robust assay. SYBR® Green chemistry performed well, its drawback being slightly lower sensitivity when compared to probe based assays. The other primer only based system that performed well were Ampliflour and Plexor, while some of the more exotic systems performed significantly below specifications given by the manufacturer.

## B2.3. Non-PCR based Alternative Analytical Methods

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Although polymerase chain reaction (PCR) has to date been the overwhelming method of choice for the laboratory based detection of GMOs because of its sensitivity, familiarity of methodology, well developed standard operating procedures and availability of suitable equipment in testing laboratories, PCR nevertheless suffers from a number of distinct disadvantages. These include the relatively high cost of equipment and of the assays themselves, potential for contamination and the sensitivity to certain classes of contaminants and inhibitors, leading to a requirement for reliable DNA purification strategies. As a result, assays need to be carried out in a laboratory and the need to accumulate batches of samples further slows the total time required for the assay chain from sampling to the eventual result in the laboratory. These issues, together with the difficulty in designing cost-effective portable devices for PCR, have driven the search for alternatives to PCR, a number of which are now becoming available that seek to overcome some of the perceived limitations of the PCR approach.

Within WP5 of the Co-Extra project, a number of new alternatives to PCR based methods have been evaluated, which offer potential advantages over PCR for speed, cost, scale or portability. The purpose of this presentation will be to review these methods and report on their suitability and potential for GMO screening applications. For more details on non-PCR nucleic acid amplification methods, the publication by partner NIB may be consulted (1), and for a review on novel analytical approaches to GMO testing see (2). The methods reported comprise two main classes. The first are true alternatives to PCR, i.e. molecular tests that, like PCR, also test for the presence of specific DNA sequences, but which employ an alternative non-PCR method of nucleic acid amplification. These include the use of strand-displacing polymerases at a constant temperature (e.g. LAMP and RDC), or the use of transcription-mediated amplification (e.g. NASBA). All these methods do not require temperature cycling, operate at a constant temperature, and offer potential advantages including cost, speed, portability and reduced sensitivity to inhibitors over PCR. The technical advantages of these approaches include the possibility to combine their use with novel reporter systems, and the use of a new bioluminescent output known as BART has been evaluated in conjunction with LAMP and RDC. The second type of method does not seek to detect DNA sequences but employs spectroscopic techniques to distinguish GM and non-GMO material. Method evaluation for their suitability for GMO detection was carried out by the partners NIB, INRA, CSL, Lumora, NIAB and CRA-W within WP5 of the EU Co-Extra Project.

In the first class, techniques known as NASBA, LAMP and RDC were evaluated, either alone or in conjunction with the BART bioluminescent reporter system. In the second, near-infrared spectroscopy was evaluated. The main characteristics of these techniques is summarised.

Loop-mediated Isothermal Amplification (LAMP), developed by the Eiken Chemical Company is a simple, rapid, specific and cost-effective nucleic acid amplification technology. Details are described on <http://loopamp.eiken.co.jp/e/lamp/index.html>. It is characterized by the use of 4 different primers, specifically designed to recognize 6 distinct regions on the target DNA template, in a process that proceeds at a constant temperature driven by a strand displacement reaction. Amplification and detection of target genes can be completed in a single step, by incubating the mixture of DNA template, primers and a strand displacement DNA polymerase, at a constant temperature. It provides high amplification efficiency, with replication of the original template copy, occurring  $10^9$ - $10^{10}$  times during a 15-60 min reaction. The primer pairs used in this amplification can be designed using a web tool at <http://primerexplorer.jp/e/>.

RDC (*Reaction déplacement chimeric*) is an isothermal DNA amplification procedure developed by Biomerieux, and is based on the use of chimeric primers consisting of an RNA stretch embedded within flanking DNA sequences. Cleavage of the hybrid duplex between the RNA region formed when the primer is hybridized to its DNA target provides the initiation for a strand-displacing polymerase. For details see US Patent 5824517 (Cleuziat and Mandrand; <http://www.patentstorm.us/patents/5824517.html>).

Both RDC and LAMP are among isothermal amplification technologies that can be interfaced with a unique reporter system known as BART (bioluminescent assay for real-time). BART is a bioluminescence real time assay developed by Lumora [<http://www.lumora.co.uk/>] that allows the quantitative analysis of DNA amplification in real time. In BART, PPi produced during DNA amplification is converted to ATP by the action of ATP sulphurylase. This ATP is then used in a coupled simultaneous reaction by thermostable firefly luciferase and luciferin to produce a light output permitting real-time analysis of amplification kinetics. A unique feature of BART is an initial burst of light, associated with the on-set of exponential amplification, followed by a rapid decrease, as pyrophosphate reaches a critical threshold. The time to reach this light peak is therefore a function of the amount of target DNA in the sample at the beginning of the reaction (time to maximum;  $T_{max}$ ), and a unique feature of the BART reporter. Quantification of BART is based on time to peak and not absolute light intensity, making it less prone to inhibition simplifying data interpretation and the hardware requirements. LAMP in conjunction with BART provides a robust, sensitive and reliable method for qualitative detection of GMOs at low levels of presence (0.1%) and has the potential for quantitative or semi-quantitative manifestations. It is also suitable and demonstrated in small, low cost devices that can be used in the field or other low-resource settings, both because of the equipment requirements and its ability to function with very simple and rapid DNA preparations, even from fresh leaf tissue.

NASBA is an isothermal nucleic acid amplification method that mimics retroviral replication and was originally applied to detection and quantification of RNA targets, but has also been adapted for DNA detection, and it was evaluated in this manifestation. Amplification occurs because the target is transcribed into RNA, which is then reverse-transcribed back into DNA, thereby providing more template copies for RNA transcription. The transcription is carried out by T7 RNA polymerase and requires the incorporation of the appropriate promoter sequence onto the template, which is achieved by appropriate primer design. This method was modified to allow DNA amplification using a two step procedure: first step with tailed primers, second step with universal primers. NASBA was developed well with performance characteristics similar to PCR, and adaptation to real-time detection using Molecular Beacons has been reported. However integration with the BART system is not straightforward due to the high concentration of ATP present in the NASBA reaction.

Near Infrared spectroscopy uses spectral properties of sample in IR to detect GMO's. The method was developed for Roundup Ready soybean (GTS-40-3-2) due to its specific characteristics. The method is non-invasive and can be applied on-site, therefore suitable for analysis of large sample lots of more expensive material, e.g. seeds.

#### References:

1. Morisset, D., et al . (2008) . Eur Food Res Technol **227**: 1287-1297.
2. Michelini, E., et al . (2008) Anal Bioanal Chem **392**: 355–367.

## B2.4. Detecting unauthorised and unknown GMOs.

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The global trade and spread of technological competence and capacity to develop genetically modified (GM) organisms (GMOs) such as plants, in combination with the cultural and regional differences in terms of suitability, need and acceptance of GMOs is a potential cause of disputes. Within the European Union (EU) as well as several other jurisdictions, no import, use or release of GMO derived material is legal without prior authorisation. Among the requirements that need to be met prior to authorisation of a GMO in the EU is the availability of a validated and specific, quantitative detection method and corresponding reference material for the GMO in question.

Field trials are a part of the performance assessment of GMOs. These field trials potentially lead to low level contamination of neighbouring fields. Birds or rodents may spread grains or seeds and incomplete sanitation or other human error may lead to unintended spread of viable material. Finally, intended distribution into the environment or food/feed chain can not be completely ruled out. Validated detection methods as well as reference materials are usually not made available in these cases.

Most of the GMOs commercialised in the world at present are herbicide resistant and/or insect tolerant. The trait genes inserted into these GMOs are usually well known and belong to a few groups: *pat/bar*, *epsps* and various *cry*-genes. Genetic elements associated with the genes to facilitate and regulate their expression are also with few exceptions well known and belong to a few groups, e.g. the cauliflower mosaic virus (CaMV) 35S promoter and terminator (P35S and T35S) or the *Agrobacterium tumefaciens* nos terminator (3'-nos). The availability of other trait genes and regulatory elements is, however, increasing rapidly. Commercial or other interests may prevent relevant information from being disseminated to stakeholders such as competent authorities and laboratories performing GMO detection.

There is no single genetic marker that can be traced as a "GMO label". Instead, it is necessary to use methods specific to particular genetic markers such as regulatory elements or trait genes (screening methods), fusion motifs between regulatory elements and trait genes (construct specific methods) or fusion motifs between inserted DNA and the recipient DNA (event specific markers). The number of GMOs that may be detected with the methods depend on the targeted genetic marker, and the analyst may need to balance broadspectrum screening against ability to specifically identify the GMOs that may be detected. Detection of unauthorised GMOs may often be achievable by using screening methods. However, with these methods it may be quite difficult to determine that the detected GMO material is coming from an unauthorised GMO. On the other hand, the absence of specific detection methods and reference materials for most unauthorised GMOs leaves few options



for identification. GMOs that can not be detected with the commonly used screening methods because the introduced DNA sequences or genetic elements are unusual (novel) may be classified as unknown GMOs. These are of course particularly difficult to detect and identify. Presence of non-declared ingredients (e.g. “botanical impurity”) may further complicate the analytical work.

Any presence of unauthorised or unknown GMO or derived material in the food/feed supply chain in the EU is by definition illegal, and may pose a risk to society and economy, the environment and/or human and animal health. Socio-economic risk is exemplified by the restrictions on import of rice from the USA to the EU as a consequence of contamination of American rice with the Liberty Link 601 rice in 2006. Risk to the environment is exemplified by potential spread of the trait gene to a wild relative of the GMO. Introducing for example an herbicide resistance or insect tolerance gene to a wild plant species may improve the fitness of the wild plant relative to competing plant species in its environment, or it may affect the diversity and/or abundance of insects that birds depend upon for feeding their brood. Risk to human and/or animal health is exemplified by the possibility that a food/feed plant used as a biogenerator for pharmaceuticals is introduced into the food/feed chain. Ability to detect, identify and characterise unauthorised or unknown GMOs is therefore necessary to be able to define, delimit, prevent and remove problems.

Traceability facilitates the identification of the origin of material, and global information networks, databases, etc. may provide information about developments of new GMOs, novel genetic elements that are potentially exploitable and authorisations outside the stakeholder’s own jurisdiction. This type of information can be used by stakeholders to improve their ability to detect, identify and characterise unauthorised or unknown GMOs, as well as to prioritise developments and applications of particular analytical methods.

Development of analytical methods and strategies for detection, identification and characterisation of unauthorised and unknown GMOs has been a major priority within the Co-Extra project. In parallel a modular decision support system (DSS) has been developed in which traceability and other information can also be taken into consideration. These developments together are expected to significantly reduce the challenges posed by unauthorised and unknown GMOs.

This presentation will give an overview of the state-of-the-art technologies and developments from the Co-Extra project relevant to the detection, identification and characterisation of unauthorised and unknown GMOs, and will also point out some of the remaining and possible future challenges of relevance.

## B2.5. New multiplexing tools for reliable analysis of GMOs

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To enforce the existing regulations on commercialisation and environmental release of genetically modified organisms (GMO), adequate tools for its detection, identification and quantification are required. The most accepted GMO detection methods are based on specific DNA sequence detection by means of polymerase chain reaction (PCR) techniques, able to detect even small amounts of transgene sequences in raw materials and processed foods. PCR assays can be used for screening purposes (e.g. targeting transgenic elements commonly used in GMOs), to detect junctions of contiguous transgenic elements, and to identify a GMO event (by targeting the junction regions between the insert and recipient plant genomic DNA or event-specific rearrangements). Additional amplification of a plant species specific gene is necessary as control. A number of these methods (including quantitative assays) are available that have been validated by official bodies or reference laboratories e.g. the EU Joint Research Laboratory.

The presence of GMO material on the market is increasing; and so is the number of GMOs approved worldwide (including stacked events) and in the pipeline. At the same time, the genetic elements introduced into new GMOs and the host plant species are becoming more diverse. This increases the cost and working power required for GMO analysis. In this context, the widely used single-target detection methods are not considered sufficient to fulfil the current and envisaged need for analysis. Consequently it is necessary to introduce new analytical technologies for reliable, low cost, high throughput, standardised GMO analysis.

The development of analytical methods and strategies for multiplex detection, identification and/or quantification of GMO has been a major priority within the Co-Extra project. The combination of two or more PCR assays in one single reaction (multiplexing) is not an easy strategy due to the interaction and competition between the reaction components and products; and the combination of high numbers of reactions is at the expense of the sensitivity and uniform amplification of the different targets. Numerous duplex reactions –often targeting the transgenic sequence and a control or two major screening elements- are available, and so are oligoplex PCRs targeting a limited number of sequences. Above a certain degree of multiplexing, novel strategies (as compared to agarose gels and real-time PCR chemistries) are required to identify the reaction products. Examples are capillary gel electrophoresis (CGE) based and hybridization in array format technologies. These approaches can allow simultaneous detection of the products of a number of oligoplex PCRs performed in parallel, resulting in higher multiplexing level, throughput and lower cost.

The use of oligoplex (and multiplex) PCR assays is foreseen as a first analysis that allows qualitative detection of GMO(s) in a sample. It can be then complemented with singleplex, validated (if possible), specific real-time PCR assays for GMO quantification when required. However, some oligoplex approaches incorporate special adaptations to achieve (semi)quantitative results, such as quantitative competitive PCR or the use of bipartite primers.

The limitations of PCR for achieving high-grade multiplexing are one of the reasons that prompted the study of alternative, non PCR-based approaches that could potentially allow multiplexing. Examples are the loop-mediated isothermal amplification (LAMP) strategy coupled to bioluminescent assay for real-time (BART) detection system; and the NASBA (nucleic acids based amplification) implemented microarray analysis (NAIMA). Near infrared (NIR) spectra of individual kernels can allow GMO detection by comparison to pre-defined patterns.

New multiplex approaches have recently been designed for simultaneous detection of very high numbers of target sequences: these can be considered as high-grade multiplex approaches. Some of

them include a first ligation step that is dependent upon hybridization of two oligonucleotide sequences to the target, subsequent amplification (with universal primers) and detection by hybridization on array support. Examples are a SNPlex method (for single nucleotide polymorphisms detection) designed to identify GMO targets; and a system based on padlock probes (circularizable probes). In a very different approach, a whole genome amplification (WGA) technique can allow producing large amounts of genomic DNA of the sample that are then hybridized to special probes in microtiter plates or microarrays to detect GMO targets (e.g. high density tilling microarray).

This presentation will give an overview of the new technologies for multiplex analysis of GMO developed within the Co-Extra project; and will also discuss on aspects such as the need and problems of validation of multiplex methods; or the difficulties in coupling a high level of multiplexing with cost-effectiveness (including the devices required) and simplicity of the method.

## Session 3: Legal, Liability & Redress Issues

### 3.1. Legal, Liability & Redress Issues

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While the legal and regulatory framework governing coexistence and traceability has been assessed more generally within the Co-Extra project, the prime focus of this conference session will be on liability issues that may arise in the context of GMO production in the food and feed supply chain.

These matters have been addressed from two angles within the framework of Co-Extra:

One team of researchers examined contractual relationships and the obligations arising there from, starting from the production to the ultimate distribution. The chain of contracts linking the various actors may include duties exceeding the requirements of local, national, or international law. It may well happen that despite compliance with the latter the more stringent contractual rules are infringed upon. The consequences of such breaches have been analysed, with a particular eye on the role of grain traders and their share of responsibility.

Another research group explored potential delictual liabilities that may arise in the course of GMO production, analysing how the current liability regimes in Europe and selected non-European jurisdictions would respond to harm caused to third parties such as consumers or bystanders, and how damage to the environment would be addressed. Specific aspects of cross-border claims were highlighted. Alternative options for compensation such as fund or other redress schemes were considered and compared to more traditional ways of indemnification. International liability regimes, in particular those possibly building upon the Cartagena Protocol on Biosafety in the future, were also taken into account.

### **3.2. Scientific expertise and the judges**

C. Noiville

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As science has become a growing foundation of decision-making, disputes more and more arise on the scientific basis of such decisions, at least when they deal with environmental or health issues. What is the quality of the scientific reports on which the disputed decision rests?

Does the present state of scientific knowledge justify this decision?

Have all relevant scientific data been taken into account?

Wasn't the previous scientific assessment too abbreviated?

We give elements in order to better understand and manage these new and decisive aspects of risk decision-making.

### **3.3. Juridical cost-benefit analysis of coexistence: uneasy this task!**

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The various analysis costs/benefits applied to the transgenic plants issue mainly concern their advantages and drawbacks in comparison with conventional plants. We also find analysis of additional costs, attributable to non-GMOs supply chains. But the existing studies do not take in consideration all of the actors - from genetic resources administrators to the society in general - nor the ensemble of parameters - from the costs of traceability to the benefits resulting from subsidizing conflicts. Furthermore, over-costs linked to coexistence are in general mixed with costs uninvolved with coexistence. Finally, a global societal analysis has up to now never been made. These are methods difficulties which we have tried to figure out.

## Session 4: Stakeholder Views in EU

### 4.1. Stakeholder views and interactions

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The activities described in this presentation are part of the Co-Extra project on “*GM and non-GM supply chains; their Co-existence and Traceability*” ([www.coextra.eu](http://www.coextra.eu)). Part of this project is organising interaction with relevant stakeholders affected by the issue of co-existence with the goal to:

- Map the opinions and attitudes of relevant stakeholders with regard to co-existence.
- Create interaction between stakeholders as such.

Within the Co-Extra project stakeholder interaction were organised on different levels:

- Interaction with a group of stakeholders on a European level through a Stakeholder Advisory Board.
- Regional stakeholder workshops in seven European countries.
- An online questionnaire

The presentation describes the outcome of regional stakeholder workshops in several European Member States and of the online questionnaire. Where the Co-Extra questionnaire tried to survey general opinions and attitudes towards co-existence Europe-wide, the regional stakeholder workshops were designed to go more in depth and get more detailed information on more technical topics. Both the workshops and the questionnaire have had outcomes of a qualitative nature. By in large these outcomes are not contradictory. There are differences between countries, and the policy contexts in those countries play an important role, but comparing the results of one stakeholder category in different countries, they tend to have similar opinions.

Some of the main results are concerning seed thresholds, costs of coexistence and traceability as well as the harmonization of coexistence rules within the European Union. There is an overwhelming wish to have GM labelling thresholds for seeds regulated and a general conviction and concern about the costs that co-existence regimes will entail in practice. Additionally, stakeholders are concerned about the practicalities of sampling and testing strategies. Guidance may be necessary here, and perhaps also a discussion on whether testing is necessary in all situations. Harmonization is seen as advantageous, but especially with the aim to prevent any unfair competition between different EU countries. Most stakeholders are not advocates of a hybrid regulatory model with rules both on the European and the country level, but some may stress the need for flexibility, especially on the practical level.

## Session 5: Decision Support Systems

### 5.1. The Co-Extra Decision Support System: A Model-Based Integration of Project Results

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Co-Extra (2005–2009) is an EU research programme on co-existence and traceability, which has involved almost 200 scientists from 54 partner organisations and has produced an extensive collection of results, such as data, scientific findings, obtained knowledge and expertise, formulated recommendations, developed methods and models, scientific publications, etc. One of the main Co-Extra aims is also to produce a *Decision Support System* (DSS). The idea of the DSS is to integrate Co-Extra project results in a form of a computer-based information system that would be operational, easily accessible for various categories of users, and could provide data and advice for various decision problems that occur in supply chains involving genetically modified organisms (GMOs). In principle, the DSS is aimed at providing support for decision-making and not to make decisions on behalf of stakeholders. It provides tools, and methods to assess various ‘decision alternatives’, to change various decision-related parameters and investigate their effects, to visualise the results of evaluations and analyses, and to maintain data related to the decisions involved.

In particular, the Co-Extra DSS addresses the following decision questions:

1. Which methods perform best or can be used at all for a given analytical or sampling purpose?
2. Will my (intermediary) product, given a current set of used procedures and materials, contain GMOs below a specified threshold level?
3. Is there any possibility that my (intermediary) product contains unapproved GMOs?
4. What are the costs associated with maintaining GMO content below some specified threshold?

These questions are general and thus interesting for various potential users of the DSS: EU policy makers, farmers, importers, transporters, feed/food producers, retailers, consumers, analytical laboratories, users of test reports from analytical laboratories, and operators and managers of official control. Although first devoted to GMO and non-GMO supply chains management, its quite generic structure may be adapted to the management of supply chains facing other quality or safety issues, such as pathogens, allergens and mycotoxins producing organisms.

*Approach.* We are using the approach of model-based DSS, which we combine with data-based DSS and elements of simulation, data analysis and communication-based DSS. *Model-based DSS* (Power, 2002) emphasize access to and manipulation of a model, for example, statistical, financial,



optimization and/or simulation models. In Co-Extra, we have primarily used two types of models: qualitative multi-attribute models (Žnidaršič, et al., 2008) and decision trees (De Ville, 2006). The models have been developed in an expert modelling way, that is, in collaboration between decision analysts and experts for a given decision problem, and using the modelling software DEXi (Bohanec, 2008).

In general, the purpose of all the developed models is threefold:

- to capture and represent expert knowledge in the form of hierarchically structured variables and decision rules, which can be reviewed, published, discussed, disputed and communicated between experts, stakeholders and other interested groups;
- to actively assess and evaluate decision alternatives;
- to analyze these alternatives using decision-analysis tools, for instance, to find the advantages and disadvantages of alternatives and analyse the effects of changes by “what-if” and sensitivity analysis.

Currently, the Co-Extra DSS consists of six decision models and a database. The models are the following:

- *Analytical Model*: aimed at the assessment of analytical methods, in particular DNA extraction and DNA analysis methods;
- *Sampling Model*: assessment of sampling plans;
- *Unapproved GMO Model*: assessing the risk of contamination with unauthorized GMO varieties based on traceability data about the product (for instance, type of product, country of origin, type and mode of transportation);
- *Transportation Model*: assessing potential GMO presence due to transportation and handling based on product traceability data;
- *Dryer and Starch Models*: assessing the effect of control parameters (such as using different strategies for handling GMO and non-GMO batches) to the collection and processing of maize.

The *Co-Extra database* stores data on food/feed products, sampling and analytical methods, and operational taxonomic units, which include GMO and taxa. This data is used as input into the models, but is also suitable for browsing, searching and creating complex queries and reports.

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## 5.2. Analytical DSS module – how to support decisions in the analytical lab

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The analytical DSS module (AM) addresses the common situation faced by analytical laboratories involved in the food/feed production chain: given some kind of food or feed “product”, they have to analyse it for different “purposes”. Common “purposes” related to GMO are the *detection* and *quantification* of GMOs in the product. This is done in a series of activities that typically involve *DNA extraction* followed by *DNA detection* methods.

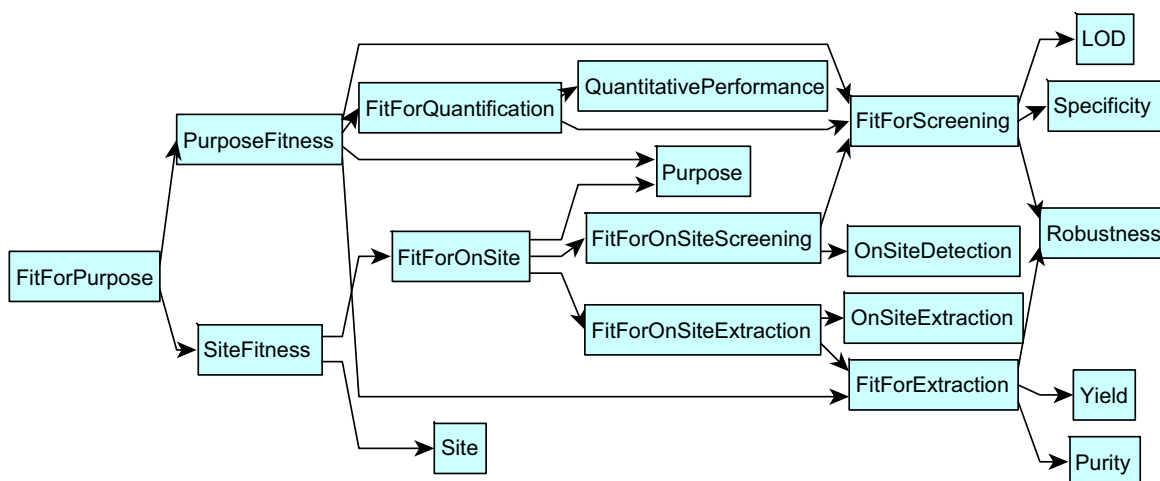
The selection of each method in the sequence depends on a number of factors. Generally, we should consider:

- properties of the product (product type, ingredients,...),
- purpose of the analysis (detection, quantification,...),
- properties of methods (limit of detection, applicability in the situation, compatibility with other methods in the sequence, ...),
- capabilities of the laboratory (available equipment, skills, ...).

Thus, this can be a difficult decision problem that requires extensive knowledge and skills. The goal of the AM module of the *Co-Extra DSS* is to provide support for the following *decision questions*:

1. Is a method “fit for purpose” in the given situation?
2. Which method is “best for purpose”?
3. Which new method is “best for further development”?

Answers to these questions are provided by means of a *qualitative multi-attribute model*. This model, which was developed in collaboration between domain experts and decision analysts, consists of hierarchically structured variables (attributes) and decision rules. Attributes represent properties of analytical methods, the hierarchy defines dependency relations between them, and decision rules determine the aggregation of attributes. For instance, Figure 1 shows a part of the attribute structure that is used to assess the “fitness for purpose” of analytical methods. This is assessed on the basis of “purpose fitness” and “site fitness”, and each of these is determined further on a basis of more and more detailed properties of analytical methods.



**Figure 1: A part of the AM multi-attribute model addressing methods' "fitness for purpose".**

The AM module of the DSS is prepared to help stakeholders in optimising the DNA-based GMO detection. For each analytical situation the user needs to define attributes related to the properties of sample to be analysed and the methods available in the lab (or available for implementation in the lab). The data are entered into a database that will allow exchange of entries between different users. Depending on the purpose of the analysis the DSS will suggest which method (combination of methods) is best to be applied in the lab. Thus it is able to help also to the labs in decisions related to development of new methods and their actual implementation in the lab.

The system was evaluated using methods currently used in detection labs, e.g. column based DNA extraction and simplex real-time PCR detection, and the methods newly developed within the traceability work packages of Co-Extra.

### 5.3. DSS modules on transportation (TM module) and on unapproved GMOs (UGM module)

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As part of the Co-Extra DSS two modules have been developed that aim to guide producers and other users to assess 1) whether their product is correctly labelled in terms of GMO (genetically modified organism) regulations and 2) whether their product might contain any materials derived from unapproved GMOs. In both cases the producer is guided to analyse the documentary data relating to the origin and the transportation of the product, or the underlying raw materials, in order to estimate the chances of the unintended presence of GMOs or the potential presence of unapproved GMOs, respectively.

The Co-Extra DSS module on transportation (*TM module*) aims to help the producer, or other Co-Extra DSS user, to estimate whether the product should be labelled as a GMO product according to EC Regulation 1829/2003, or not. This Regulation on genetically modified food and feed allows the presence of GMO-derived materials in GMO-free batches up to the level of 0.9 %, if this presence is adventitious and technically unavoidable. In that case the product does not have to be labelled as a GMO-product. In all other cases the product ingredient(s) that are GMO-derived will have to be labelled as such. The TM module, whose structure of attributes is shown in Figure 1, assesses available data on origin and logistics of the different (raw) materials that constitute a final product in order to determine whether the product should probably be labelled or not. This assessment relates primarily to documentary data, but may also comprise analytical data on individual components, if available. The analytical data of individual components as such will not always be sufficient as more ingredients derived from the same crop species may be included in the final product. In that case all these components should be included in the assessment for correct labelling. Relevant documentary data will include, amongst others, data on the country of origin, the number of harbours and bulk carriers included in the transportation system, and information on whether coexistence measures have been implemented in the countries involved.

The Co-Extra DSS module on the potential presence of unapproved GMOs (*UGM module*) aims to help the producer, or other Co-Extra DSS user, to determine the chance of unintended commingling of any (EU-)unapproved GMO in (one of the raw materials constituting) the final product to be marketed. The basis for this assessment is the same EC Regulation 1829/2003 that stipulates that unapproved GMOs are not allowed in food and feed products that are brought onto the European market. If unapproved GMOs are detected this may therefore have severe economic implications for the producer or importer of a particular product. The UGM module (Figure 2) assesses available logistical documentation on the individual ingredients of the final product to determine the chance that any (unintended) commingling with unapproved GMOs has occurred during growth, harvest, transportation, storage and processing of the final product. The UGM module also assesses both documentary as well as analytical data that is available on the final product and the constituting ingredients. Relevant documentary data will include, amongst others, data on the country of origin, the production area of approved GMOs in this country, the transportation system used for the product or underlying raw materials, the logistical route and number of harbours and silos involved in the supply chain.

Both modules, that complement each other, will be explained and illustrated on the basis of a number of example products.

Attribute	Description
<b>GM_Presence</b>	Transportation Module: Assessment of GM presence due to transportaiton
<b>TraceabilityData</b>	Risk due to traceability data
<b>Products</b>	Risk due to product characteristics
<b>CropRisk</b>	Crop/product type
ProductType	Product type
CropSpecies	Crop species
ProcessingLevel	Processing level
<b>Countries</b>	Risk due to the properties of countries and regions of origin
NumberOfCountries	Number of countries involved in storage
CountriesAtRisk	Are there countries at risk involved?
CoexistenceMeasures	Are coexistence measures in place in countries?
<b>Transportation</b>	Risk due to transportation route
<b>Storage</b>	Risk due to storage
DedicatedSilos	Dedicated silos used for non-GMO?
<b>Carriers</b>	Risk due to carriers
NumberOfHarbours	Number of harbours involved
DedicatedCarriers	Dedicated carriers used for non-GMO?
<b>AnalyticalData</b>	Analytical data available about unintended admixture
AnalyticalDataAvailable	Is analytical data available?
AnalyticalData	Analytical data, if available

Figure 2: Attribute structure of the TM module.

Attribute	Description
<b>UGM</b>	Detection of Unapproved GM using Traceability Data only
<b>GeographicalOrigin</b>	UGM risk related to the geographical origin of the product
EU	Does the product originate in an EU country?
GM_Region	Does the product originate in a region of large GMO production?
<b>SystemsUsed</b>	UGM risk due to used traceability systems
TraceabilitySystemInPlace	Is a traceability system in place?
IP_GMO	Are IP systems for GMO being used?
IP_Other	Are other IP systems being used?
AnalCtrl_Systems	Are there systems used that include analytical control?
PrivateContracts	Are there any private contracts?
<b>Logistics</b>	UGM risk originating in logistics
<b>Log_Complexity</b>	UGM risk due to logistics complexity
Interactions	Number of interactions in the supply path
Companies	Number of companies involved in logistics
<b>Log_Storage</b>	UGM risk due to storage used
Harbour	Has the product been shipped through harbor(s)?
Silo	Has the product been stored in siloses?
<b>MethodsUsed</b>	UGM risks based on the appropriateness of used methods and available results
<b>AppropriateMethods</b>	Have appropriate methods been used?
AppropriateSampling	Have appropriate sampling methods been used?
AppropriateAnalysis	Have appropriate analysis methods been used?
<b>AnalyticalResults</b>	Risks according to analytical results
ResultsAvailable	Are analytical results available?
Results	Analytical results, if available
Certificate	Regulation-based certificate with relation to GMO;s under emergency measure

Figure 3: Attribute structure of the UGM module.

## Session 6: Experiences from third countries

### 6.1. Benefit-Cost Analysis, Food Safety, and Traceability

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Quantitative methods for analysis of environmental policy and other decisions are predicated on the need to make tradeoffs among valued outcomes. In general, no policy is best for everyone in the population, and no policy is best for all attributes of concern (e.g., risks of multiple health and environmental consequences, resources devoted to reducing risks). In determining whether one policy is better than another, it is necessary to evaluate whether the harms imposed on some people (e.g., costs of compliance) are offset by the benefits conferred on others (e.g., reduced health risk). Similarly, one must determine whether the losses on some attributes (e.g., the resources devoted to compliance that cannot be used for other social purposes) are offset by the gains on others (e.g., reduced health risk).

Benefit-cost analysis (BCA) is intended to predict whether a society would judge itself better off with a policy change, in the sense that individuals who benefit from the policy could compensate those who are harmed (with money) so that everyone would prefer the policy change with compensation to the status quo. BCA requires that all (significant) effects on all affected individuals be quantified in monetary terms. The value of a benefit to an individual (e.g., reduced health risk) is defined as the maximum amount of money she could pay to receive that benefit and still judge herself better off than if she did not receive the benefit and did not have to pay. Analogously, the value of a harm to an individual is the minimum amount of monetary compensation he would require in order to accept the harm and judge himself better off than if he did not suffer the harm and receive the compensation. By summing these monetary values across the population, one can calculate the net benefits and so determine whether there is a surplus of the value of benefits over the value of harms. If so, then in principle compensation could be paid in such a way that everyone in the population judges him or herself better off.

The monetary value of a change in health risk may depend on characteristics of the risk in addition to the probability and severity of health effect. People tend to be more fearful of, and demand more government regulation of, risks that are viewed as dreaded or uncertain. Dreaded risks are those perceived to be uncontrollable and involuntary (to the individual), potentially catastrophic, affecting future generations, and where the potential harms are not distributed equitably in relation to the benefits of the risk-producing activity. Uncertain risks are those that are unobservable, newly recognized, have delayed consequences, or are not well understood by science.

Monetary values of health risk can be estimated using revealed- and stated-preference methods. Revealed-preference methods are based on observing the choices people make in which they implicitly or explicitly trade changes in health risk against money. People who choose a more expensive, safer food are assumed to value the increased safety at more than the incremental cost, and those who choose the less expensive, less safe food are assumed to value the risk reduction less than the incremental cost. A critical assumption of revealed-preference studies is that consumers understand the differences in risk, cost, and any other important attributes among alternative food types. Studies have been conducted comparing organically and conventionally produced foods and experimental studies have varied the risk associated with microbial contamination.

Stated-preference studies are based on surveys, in which respondents are asked what choices they would make in a hypothetical setting. Stated-preference studies have addressed risks associated with a variety of food-borne risks including pesticides and microbial contamination. Compared with revealed-preference studies, a strength of these studies is that respondents can be asked about hypothetical foods that are not yet available and can be informed about the risks and other characteristics. A weakness is that survey respondents have less incentive to consider the choice carefully, as they do not have to pay the costs and face the risks as they do in a revealed-preference study.

## 6.2. Segregation Measures for (Non-) GM crops and their Implications for Supply Chains in Japan

Masashi Tachikawa

Ibaraki University, Japan

In Japan, the decision to introduce mandatory labelling in August 1999 became the watershed of non-GMO identity preservation business, although implementation of labelling scheme was designated to start April 2001. Just after the decision, all kinds of food item under mandatory labelling system were changed to be manufactured using non-GM soybean and maize. Japanese GMO labelling system is different from that of European and only food items which maintain rDNA or protein intact and detectable are subject of mandatory labelling. Therefore, food items such as refined oils, sweetener and soy sauce are exempted from labelling. In contrast to EU system, feed is totally excluded from labelling.

The original intention of introduction of mandatory labelling is to enable consumers to allow choosing their product based on labels. However, the result is that complete replacement of GMO with non-GMO was happened for food items which are subject to mandatory labelling on the one hand and continuous dependency on GMO for those which are exempt from labelling on the other. Here Japanese consumers cannot practice their right to choose for GMO product, and GMOs became invisible from consumers' viewpoint while some items, typically soy oil, are still heavily dependent on GMOs. This invisibility of GMOs on our table keeps consumers' uncertainty to linger, since no material experiences have been accumulated regarding this issue among consumers. This is an unintended paradoxical consequence of introduction of mandatory labelling in Japan.

The cost of identity preservation of non-GMOs and their product occurs at various stages of food chain, and it depends on case by case (roughly speaking, they sum up about 15% to 20% of total cost). Cost structure is very difficult to assess, and might be different from company to company. In this paper, I would like to elucidate how these cost are being managed for each food item by manufacturers, such as corn starch, soy food etc. Basically, these IP costs of non-GM products has not been transferred to the price of final products, but rather absorbed by manufacturers. This inability of manufacturers transfer cost to final product could be explained as the increasing market power of retailers and deflational Japanese economy almost over a decade. The exception is non-GMO dairy products manufactured using non-GMO feed. As the dairy product is exempted from mandatory labelling, this non-GMO labelling is totally voluntary, and manufacturers try to appeal this kind of product as special differentiated product.

Several years have passed since 2001 and there seemed no particular factor to change this situation, two separated markets of GMO and non-GMO, except increasing percentage of GM adoption within US farmers. However, US biofuel demand and related subsidies has completely transformed above picture for non-GM market, in particular for maize. The market situation when the Japanese government decided to introduce GM mandatory labelling in 1999 was that the maize price was so low that farmers were willing to make every effort to get additional premium, such as IP for non-GMO. This situation has completely changed because of soaring market price for commodities. Without any additional effort, US farmers are now enjoying high market prices. It is widely recognized that non-GMO procurement is very difficult to sustain for a long term. Soaring grain prices give a large cross pressure upon food manufacturers from both ends of food chain, and eventually result in further cost-price squeeze.

Another important issue of segregation today is low level presence and commingling of unauthorized events in grains for food, feed and processing. Based on the series of contamination, it is widely shared that some kind of risk management measures need to be taken to avoid this kind of contaminations and following disruption in food chain. In the similar vein, US government has



proposed policy on low level presence of GMOs and early notification of risk information to government agencies. US biotech industry organization has also begun its initiative on quality control and risk management system called “Excellence Through Stewardship (ETS)”. Along with these initiatives, Japanese government now takes one step further to facilitate coordination with US government, biotech industry and traders to elucidate each role to minimize this kind of disruption. However, this initiative of Japanese government is limited to “feed” only. In this paper, I also describe this activities and implications on segregation and traceability.

### 6.3. Co-Existence and traceability: Costs and benefits in food and feed supply chains

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European Union (EU) traceability requirements impose added costs and risks on suppliers. This is true for prospective deregulated traits grown in other countries. In this paper we describe the costs and benefits of traceability, as well as the operational implications of conforming to these requirements. We draw on several sets of results that analyze traceability costs, strategies and risks respectively. IN particular, we use results from stochastic simulation models to determine optimal testing strategies and marginal costs to conform to EU traceability requirements for exports of non-genetically modified (non-GM) wheat from the United States. The optimal strategy is chosen to maximize an integrator=s utility. Cost components include certified seed, certification and auditing, testing, traceability, quality loss, and a premium for the added risk of a dual traceability system over a single non-traceability system. Adventitious commingling risks are defined stochastically. Results indicate that traceability requirements can be conformed to with reasonable buyer and seller risk at a total cost of \$18/non-GM mt.

## 6.4. A Company Perspective

Randal Giroux,

Cargill Incorporated, USA

Effective coexistence is one measure to assure the production and processing of abundant supplies of safe and nutritious foods on a sustainable basis, while allowing customers and suppliers to be free to choose whether to use conventional, organic, or agricultural biotechnology products consistent with underlying consumer preferences and choices. To enable effective commercial coexistence, there must be a recognition that this is a dynamic, evolving, and complex marketplace involving diverse agricultural systems.

Several countries have adopted all three agricultural systems and each has evolved towards some form of co-existence, that is commercially-relevant and effective for their needs. It must be recognized that the concurrent use of different production systems can sometimes limit individual choices of both farmers and retail consumers. While true consumer demand eventually influences what farmers grow, sometimes there are temporary market failures in meeting emerging demand for a particular crop or product.

In developing coexistence, some markets have discovered commercial realities that can either enable or inhibit coexistence. If the desire to develop coexistence continues, markets will continue to evolve towards enabling key features and addressing the inhibitors. Key features enabling coexistence include:

- The development and availability of identity-preservation (IdP) systems and test methods to meet *market-based thresholds* for adventitious presence that are appropriate to specific applications and needs;
- The willingness of customers and retail consumers to pay a *premium for differentiated food products*, e.g. organic, non-GM, and other specialty products; and,
- Commercial agreements (contracts) based on *clear, verifiable and achievable specifications* with limited government mandate;
- Several key factors also pose a challenge to coexistence including:
- Failure to adequately contain *regulated GM events* or products being developed through breeding programs and/or field trials associated with product development.
- Adventitious Presence (AP) policies for LLP and food/feed labelling that are not commercially-achievable.
- Asynchronous approvals and *zero tolerance policies* can have significant upstream and downstream effects. Exports of an entire crop (GM, organic, and non-GM) can be placed at risk.
- Compliance and enforcement protocols that are not based on consistent standards and have not been suitably validated or demonstrated they are fit for purpose as the product moves through the food and feed supply chains

This presentation will provide a general overview of commercial perspectives on managing coexistence and some lessons we have learned in implementing these systems into both domestic and global food and feed supply chains.

## 6.5. Protecting European quality agriculture: Non-GM feed supply and production

### The action of the European GM-Free Regions 2005 – 2010

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### Europe's agricultural policy at a crossroads

Since the 1958 Stresa conference, Europe has built a unique tool to promote its agriculture: the Common Agricultural policy. During the 70ies and 80ies, Europe became self sufficient and a major player on world food markets: the European budget being largely directed towards the CAP with more than 50% of the total spending.

In the 90ies, the context began to change:

- The historical disagreement between USA and Europe about subsidies changed into a North – South conflict, the Cairns Group of 18 major exporting countries opposing to the USA-Europe subsidy policy. The GATT and later the WTO supplied its members with a efficient action and negotiation platform.
- The extension of the EU with the integration of new members questioned the opportunity to have half of the European budget directed towards CAP.
- European agriculture realised, under the pressure of Mediterranean countries, that quality agriculture products had to be promoted and issued the AOP-IGP regulation including a “cultural” content to foodstuff. Northern countries concentrated on organic farming and also pushed towards a common policy on the subject.

### Quality agriculture in Europe and in our network

One of the main aspects of the GM-Free regions network (**44 regions in Austria, Belgium, France, Greece, Italy, UK, Spain**) is the very strong presence of quality agricultures:

- To keep a high quality standard products enabling the sector to employ numerous farmers at work and a lively rural sector.
- To manage landscape and environment either because there are important needs and/or because it's a component of the region's image.
- To be able to face a perspective of progressive deregulation of world food markets and competition with globalized foodstuff.

### CAP Reform outlook : towards a strategic alternative

Although the CAP has been frozen for the next few years, after 2010 one can expect important changes. Following the intense lobbying by the Cairns Group but also other sectors involved in the international negotiations, many productions will have an easier access to European markets: dairy products, poultry, beef, most of which are produced with GM feedstuff. Direct subsidies will be questioned and most probably reduced unless they show unquestionable advantages on environmental (and may be social) issues.

Regional agricultural networks in all the European regions will be faced with stronger competition and only very few alternatives.

Either:

- **Try to compete with low price products coming from abroad.** This first strategy can only be met with industrialisation of European farming: low employment rate, large production units in well organised territories close to logistic facilities and probable use of GMOs in feedstuff. If this model is chosen, our regions' agriculture will not be able to sustain environmental, social or cultural goals. Their specificity will disappear as well as a significant part of rural employment.

OR

- **Promote specific agricultures to avoid direct confrontation with globalized foodstuff:** This second strategy relies on a total quality management scheme including a strong territorial link with social, landscape and environment management issues; it also means that no genetically modified material should be used throughout the production process. AOP-IGP, organic and also some private labels are concerned by this market share of the European food market.

### **Feedstuff issue and specific agricultures – step 1 accomplished**

Although some countries of the network have taken steps towards self reliance in feedstuff, the ability to keep an imported non-GM feed supply is crucial for quality foodstuff production. Today all the regions of the network import soy to increase protein rate into feedstuff and if Europe imports more than 37 million metric tons of equivalent soy cake one can guess that our regions' potential, due to their strong agricultural sector, is at least 10 million metric tons; part of which is being directed to quality agricultures.

This is the reason why our network has launched a specific action on feed :

- **October 2005**, mission of 11 regions to Brazil, meeting with Federal Ministers of Environment (Mrs Silva) and Foreign Trade (M Furlão), letters of intentions with the State of Paraná, stakeholders in Paraná, Santa-Catarina, Goias, visit to producers...
- **December 2007**, 1<sup>st</sup> global non-GM feed global meeting organised by our network at the Committee of the Regions in Brussels gathering soy producers from Brazil, Canada, China, India, USA as well as with 117 European businesses collecting meat & milk from more than 680 000 farms, 48 regions, 21 members of the European Parliament...
- **October 2008**, 2<sup>nd</sup> global non-GM feed global meeting organised by the non-GM Trade in Brussels. Strong attendance from Northern Europe traders official announcement of the creation of the Brazilian non-GM soy producers' association (ABRANGE)...
- **January – February 2010**, 3<sup>rd</sup> non GM feed global meeting to be organised by our network at the Committee of the Regions focussed on the use on non-GM and its link with quality agriculture. Partnership with AREPO (Association of Quality production Regions of Europe).

In just 3 years our network has registered valuable successes. Besides raising awareness in the European regions and creating a market intelligence network worldwide, our message expressed right from 2005 about the necessity for Brazilian production to organise has gone through (creation of the ABRANGE). Indian soy production (100% GM Free) is now supplying the EU market, and the perspective of a complete drought in non-GM soy supply to Europe seems to have been avoided.

### **The next steps – the relationship with consumers**

The issue for our network, now that the non-GM soy supply seems secured on the long run, is to value the efforts of non-GM soy producers (Brazil, India, Canada, USA, China) and of non-GM soy users (European quality productions).

The network has observed with the upmost attention the German non-GM feed label, is supporting it's own producers to follow suit and will lobby the European Commission to facilitate the implementation of this kind of tool. That is why non-GM feed supply in the next network's business meeting will not be the main subject and regions will prefer to focus on how to value non-GM feed use through regional strategies on feed autonomy or success stories of productions that have banned GM feed component imports or productions.

## 7. Integration of Co-Extra results in EU tools for coexistence & traceability

Guy van den Eede

European Commission – Joint Research Centre, Italy

The European Network of GMO Laboratories (ENGL), chaired by the Joint Research Centre of the European Commission, is a pan-EU Network of enforcement and/or (national) reference laboratories that deals with all technical issues related to the enforcement of the GMO regulations in Europe. Its activities have had a significant impact on the technical capacities, not only of the participating laboratories, but also on the capacities of GMO test facilities in general.

This leading role has been acknowledged by the European Commission and the European Parliament and has nominated the JRC as Community Reference Laboratory for GM Food and Feed and has entrusted a supportive role to the ENGL. Thanks to the JRC, the EU regulations have inscribed the obligation for notifiers to provide detailed information as well control samples and reference materials, which has had a major impact on testing. Ten years ago it was unforeseeable that all information about event-specific methods is published on the Internet even before the GMOs are approved.

The discussions within ENGL are of the highest possible scientific quality: indeed the Commission trusts that the JRC and ENGL base their solutions and proposals for harmonised testing on technical solutions based on the best knowledge available and on science of the highest quality. Therefore it is imperative that its members, as well as the JRC, are not only aware of the scientific developments but are part of science projects of excellence themselves.

ENGL has been already well represented in FP5 (QPCRMFOOD) and it has been a substantial associate in launching Co-Extra and has been a privileged partner in having access to the information, based on an agreement between Co-Extra and ENGL.

Many of the advancements of Co-Extra have already been discussed in ENGL and will certainly impact the activities of the network.

Today we see also a change of practice moving forward from validation of methods for notification purposes towards validation of approaches for control purposes, for instance by looking at matrix approaches and concomitant decision trees and by applying new ready-to-use qualitative tools, further improving the harmonisation throughout the European Union and beyond its borders.

During the presentation a review of the achievements will be made. However, it is imperative for the functioning of ENGL and the whole enforcement process as a whole that the consortium can continue to be involved in ambitious research programmes and can remain abreast of science and technological capacities in order to continue to fulfil its role and to demonstrate to the consumers and stakeholders that the EC is capable to implement a stringent regulation in a very complex technical area.

## 8. Summary of main Co-Extra deliverables & results, perspectives, information dissemination & application.

Yves Bertheau

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Generally speaking, GMO production and use is a quite controversial socially debated item around the world. This worldwide controversy is also facing the obligation of free trade as made mandatory by international treaties like the WTO. It was not the purpose of Co-Extra to participate to these debates but, as a EU policy support research program, to provide all technical, economic, scientific and legal basis for providing the European stakeholders with accurate data for making decision and keeping the freedom of choice to European producers and consumers.

Herewith you will find the most important results and messages from the several Work-packages of Co-Extra, both in terms of scientific results and practical implementation issues and solutions. Some issues are still pending and will need further research, some of them also depending on the decisions to be taken by policy makers on, for instance, seeds thresholds and fields' co-existence to be harmonized or not to the European level.

As a summary of the Co-Extra results, this summary does not show all results, but more details can be found on the Co-Extra website ([www.coextra.eu](http://www.coextra.eu)), particularly in the on-line deliverables and in the peer-reviewed papers published by Co-Extra members.

### Co-existence in the fields

The first aim of Co-Extra on that issue was to test the stability and reliability of biological containment tools like cytoplasmic male sterility (CMS) in maize, cleistogamy in oilseed rape and plastid transformation in tobacco. Therefore, gene flow parameters of CMS maize and cleistogamous oilseed rape have been studied over the 4 last years, under field conditions located at different sites in Europe:

- The Co-Extra data demonstrate that stable cytoplasmic male sterility in maize is an effective way to reduce or even eliminate GM pollen-mediated gene flow to adjacent fields if stable T- and C-cytoplasms are used. Furthermore, appropriate combinations of CMS hybrids and fertile pollinators used as an agricultural bio-containment system can lead to a significant gain in yield.
- Cleistogamous oilseed rape as a biological mitigation technique has a major potential for limiting cross-pollination due to the strong reduction of the pollen cloud.
- Moreover, data mining was performed to gain information about the suitability of chloroplast transformation as a containment strategy. The outcome is that plastid transformation provides a highly effective tool to decrease pollen-mediated gene flow from transgenic plants. However, in cases where pollen transmission must be prevented completely, stacking with other containment methods might be necessary to eliminate the residual outcrossing probability.

The second aim of Co-Extra work on coexistence in fields was to gain information about the major drivers of maize pollen flow over fragmented landscapes, through field experiments and modelling. Various factors involved in maize pollen emission and pollen flow were analysed through existing data analysis and field experiments. Tools modelling velocity and pollen concentrations over heterogeneous fields were also developed to assess the cross-pollination rates between GM and conventional maize over large distances and in fragmented landscapes. Using new and previously gathered data a statistical model of pollen emission in relation to microclimate and a physical model



of pollen flow based on fluid mechanics were successfully validated. These results apply on single event transformations.

- The Co-Extra data demonstrate that practical and technical knowledge on GM cross pollination in maize is highly accumulated. Models have been validated for large distances and fragmented landscapes.
- Technical measures could ensure that coexistence at the 0.9% labelling threshold for corn hybrids would be achievable on a long-term basis, as far as seed lots are pure enough. Co-existence for maize grain production is feasible and highly dependent on local conditions (e.g. cropping systems, landscape patterns) and on the evolution of practices (e.g. rate of adoption of GM varieties in a region and crop management). Furthermore, various possibilities can be used in different situations (e.g. time-lag of flowering vs. isolation distances) and local operators should be able to choose themselves the best solutions depending on the local constraints. The issue of farmers using farms saved seeds and corn populations instead of hybrids was addressed in a part on legal issues.

The third aim of Co-Extra work concerns seeds. Seed lots are the starting points in an ever increasing supply food chain; therefore field experiments of maize seed admixture have been conducted to evaluate the effect of seed thresholds on the final out-crossing rate in the harvest product.

- The main sources of adventitious presence in non-GM maize are seed impurities, GM cross-pollination, and GM kernel transfer via machinery. The average potential rates of adventitious presence occurring at various stages during farm production are relevant to the 0.9 % threshold set by the EU labelling legislation.
- The Co-Extra data demonstrate that the final GMO rate in the harvest product is similar to that of the seed admixture for current GM varieties (but will differ with stacked GMOs) and highly dependent on local conditions (flowering coincidence, the site and climatic conditions).
- Co-Extra has also investigated the impact of gene stacking on adventitious GM presence due to pollen flow and seed admixture as well as its translation in terms of percentage of GM-DNA in a non-GM harvest. We established, in the case of GM varieties bearing one to four stacked events, the relationships between the cross-pollination rate between GM and conventional fields, the percentage of GM kernels and the percentage of GM-DNA in a non-GM harvest as well as the relationships between the rate of seed admixture and the percentages of GM material in a non-GM harvest. Thanks to these relationships, we substantiated, through several examples, the fact that the number of events and the stacking structure of the emitting fields impact the ability for a non-GM maize producer to comply with given GM kernel or GM-DNA unit based thresholds.
- On a legal and economic point of view, public research policies should be developed for instance on, the breeding of conventional varieties. Moreover, the genetic resources, as those under the auspices of CGIAR, should be preserved. Accordingly international technical protection measures should be put in place, with indemnification, compensation systems for hosting countries.

Considerations derived from Co-Extra work:

The pollen flow to be expected to occur during the growth of crops is indeed highly dependent on the crops' biology:

- The seed purity<sup>10</sup> is of utmost importance for ensuring coexistence in the fields. Any seed threshold (not yet determined at the EU level), should be lower than the labelling threshold but also leave enough leeway to make it possible the coexistence at the field level. There is a trade-off between the seed purity and the adventitious presence in the harvest: the higher the seed

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<sup>10</sup> A seed threshold has not yet been set up at the EU level,

purity, the lower the adventitious presence or the easier to ensure coexistence particularly when taking into consideration the threshold, lower than the labelling threshold of 0.9%, requested by the companies. This practice of using a practical threshold lower is commonly observed in quality control of production in other supply chains when a threshold is required for quality or safety purposes. For those supply chains which claim for thresholds lower than the official labelling threshold, the seed purity will be particularly critical.

- The techniques and procedures for obtaining seeds with low levels of admixture are already available since the GMOs' seeds sold in numerous countries are also used with high levels of purity. As observed in other research programs such as the INRA research program held in 1999-2000, a low level for seeds threshold admixture might increase the prices of seeds, which is however not impacting the final prices,.
- New sampling plans have to be tested for taking into account the still to be decided seed threshold and the practical threshold. So far, most of the studies of other research national and European programs have focused on an expected seed threshold around 0,5% and a kernels labelling threshold of 0.9%. The results of the Co-Extra first study for reaching such a 0.1% threshold are expected soon.
- Biocontainment measures may facilitate the implementation of individual farm coexistence, provided models taking into account the several environmental conditions and the farmers' choices (such as individual choices of growing or not GMOs, late arbitrages according to expectable markets trends, etc.) factors involved are carefully considered.
- The practical implementation of biocontainment measures does however raise several issues:
- Till now, one of the rather stable CMS of corn type (T type) is one of those already observed for hybrid productions, thus with a high sensitivity to a fungal pathogen from which an epidemic in the 70's had huge economic impact on seed production. Its use might be limited to the growth of small-scaled transgenic fields, e.g. for the synthesis of pharmaceuticals.
- The practical implementation in farmers' fields of such mixtures of CMS corn and fertile varieties should be further studied, though higher yields can be expected as observed with the Hybrid Plus technology studied in the Co-Extra project.
- There is no indication of the rapid commercialization by the seed companies of corn varieties with CMS or oilseed rape with cleistogamy traits. Accordingly, the use of biocontainment methods is depending on the future release of biocontained varieties by seeds companies. The interest of the seeds companies to release these biocontained varieties is questionable, since more costly, as well as it could favour the development of hybrids by farmers and reveal some know-how to their competitors.
- Due to the effect of the definition of the DNA unit as recommended by the EC, the increasing number of stacked genes will rapidly increase the GMO content, measured as HGE11. Accordingly, it may be recommended to use the biocontainment methods to stay on the safe side of the GMO content.
- The famers using farms saved seeds should benefit from the same protection measures, such as long distance isolation, that the farmers professionally producing for seeds companies. Farmers who produce farms saved seeds should of course be notified, and GM crops should be produced with the same minimum distances to avoid any cross-pollination with farm-saved seeds.

In conclusion, according to the results of SIGMEA<sup>12</sup> models and the results of Co-Extra, particularly those concerning the practical contractual threshold used by the stakeholders, and the available techniques and information systems, coexistence in European fields, whose size is on average rather

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<sup>11</sup> Haploid Genome Equivalent

<sup>12</sup> Sustainable Introduction of Genetically Modified Crops into European Agriculture, FP6 research program.

small, would be possible only by using large isolation distances (together with strong information system of farmers) or in dedicated production areas, be it GMO or non-GMO.

The validated biocontainment techniques may provide an effective tool to increase the biosafety of transgenic plants and might be used to reduce dramatically for instance implemented isolation distances. However, in cases where pollen transmission must be prevented altogether (e.g. GMO used for non-alimentary purposes), stacking with other containment methods will be necessary to eliminate the residual cross pollination risk.

## Co-Existence in the supply chains

### Supply chains management

Generally speaking, the European companies have not yet been facing with coexistence as the European GMO production is rather limited and mostly, if not completely, used in feed production. As animals derived products are not labelled, coexistence is currently not an issue. Third countries, with very large fields have implemented efficient traceability and products segregation for exports towards the countries, like EU, with a labelling threshold.

From interviews conducted with European and third countries companies involved in commodity supply chains, it can be stated that a vast majority of stakeholders, if not all, is using a practical threshold which is lower than the labelling threshold (generally from 1/3<sup>rd</sup> to 1/10<sup>th</sup> of the labelling threshold, more generally 0.1% of DNA based unit GMO content). These observations confirm those made since 2001 in other studies on GM and non-GM supply chains (such as third countries IP<sup>13</sup> systems). This practice is similar to the ones used in other supply chains management (mycotoxins, allergens, pathogens, etc.). This very common practice of using a practical threshold lower than the official one (for quality or safety purposes) can be explained by the assurance required by stakeholders to protect themselves against sampling and analytical measurement uncertainties in front of contracts or State controls. In addition, this practice is rather easy to implement today because the GM pressure is today very weak. It has been difficult to assess what would be the behaviour of stakeholders under different scenarios (combining different hypotheses on seed thresholds, non-GM demand or GM pressure).

This practical threshold contractually used by the stakeholders conditions the whole supply chain management and thus the farms' outcomes and seeds' threshold(s), still to be defined. This is partly due to the absence of European definition of GMO free. All EU members States having legally defined GMO-free products are using the 0.1% threshold.

In addition to the analysis of their current strategies, Co-Extra has explored how stakeholders could be coping with coexistence along supply chains, where GM crops be developed in the EU.

- In principle, stakeholders can use three different segregation strategies to cope with coexistence along supply chains:
  - If they have dedicated factory plants (strategy 1), they can separate GM and non-GM material but this may lead to increased costs (transportation or under-utilisation of some plants if the market demand changes).
  - They can also use separate production lines in the same factory plant (strategy 2), which is more flexible than dedicated plants but not always feasible (for example starch factories use single production lines);

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<sup>13</sup> Identity Preservation

- The temporal specialization of process lines (alternating between GM and non-GM batches) is more flexible, but requires regular cleaning of equipment or downgrading of non-GM batches (strategy 3). Downgrading involves removing non-GM batches that do not meet a targeted threshold for GM presence, and are therefore diverted into the GM supply chain.
- In general, segregation of GM and non-GM supply chains is technically feasible, but the organisation of the chain, from the upstream farmers to the downstream stakeholders, plays a critical role in maintaining/improving the probability of compliance with the official EU labelling threshold level of 0.9% (with a practical threshold between 1/3 and 1/10 of the labelling threshold). On the contrary, upstream farm batches may comply with the threshold but, if chain management strategies are not appropriate, the level of compliance of the final product may be very low.
- Models have been developed by Co-Extra to assess the effect of various variables on the GM adventitious presence in non-GM batches and the probability of compliance of non-GM batches with a given threshold, at each step of supply chain (from the field level to the end user). These models can be used with the 0.9 % labelling threshold as well as with lower thresholds such as the ca. 0.1% practical threshold used by the stakeholders.
- The supply chain simulation model (based on the example of the starch supply maize chain) can test several management scenarios and compare the various strategies (i.e. automatic downgrading *versus* each batch processed subsequent to the processing of GM material is automatically put into the GM supply chain if a PCR test indicates the batch does not comply with the required threshold).
- By using gene flow models, it is possible to estimate the adventitious presence of GM material in non-GM maize at the farm gate. The Co-Extra results show that this information helps in the implementation of an automatic downgrading strategy and may therefore save further PCR testing. This requires strict vertical organisation but can increase overall profitability.
- As the “non-GM” characteristic is not observable by the final consumers, public regulation is necessary to enforce the compliance of final products to the compulsory labelling threshold. This compliance can be obtained through public controls and penalties costs in case of non compliant non-GM products (*ex post* regulation). It can also be obtained through testing and sampling rules imposed to private stakeholders (*ex ante* regulation).
- When GM and non-GM materials are processed in the same production line (strategy 3), from an economic point of view there is a trade-off between the level of compliance of the final product and the number of downgraded non-GM batches. This trade-off depends upon both the relative value of the penalty cost incurred as a consequence of non-compliance (when a non-GM batch does not meet the threshold) and the non-GM price premium in the marketplace.
- Co-existence between GM and non GM products seems difficult to implement within the same supply chains when the GM pressure is high. It is only viable from an economic point of view if there is a price differentiation between both products in the marketplace. This is not always the case, and therefore some stakeholders have stopped segregating GM and non-GM compound animal feed-stocks (because products derived from animals fed with GMO's are currently not labelled).

### Documentary traceability

Documentary traceability (ISO 20005:2007) is an important pillar of the European system of GM and non-GM coexistence system. It allow the cost-effective management of supply chains, by using data from rather raw materials, more easily analysable, in terms of sampling and detection procedures, provided critical points are identified along the supply chains and analytical controls are appropriately made.

The concept of “co-existence” is always directly related to the concept of “segregation”, which is the shape that the organization of the supply chains essentially takes to make coexistence possible. The term “coexistence” is linked with different meanings, which are sometimes confused in several studies. The first one concerns the links between co-existence and segregation and competition strategies. The second one is mostly linked to the problem of co-existence and segregation in relation with differentiation trends and GM events multiplication.

- The work on documentary traceability shows the existence of three typical forms of organization systems for the supply chains in the case of non-GMOs:
  - The first one is a long and “containerized” supply system, which can be observed in Argentina and Brazil, using the ocean transport (generally called “hard IP<sup>14</sup>”).
  - The second system is a long bulk supply system, also using sea transport. This system, used in Argentina and Brazil to guarantee the European importers with the grains type, is an IP system of segregation.
  - The third system is an intra-European system.
- Since the enforcement of the Regulations 178/2002 and 1830/2003, traceability and labelling are required for GM food and feed products in Europe. In Argentina and Brazil traceability of GM food and feed is optional and not officially required, Labelling is officially required in Brazil. The quality systems and the certification are a voluntary action of a part of the companies or cooperatives, most of whom are attempting to export their products, directly or by the intermediate of grain traders such as ADM, Bunge, Cargill and Dreyfus companies.
- The experience on co-existence and traceability, gathered in the Co-Extra Project is of particular relevance to the stakeholders and entrepreneurs, willing to implement new supply chain and quality system. However, these observations have little application for co-existence between farmers, due to the quite larger size of numerous farms in those exporting countries.

### **Economy of supply chains**

The interaction of Co-Extra partners with the companies has been rather difficult and thus the retrieval of quantitative data has been almost impossible.

Generally speaking, the cost-reduction impact of general European directives and regulations, such as the 178/02, making mandatory the implementation of traceability in European supply chains, is not properly estimated by the companies. Moreover, the positive impact of already-implemented traceability and controls, due to both the general, or GMO specific directives and regulations, on e.g. companies’ image, decreases of market withdrawals or recalls, welfare, or development of markets niches, impact of GMO and non-GMO supply chains organization on products related to safety issues (e.g. management of products for allergens or mycotoxins), is also not properly estimated. On several occasions, the use of analytical controls was over-estimated since low-cost documentary traceability is always used. Several third countries have already put in place efficient segregation strategies of GM and non-GM products, in order to gain new markets, which can be used for any value-added supply chains.

This situation may be due to either a lack of analytical analyses of the impact of these different legislations frames or to a willingness of companies to disclose such results, maybe for concurrence related issues, or both.

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<sup>14</sup> Identity Preservation (meaning management of non-GM products)

We can translate this lack of accurate data as a lack of companies' willingness to carefully carry out cost-benefit analyses on coexistence in order to increase companies' profits.

Coexistence of GM and non-GM supply chains is possible only if all stakeholders can valorise their production. This is particularly important for animals-derived products which are not labelled, according to whether that animal was fed with GM or non-GM products. Accordingly co-existence can be insured in the EU only if GMO-free labelling is possible, including animals fed with non-GM products.

According to the results of the analysed food supply chains, only additional costs can thus be expected by organising co-existence between GM and non-GM products in the value chain from production of farm crops up to the production/processing levels of the single supply chains and by maintaining mandatory (or voluntary) thresholds and regulations. Depending on factors like crop requirements, farming, storage and elevating systems, processing strategies, monitoring managements etc, the total additional costs of co-existence and product segregation, for some systems, can increase to 13% of the total product turnover at the gates of rapeseed oil mills or starch industry processing wheat and maize.

However, for most value chains the question of co-existence is a theoretical one at the moment. The implementation and permanent running of co-existence and segregation systems in the food industry can decrease the additional costs due to savings e.g. in the testing requirements of raw materials or routine procedures during the documentation process.

The segregation, traceability and labelling systems for maintaining the GMO threshold below 0.9% hardly provides any significant additional benefits for producer, retailer or consumer (as this would be the case e.g. in organic production, fair traded products etc.). Thus it is possible that no actor of the value chain may be willing to pay the incurred costs of co-existence measures occurring along the line of the supply chain.

Since European consumers, of the countries studied, rarely accept genetic modifications in food products, they are unwilling to pay extra money for product differentiation in the sense of a labelled food product that contains GM materials below the labelling threshold of 0.9%. Besides farmers and seeds companies' production- and crop-related benefits by genetically modified crop varieties like pesticide resistances, anticipated higher yields or increased contents of substances, the benefits for the consumer are quite vague, intangible and hardly convincing. As shown in the consumer surveys in the countries analysed, the putative health or environmental benefits of GM crops are mainly unknown, uncertain and the consumers sees no reason for spending more money on these products.

- More consumers in Denmark, Germany and Poland thought eating GM foods might harm them than did those in GB and Spain. Relatively few consumers, in each study country, agreed strongly with the statement that GM technologies will lead to healthier food and to cheaper food.
- Apart from Spain, consumers in the four other study countries required 'compensation' in order for them to choose GM food products. Furthermore, the level of 'compensation' has to be higher when GM technology is associated with environmental benefits, than when it is associated with health benefits.

The Co-Extra results of consumers' propensity to pay for non-GM products should be usefully compared to those obtained in the consumers' survey carried out under the coordination of the King's College<sup>15</sup>.

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<sup>15</sup> <http://www.kcl.ac.uk/schools/biohealth/research/nutritional/consumerchoice>.

## Traceability and controls in supply chains

By traceability we understand below both the analytical traceability, carried out by analytical methods, and documentary traceability according to its usual standardized meaning (ISO 22005:2007).

The results described below strongly benefited from the involvement of the JRC<sup>16</sup> (IRMM and IHCP institutes) and of numerous ENGL<sup>17</sup> members as Co-Extra partners.

Efficient and cost effective sampling and testing approaches are needed in order to implement co-existence and traceability, stakeholders need first reliable sampling procedures to obtain representative samples secondly validated methods and finally novel methods due to the increase of the number of GM crops.

Sampling represents the initial step and in most cases the major crucial step of the analytical chain particularly when targets or analytes are not homogeneously distributed as for GMOs (see e.g. the Kelda project<sup>18</sup>). The analysis of samples not representative of the lots to be analyzed for compliance could get to wrong decision and then to waste of cost and efforts. Development of sampling methods has been an important goal within the Co-Extra project.

- Dedicated software tools to support sampling and sub-sampling plans aimed at GM detection through the food and feed chain were developed: SISSI a novel approach to estimate the optimal sample size in experimental data collection and OPACSA (OPTimal ACceptance Sampling by Attributes) a new statistical optimisation software including a cost function to find the cheapest and most reliable mode of analysis by sub-sampling. It has to be outlined that the EC recommendation for sampling is also based on such sub-sampling strategy and thus could be adapted for using the OPACSA cost function and optimisation.
- In certain cases of co-existence it is also important to determine, before harvesting in the field, the level of adventitious presence of GMOs in a non-GMO field. Based on the predictions of spatial variability of out-crossing rate, different sampling schemes were developed and validated. After an initial work focusing on the 0.9% labelling threshold, new work has been started for a 0.1% level.
- General control plans should be undertaken where several analytes could be sampled, with low-cost sampling methodologies. In this regard, the current sampling methodologies for mycotoxins (the more heterogeneously distributed analyte in a lot) could fulfil the requisite of a representative sampling also for GMOs and derived products. An important experimental work is currently under way to test this assumption.
- Models have been developed by Co-Extra to assess the effect of various variables on the GM adventitious presence in non-GM batches and the probability of compliance of non-GM batches with a given threshold, at each step of supply chain (from the field level to the end user).
- The examination of several data sets of results of the measurement of the quantity of GMOs in flour by PCR-based methods collected through inter-laboratory studies showed that the use of the log-normal transformation is necessary to correctly estimate measurement uncertainty of the whole detection process. Uncertainty Profiles built from estimates of measurement uncertainty generally give a range of 50 to 200% of assigned concentrations for materials that contain at least 1% GMO. This range of 50 to 200% is consistent with European Network of GMO Laboratories and the EU Community Reference Laboratory (ENGL and CRL) validation criteria and can be used as a fitness for purpose criterion for measurement methods. The effect of this on

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<sup>16</sup> Joint Research Center of the European Commission (Geel, Belgium and Ispra, Italy)

<sup>17</sup> European Network of GMO Laboratories.

<sup>18</sup> [http://bgmo.jrc.ec.europa.eu/home/sampling\\_KelDA.htm](http://bgmo.jrc.ec.europa.eu/home/sampling_KelDA.htm)

the enforcement of EU labelling regulations is that, in general, analytical results need to be less than 0.45% to demonstrate compliance and greater than 1.8% to demonstrate non-compliance with a labelling threshold of 0.9%. These results explain the observation made in Co-Extra that companies involved in the food and feed supply chains are using a contractual practical thresholds around 0.1% for complying with the European labelling threshold for GMOs, which is set at 0.9%.

- Within the project a framework for the analysis of control plans, defined as a test procedure combined with a sample acceptance limit, has been developed in order to enable stakeholders to make objective choices about the effort that should be put into sampling and testing in order to make objective choices of sampling and testing strategies. The main factors that can affect the reliability are the GMO heterogeneous distribution in the lot and the effect of analytical uncertainty.

The use of GMO is subjected to legal constraints, either within by a “deregulation” system (e.g. USA) or an authorizing (e.g. EU) framework. To assess compliance with national and international requirements there is a continuous and increasing need for reliable and cost- and time-effective analytical methods in all areas of analysis.

The reliability of a method is first determined by the validation process, which is the procedure providing evidence of suitability of an analytical method for its intended purpose. All laboratories in charge of GMO detection are working under a quality system within an accreditation scheme for which the compliance of the laboratories’ measurement uncertainties (repeatability and reproducibility) with those obtained in validated method is mandatory. Accordingly, the validation of analytical methods and the implementation of the validation process, have been key goals within the Co-Extra project.

- Within method validation two basic concepts are prevalent:
  - the global approach, prevalent in the USA and in other detection areas, in which the whole process from the product to the final measurement outcome is to be validated as a whole and
  - the “modular approach”, in which the analytical methods are considered as separate “module” ([sub] sampling, homogenization, analyte extraction, etc) and each of these can be validated independently. As such, the “modular approach” provides a good basis for developing a cost-effective validation process by the stakeholder and for its further flexible implementation in routine laboratories. For this purpose performance criteria and statistical evaluation tools (such as AMPE: Analytical Method Performance Evaluation software and 'Decision Support System' ) have been proposed.
- The number of GM crops worldwide is increasing continuously and a corresponding increasing of approved and non approved GMO is an obvious need for screening tools<sup>19</sup> for simultaneous detection of different GMOs in a sample in one step. The DualChip<sup>®</sup> GMO micro-array is a novel multiplex screening method for the detection and identification of GMO, based on the use of multiplex PCR followed by hybridization on a microarray. The validation of this novel method was performed within the framework of Co-Extra, according to ISO 5725 standard. Furthermore due to the complex nature of a microarray experiment results in many potential sources of variability, a fuzzy-logic validation based approach was successfully applied to the analysis and data interpretation of the chip validation exercise. This micro-array can be used not only for GMO screening and identification but also, by using its software using the “matrix approach”, permits suspicion of the presence of unexpected GMO (generally unapproved in the EU).

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<sup>19</sup> Detection targets present in several GMOs.



The current legal frame resulted in the establishment of the CRL<sup>20</sup> for validating GMOs notifiers' identification methods of GMOs with the support of ENGL<sup>21</sup>. However, the current mandate of the CRL is restricted to the identification quantitative uniplex PCR<sup>22</sup> methods provided by the notifiers while the routine laboratories are also using screening methods and attempting to decrease the analytical costs and analysis duration by multiplexing the PCR.

Event though, most of the analytical controls are made on raw products and that the documentary traceability is mostly used for the remaining of the supply chains, the analytical traceability may impact the costs and time (important for instance when downloading a shipment before entrance of the products into the EU) of controlling the GM and non-GM products, by their development costs, routine use, discrepancies between laboratories or finally by their implementation in accredited laboratories. Thus several improvements towards more efficient and effective analytical traceability were made in the frame of Co-Extra:

- Development of screening methods, as opposed to the event specific<sup>23</sup> methods, is not covered by EU legislation and it represents an additional burden to analytical laboratories. To improve GMO coverage by the screening step of analysis, new screening methods were developed and are ready for implementation, even though validation has yet to be put in place. To recognize false positive results in screening step of detection, methods to detect sequence donor organisms were developed, e.g. method for detection of Figwort mosaic virus, donor of P-FMV sequence introduced into several commercial GM crops.
- To improve cost- and time-effectiveness of GMO detection several methods were developed in multiplex format. Most are quantitative real-time PCR based, but some alternative systems for detection of PCR produced products were also tackled; such as capillary gel electrophoresis (CGE) which separates and identifies PCR products based on length and fluorescent tag. Two real-time PCR multiplex systems are already available as commercial kits and pentaplex<sup>24</sup> PCR-CGE method for identification of 4 most common GM maize lines is being fully validated within the project. Also, a duplex system for on-site detection and quantification of GT73 oilseed rape is ready-for-use by the control laboratories.
- A lot of effort was put into improvements of performance in GMO detection. SIMQUANT, a 'most-probable-number<sup>25</sup>' statistics combined with real-time PCR approach, was developed and is showing up to 100-fold improvement in limit of quantification. Together with new protocols for DNA extraction from highly processed samples, the efficient control of GMO presence is now possible in most of the processed soybean lecithins and oils.
- Notifiers are providing the CRL with numerous taxon<sup>26</sup> identification methods to be used for the relative GMO content quantification. The reliability of the relative GMO quantification (expressed in % of GMO content) was addressed through detailed evaluation of characteristics of reference gene methods. Besides strong relationships with EuropaBio<sup>27</sup> for harmonizing the taxa reference genes, a guidance document on how to develop and appropriately test new reference gene methods was prepared. Similarly, a solution for independent, cheap and reliable

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<sup>20</sup> Community Reference Laboratory, Joint Research Center, Ispra, Italy

<sup>21</sup> European Network of GMO Laboratories, <http://engl.jrc.ec.europa.eu/>

<sup>22</sup> Polymerase Chain Reaction, <http://en.wikipedia.org/wiki/PCR>

<sup>23</sup> GMO identification method

<sup>24</sup> Five (5) PCR in a tube

<sup>25</sup> Statistics based technique used in microbiology

<sup>26</sup> Generally a species like corn. Can be a lower taxonomic level as for instance sugar-beet.

<sup>27</sup> European association of biotech companies.

reference material were searched for and found in the form of plasmids and genomic DNA. To improve reliability of GMO quantification a guidance document on different options for systematic DNA quality control was prepared. Different analytical approaches of real-time PCR results and different quantitative real-time PCR machines and real-time chemistries were tested to find possible sources of bias in GMO quantification and to facilitate implementation in routine laboratories of alternative, more cost-effective, detection methods. They were all found to be minor, compared to the bias introduced through less reliable reference gene methods and the effect of low DNA quality.

- Several non-PCR based approaches were also evaluated within Co-Extra to check for their performance. Among several tested, loop-mediated isothermal *amplification* (LAMP) combined with **Bioluminescent Assay in Real-Time (BART) detection system** is promising system for potential broader use in GMO detection its sensitivity and quantification is similar to PCR, but is less sensitive to inhibitors, cheaper. The machine for on-site detection is available. An alternative for implementing on-site detection, such as cooperatives, was also successfully studied.
- During the process of focus groups with stakeholders, the question of how to deal with “botanical impurities<sup>28</sup>” was raised. A document was established summarizing all our knowledge. There is unfortunately no easily applicable technical alternative to the microscopic counting of representative sub-samples. Accordingly, the current practices of adding non-GM products of such a botanical impurity, should continue, even though rather expensive.

The increasing number, diversity and complexity of GMOs authorised within and/or outside the EU calls for more rational strategies to be applied for GMO detection. Stacking of added “effect” genes (traits) and possible presence of unauthorised GMOs pose two particular challenges in this context. Within Co-Extra, several new multiplex methods, detection technologies and strategies have been developed in response to this.

- Efficient screening based on the “*matrix approach*”, whose concepts were defined in the previous EC FP5 GMOchips<sup>29</sup> research project, can be used to identify the most likely sources of observed transgenic material in a sample. The “*matrix approach*” is highly flexible, as individual screening modules may be added or substituted, depending on needs, availability and validity of modules. Furthermore, both protein and DNA based analytical methods can be exploited with the “*matrix approach*”.
- Multiplex<sup>30</sup> detection was mainly achieved through development of well performing oligoplex amplification reactions (2-10 PCR targets amplified simultaneously), where the amplified targets were successively pooled and identified simultaneously, e.g. by array hybridisation or colorimetric capillary electrophoresis. This strategy increases flexibility compared to multiplexing at the amplification step, because target interference (DNA sequences) is mainly a problem during amplification. Expanding the diversity of targets that can be detected in a multiplex assay is much easier when optimisation can be focused on oligoplex amplification modules rather than on a more complex multiplex reaction.
- Unauthorised GMOs have been observed several times within and outside the EU. A review of the sources and the legal status of various types of unauthorised GMOs and a proposal for a terminology for their classification were produced in Co-Extra. Detection of some unauthorised GMOs may be achieved with the “*matrix approach*” within the same screening strategy that may be applied for routine GMO testing, depending on the specific screening modules applied and the diversity of GMOs in the sample. Another method usable in routine for detecting the unapproved GMOs, the differential quantitative PCR, was also developed. Its main interest is

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<sup>28</sup> For instance: 1 kg of GM soybean in a 40 000 metric tons shipment of non-GM corn.

<sup>29</sup> <http://www.bats.ch/gmochips/contact/index.html>

<sup>30</sup> Several PCR carried out in the same tube, as opposed to uniplex PCR (1 PCR per tube).

that this detection uses only a statistical test on routinely used (screening *versus* identification) detection method data. This method is currently under validation through a ISO 5725 organised ring trial. These two main detection methods to be used for detecting EU unapproved GMOs, are accessible to routine laboratories. However, other unauthorised or unknown GMOs may require more sophisticated technologies that were also explored within Co-Extra, such as analysis of total genomic DNA on high density microarrays without selective amplification or high throughput mRNA sequencing.

- Stacking of “effect” genes (traits) has become increasingly popular over the last few years (see its impact on relative, DNA unit based, GMO content above). As a consequence, identification and quantification of GMOs may become less accurate and this in turn may affect the legal compliance of a food or feed product (single GMOs may be EU approved while their stacked counterpart may be not). How gene stacking can be defined and achieved, and its various implications including some legal implications were reviewed in Co-Extra. Some proposals for terminology and solutions to cope with the challenges posed by gene stacking were also presented in the review. A statistics based detection method is currently proposed, however with a probable higher analysis cost.

The polymerase chain reaction (PCR) has several limitations, such as the need for specific primers, limited potential for multiplexing and need for thermal cyclers. Alternative technologies that do not depend on the use of PCR were explored within Co-Extra.

- Multiple displacement (MD) amplification is an isothermal amplification method that may be used to create large quantities of a sample DNA, e.g. for preparation of reference material from limited source material, or to reduce the interference of impurities and DNA damage on microarray hybridisation. Co-Extra showed that, because the MD amplification may introduce some bias, i.e. alter the relative copy number ratio of various DNA sequence motifs, it should thus not be used for the preparation of (reference) materials for quantitative analyses.
- NASBA<sup>31</sup> implemented micro-array analysis (NAIMA) combines the isothermal NASBA technique with multiplexing, potentially resulting in simultaneous amplification of multiple targets that can be identified subsequently, e.g. via microarray hybridisation.
- The interest of direct analysis of genomic DNA via micro-array hybridisation was demonstrated without prior amplification or with MD amplification of the genomic DNA. The main advantage of this strategy is that the number of targets that can be analysed simultaneously is extremely high ( $> 10^5$ ), and that very few assumptions need to be made regarding the target sequence prior to analysis. Such strategy might thus be used for detecting EU unapproved GMOs.

Target specific bias could have severe impact on the reliability of GMO analyses. Co-Extra therefore investigated possible pre- and post-harvest sources of target specific bias. Pre-harvest sources of bias included the frequency and location of substitutions and insertions/deletions in selected DNA sequence motifs targeted in GMO analyses. Post-harvest sources of bias included a number of physical and chemical processing such as heating, low pH and UV-light. The results indicate that bias can be a problem for some product types. For these types of products it is proposed that control reactions are performed to assess if bias is likely and to determine the range and direction of bias. Notably, bias may be more pronounced with some than with other analytical modules (DNA extraction protocols and specific PCR assays). The modular approach for GMO analysis, which was subject to study in the WP4 part of the Co-Extra project, therefore requires that possible bias is covered in validation of the analytical modules. A strategy to implement this was developed in collaboration between two work-packages of Co-Extra.

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<sup>31</sup> [http://en.wikipedia.org/wiki/NASBA\\_\(molecular\\_biology\)](http://en.wikipedia.org/wiki/NASBA_(molecular_biology))

## Legal and policy issues

Co-Extra was attempting to address the issues of stakeholders not only from a technical or economic point of view but also from a legal point of view, taking regard not only of generally applicable regulations governing GMO approval and use, but also of contractual modifications thereof.

The coexistence project is a new modality of government of techniques; it is particularly important concerning new technologies which until now have been managed only in reference to potential or proven risks. This has meant that it tends to prevent the involuntary spread of technology causing the elimination of other technologies.

This government of techniques' modality could be linked to an objective of technological pluralism such as the "energy mix", which could be useful regarding nanotechnologies for example. The project itself is difficult to carry out; it is even harder to find the proper rules to make it sustainable.

Co-Extra shows how European authorities have reached this solution aiming at ending the crisis generated by the public's distrust regarding GMOs food and feed. A Co-Extra study analyses the three government modalities that have been tried out to this day: the "*Law of the Alliance*" which designates a supple regulation conceived by experts, industry and administration; "*Law as seen by the Rulers*", represented by the 90/220 directive, based on risks assessment without managing farm-produced products' supply chains; the "*Law as seen by the ruled*", implemented by the 2003 (1829/03 and 1830/03) regulatory package.

It is finally proved that coexistence is a "more in depth" form of traditional freedom of commerce and industry; it lies on a paradox: to insure all a certain freedom, it is necessary to impose strong constraints and a certain mutual tolerance.

Accordingly, Co-Extra is considering important:

- To officialise the technological pluralism as a global project allowing the reconciliation of *knowledge society* and *risk society* by the promotion of a mechanism insuring public *confidence*.
- To conceive rules so that this *pluralism be sustainable*.
- The coexistence strategies must from now on be thought of from the supply chain level and not only from field coexistence (present regulation).
- It is essential to insure a better distribution of supply chains' segregation costs by establishing a main principle; those introducing a new technology will take in charge the costs of segregation from the field to the consumer (Neighbourhood disturbances theory).
- It is important to quickly solve the question of various types of unknown or unauthorised events.
- Concerning seeds, it is important to quickly solve the matters of 1) the question of fortuitous present threshold 2) the one of the farmer's right to use « farm saved seeds »-but these seeds risk having an increasing level of unwanted GMOs in some species. 3) the question of the availability of conventional seeds which have been the object of a traditional technology of plant breeding to benefit from genetic progress.

As science has become a growing foundation of decision-making, disputes more and more arise on the scientific basis of such decisions, at least when they deal with environmental or health issues. What is the quality of the scientific reports on which the disputed decision rests? Does the present state of scientific knowledge justify this decision? Have all relevant scientific data been taken into account? Wasn't the previous scientific assessment too abbreviated?

A Co-Extra deliverable gives elements in order to better understand and manage these new and decisive aspects of risk decision-making.

Two main recommendations are formulated which can have direct impact on coexistence matters.

- As risk decisions are more and more submitted to courts (national, European and international), it is of utmost importance to have a clear vision of what is required by the judges in terms of risk assessment.
- As the judge's role vis-à-vis science is growing, courts endorse a more disputed role of "arbitrator of good scientific reports", which raises deep stakes that need to be correctly understood.

About liability and redress mechanisms:

- The legal framework affecting coexistence and traceability was analyzed from various perspectives. European, non-European and international approaches to regulating biotechnology in the food and feed supply chain were compared, including contractual duties and possible liability issues that may arise. Complications arise in particular in international settings with differing national systems, and such problems are aggravated by the fact that market participants may develop overlapping contractual regimes deviating further, even though it may be easier for vertically integrated companies. It shows the unifying effect of EU laws on a side and of private standards on the other side.
- While it is still unclear how losses caused to third parties will be resolved, particularly in cross-border cases, the solutions offered by each country's laws are strongly influenced by its political attitude towards GM farming in general, and may amount to a *de facto* obstacle thereto.
- The survey of legal, technical and political issues arising from co-existence and traceability in third countries identified some examples of workable systems and best practices that EU Member States may use when implementing co-existence and traceability rules.
- The analyses clearly showed large diversity in the extent to which third countries are considering introducing or in fact implementing co-existence measures, i.e. to maintain three supply chains. For candidate countries especially, a workable and reliable EU model would be highly appreciated.

## Co-Extra communication with stakeholders

### Stakeholder opinions and attitudes on coexistence of GMOs with conventional and organic supply chains

#### *Main outcomes of national stakeholder workshops and online surveys:*

Seven stakeholder workshops were organised on the issue of co-existence in seven EU countries, and an online questionnaire was launched to survey the general attitudes and opinions towards co-existence. Among a broad spectrum of attitudes and information needs of stakeholders the following are the most dominant:

- There is an overwhelming wish to have the GM labelling thresholds for seeds regulated. This is over different countries and different stakeholders. Without these thresholds it is difficult to set practical co-existence measures.
- There is a general conviction and concern about the costs that co-existence regimes will entail in practice. Most stakeholders are of the opinion that co-existence measures will entail costs – as any regulation will entail costs – but there is difference of opinion on how significant these costs will be.
- There is a concern about the practicalities of sampling and testing strategies. Guidance may be necessary here, and perhaps also a discussion on whether testing is necessary in all situations, or that in many situations sampling will do, followed by testing if a problem has arisen.

- A common concern on how to deal with unauthorized events. Nobody would like to be confronted with an unauthorized event – especially one that is not authorized anywhere in the world – and there are questions on whether it is possible to prevent contamination with such events at all times.
- Especially from the side of the NGOs and organic farmers: a discussion on the legal meaning of the concepts of ‘adventitious’ and ‘technically unavoidable’. There is general recognition of the fact that the 0.9% is a labelling threshold. But there is difference of opinion on what the consequences of these concepts are for the design of co-existence measures. What should practical co-existence measures be aiming at?
- Most stakeholders are not supporters of a hybrid regulatory model with coexistence rules both on the European and the country level, but some may stress the need for flexibility, especially on the practical level.
- Many stakeholders recommend to monitoring the development of practical co-existence measures and compensation schemes in the different EU member states, with an eye on harmonization and the prevention of competitive advantages and disadvantages for particular farmers.
- Farmers are inclined to see co-existence regulatory frameworks as yet another set of requirements that will increase the amount of paperwork that they have to do. They are not in favour of having to be certified or licensed to be able to grow GM crops.
- The questionnaire also shows that although co-existence is an economic and choice issue, some stakeholders perceive, present or use it as an environmental or social issue, especially those stakeholders having a more negative opinion about GMOs.

### Co-Extra data integration

Numerous data are issued from Co-Extra work and thus can only with difficulty be made available to the stakeholders, or the control routine laboratories. Accordingly a large part of the Co-Extra work was dedicated to the integration of data into a tool rather more easily usable by stakeholders. This work was focused onto a quite user-friendly DSS<sup>32</sup>.

The outcomes of Co-Extra provide a whole range of stakeholders: farmers, EU policy makers, importers, transporters, feed/food producers, retailers, consumers, analytical laboratories, users of test reports from analytical laboratories, operators and managers of official control with science-based, ready to use information.

The Co-Extra Decision Support System integrates some results of the Co-Extra project (such as collected data, scientific findings, obtained knowledge and expertise, formulated recommendations, developed methods and models, etc.) in a way that is potentially useful for different types of stakeholders.

The DSS provides data and advice for various decision questions that occur in supply chains involving GMOs, for instance:

- Will my (intermediary) product, given a current set of used procedures and materials, contain GMOs below a specified threshold level?
- Is there any possibility that my (intermediary) product contains unapproved GMOs?
- Which methods perform best or can be used at all for a given analytical or sampling purpose?

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<sup>32</sup> Decision Support System

- What are the costs associated with maintaining GMO content below some specified threshold?

We are using the approach of model-based DSS. In collaboration between experts and decision analysts, we create qualitative models that:

- capture and represent expert knowledge in the form of hierarchically structured variables and decision rules,
- are able to assess and evaluate decision alternatives, and
- provide decision-analytical tools to analyze these alternatives (for instance, finding the advantages and disadvantages of alternatives, and analyzing the effects of changes by “what-if” and sensitivity analysis).

Currently, there are six models implemented or under development:

- *Analytical Model*: aimed at the assessment of analytical methods, including DNA extraction and DNA analysis methods;
- *Sampling Model*: assessment of sampling plans;
- *Unapproved GM Model*: assessing the risk of contamination with unauthorized GMO varieties based on traceability data about the product (for instance, type of product, country of origin, type and mode of transportation);
- *Transportation Model*: assessment of potential GM presence due to transportation based on product traceability data;
- *Dryer and Starch Models*: assessing the effect of control parameters (such as using different strategies for handling GM and non-GM batches) to the collection and processing of maize.

All together these modules are currently pre-validated by Co-Extra partners. A second step of validation should be started as soon as possible with ENGL members and some stakeholders before any release.

## Conclusion

Co-Extra is the largest EC granted project on co-existence and traceability of GM and non-GM supply chains.

Co-Extra focused on GMO and non-GMO supply chains. But the number of supply chains susceptible of being implicated is potentially unlimited, even if very small today. They will all be different from one another. It is therefore impossible to have an exhaustive count. As a matter of fact, traceability is the segregation tool, which itself is the tool for coexistence. Traceability has been studied for what it is, a complex regulation, but also for its economical and social function: allowing trust to establish itself among activities suspected for presenting risks, -rightly or wrongly. We here show that, at the intersection of *knowledge society* and *risk society*, juridical systems are trying to establish a *confidence society* to be the link between the other two.

Having as an aim to develop practical implementation of the techniques developed, Co-Extra was the first attempt to take into account the several stakeholders’ practices, from seeds to shelves, through consumers’ survey, companies interviews and stakeholders’ focus groups. Co-Extra first apprehended the current practices in the EU and third countries, the bottlenecks and then proposed solutions. Co-Extra described thus processes, developed models and tested strategies.

Besides experimental work, economic and e.g. pollen flow modelling, whose information can be used for optimising segregation strategies down-stream, Co-Extra has released numerous technical and legal results all aiming to favour coexistence and traceability at the lowest cost.

Such consideration of both coexistence and traceability and their respective impacts has been taken into consideration for the first time in a European research program devoted to the coexistence of GM and non-GM products.

Co-Extra has also developed new detection strategies such as for detecting stacked or unapproved GMOs. Due to the large number of questions Co-Extra embraced, a Decision Support System has been developed to integrate those data and facilitate their use by stakeholders including laboratory analysts. Its full validation still remains to be carried out after the current pre-validation.

Some issues, such as how to deal with “botanical impurities” in routine analyses, are however still pending.

We can outline the confirmation that stakeholders are using a practical threshold (generally at 0.1%) well below the 0.9% European labelling threshold, as used in other areas having a safety or quality threshold. This observation of the true life, of the reality, of the day-to-day stakeholders practices, shows that the co-existence between farmers is possible only by using large distance of isolation or production (GM or non-GM products) dedicated areas, as determined by the models developed in EC-funded SIGMEA project. The technical and legal definitions of such production dedicated areas remain to be done. Biocontainment methods can be helpful but this depends on their rapid commercial availability when proved to be stable and effective.

Generally speaking, the methods, strategies, tools, models developed in Co-Extra for GM and non-GM supply chains co-existence and traceability will be used in the management of numerous other supply chains, value added or not niche markets, harmful products such as allergens and mycotoxins producing organisms or pathogens.

Thus again a GMO based work provides a good cost-benefit ratio, as previously done for instance with PCR applied to the whole supply chains in 1999 or standardization of PCR requirements, for developing safer and better food and feed supply chains.

As for the former FP5 research programs, such as QPCRGMOFOOD<sup>33</sup> and GMOchips<sup>34</sup>, we could expect that Co-Extra would have a rather important impact not only on the national and EU legislative frames but also on supply chains management.

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<sup>33</sup> <http://www.vetinst.no/eng/Research/EU-projects/QPCRGMOFOOD>

<sup>34</sup> <http://www.bats.ch/gmochips/>



## Poster Abstracts

### **P1. A cost-effective P35S/Tnos multiplex screening assay with internal positive control**

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Although Real-Time PCR methods for the event-specific identification of most commercially relevant GMOs are available today, “traditional” screening methods are by far not out-dated. In contrast, against the background of a growing number of approved and commercialized GMOs these are still very valuable tools for a cost-efficient initial screening step and an indispensable prerequisite for economically bearable analytical strategies. Examples for widely used screening targets are p-35S and t-nos. As an improvement of existing screening systems a robust and userfriendly multiplex assay has been developed for these important screening targets. Specifically the feature of a built-in “IPC” amplification control (Internal Positive Control) constitutes a significant improvement of quality as it provides a cost-efficient inhibition control for highly reliable exclusion of false-negative results which is specifically important under routine conditions where impurities in sample DNA can't be eliminated completely. In addition the new format saves consumables and allows higher throughput on limited PCR instrument capacity.

A programmed spreadsheet tool facilitates automated data evaluation based on precisely defined acceptance algorithms and eliminates individual and thus subjective evaluation of results largely. This new p-35S/t-nos triplex assay format however - due to its complexity - requires specific and stringent validation in order to proof its performance: In addition to “classical” method parameters of simplex methods like specificity, sensitivity or robustness, further multiplex-specific performance parameters had to be thoroughly addressed. Most importantly non-interference and non-competition of the different PCR systems, as well as sensitivity under strongly asymmetric target concentrations had to be validated. Experiments were carried out on ABI 7500 SDS, Stratagene Mx3005P and Bio-Rad iQ5 because multiplex assays call for instrument specific validation. The system proved to perform to specifications on all platforms tested.

#### **Acknowledgements:**

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.

## P2. The problem of when to label in presence of low amounts of transgenic material : the case of botanical impurities

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Within the Co-Extra project a deliverable was dedicated to the problem of presence of botanical impurities in feed material with respect to enforcement of the GM legislation linked to labelling.

A first section is devoted to explaining the source of the problem, which fundamentally is linked to incompatibilities between different legal texts. At a first hand there are regulatory provisions made about the requirement of purity, essentially of feed material [1, 2]. As long as the purity level is above 95% (mind that there are some exceptions to this figure with somewhat lower rates) the feed material is supposed as pure and there is no need to mention in a label the nature of botanical impurities which by definition are harmless components in the feed. At the other hand there are the regulatory provisions for GMO labelling if the tolerance level for adventitious and technically unavoidable contamination is exceeded [3]. The problem arises from the fact that for the latter tolerance threshold the unit is generally considered as expressed “per ingredient but grouping all those of a same plant species” and not as is the case for purity requirements over the total weight of the feed material.

Although the theoretical solution of expressing results of GM botanical impurities towards the whole mass of the feed material would solve the problem from a legal viewpoint (such exceptions are foreseen, see [4]), we show that by this way technical problems do remain.

Because PCR is only able to express a content level “per ingredient”, the following formula should be used to transform this result into a content expressed as a mass fraction of the feed ingredient:

$$C_{GMBI} = 100 \times m \times c$$

With :

$C_{GMBI}$  giving in % the GM content of the botanical purity in terms of mass towards the all mass of the feed material,

$m$  representing the mass ratio of the botanical impurity (i.e. 5,0% becomes 0.050) in the feed material,

$c$  representing the content in GM material of the botanical impurity as measured by PCR (i.e. 1,0 % becomes 0.010), thus a content per ingredient and not towards the all mass of the feed.

A second part of the deliverable handles the scientific possibilities with respect to the terms used in the above formula. A special focus is given to the techniques that are required for measuring the mass fraction  $m$  of the botanical impurity in the product. From the review of techniques made, it clearly appears that with present-day technology there is a technological gap in how to quantify with a sufficient reliability the mass fraction of a botanical impurity in a product. Presently, classical optical microscopy is in fact the main technique used to assess  $m$ . The problem of unit type to consider for the determination of the relative GM material content ( $c$ ) within the botanical impurity is also assessed with its impact on the final result of the equation that is considered as the solution to the problem. The best way appears to be working in the recommended unit of copy % per haploid genome equivalents [5] that should then be converted through fixed conversion factors (defined per species for instance) into relative mass fractions.

A discussion also compares in how far these concepts applicable to feed are transposable to food and seeds. The situation in seeds is very comparable to that of feed materials. While in the food sector, the problem is handled in a totally different way because purity levels of ingredients are not defined by law but through contracts.

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**Acknowledgements:**

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.

### **P3. NIR imaging and chemometrics in support to the detection at the single kernel level of GMO**

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Since 2000, the Walloon Agricultural Research Centre (CRA-W) has acquired expertise in the development of analytical methods based on NIR hyperspectral imaging for particles and single kernel analysis. The instrument used is a MatrixNIR® Chemical Imaging System (Malvern instruments Ltd) recording sequential images with an InGaAs array detector (240x320 pixels) active in the 900-1700 nm range, that means 76 800 spectra per image.

In the framework of the [Co-Extra FP6 project](#) (GM and non-GM supply chains: their CO-EXistence and TRAcability), the CRA-W is in charge to investigate the potential of NIR hyperspectral imaging together with chemometrics for GMO (Genetically modified organisms) detection. Soybean and barley samples coming from different origins and some being transgenic have been analysed for this purpose.

The aim is to produce a methodology in order to investigate the potential of NIR imaging together with chemometrics for GMO (Genetically modified organisms) detection. The data treatment of the spectral data collected corresponds to unsupervised (PCA) and supervised (PLS-DA) techniques. In all data sets the results have shown that a good discrimination could be performed according to the variety and the presence of GM. However with barley it is impossible to differentiate the transgenic lines from the non-transgenic ones when a large diversity of varieties of different origins are considered. From the pattern recognition point of view, more interesting approaches in order to make estimations of the statistical properties based on the images combined with the spectral information has been identified. From the results obtained it appears that next to a merely a qualitative detection, there might be a potential to quantify the GM content in Roundup Ready soybean at the kernel level. There is at least a correlation but more work is being done to document this completely and to improve the correlation.

#### **Acknowledgements:**

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.

## P4. Performance of TaqMan<sup>®</sup>, LNA, Cycling Probe Technology, Lux and Plexor real-time PCR chemistries in quantitative GMO detection

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The real-time polymerase chain reaction is widely used for detecting and quantifying genetically modified components in food and feed. To comply with various applications the number of different Q-PCR detection chemistries is increasing, reaching more than 20 at the moment. Vast majority of the laboratories still uses TaqMan or SYBR Green only and few comparisons were done about the alternative chemistries. In these study Lux, Plexor, Cycling Probe Technology (CPT) and LNA were extensively evaluated and compared using TaqMan chemistry as a reference system. It should however be taken into account that both TaqMan<sup>®</sup> methods were already proven to be robust and reliable through use in routine GMO detection, while the alternative methods were developed and optimized only to the degree described in this poster.

For each chemistry amplicons were designed on the maize invertase as a reference gene and on the 5'-junction in MON 810 event. Assays were optimised and compared for their efficiency in PCR amplification, limits of detection and quantification, repeatability, accuracy and specificity. In addition the time investment and costs issues were evaluated. Even though each assay provided satisfactory performance, results suggest some are more suitable for quantitative analysis than the others. Of the probe based methods, LNA<sup>®</sup> chemistry is the most promising, with excellent quantification limits and efficiency. Very good repeatability, even for low copy numbers, is reflected in high precision and accuracy of measurements. LNA<sup>®</sup> methods can be easily transferred from the widely used and certified TaqMan<sup>®</sup> methods should this prove beneficial for some applications. Because LNA<sup>®</sup> probes are much shorter they could be especially appropriate where high specificity is needed (e.g. only one nucleotide difference in the sequence). They are also likely to be used where the sequences are such that the design of a common TaqMan<sup>®</sup> probe is difficult or even impossible, for example in detecting junctions between GM insert and plant DNA.

Due to some performance characteristics it is not likely that Lux<sup>™</sup> or Plexor<sup>™</sup> chemistries would replace the probe based chemistries in the quantification of GMO content, especially for samples with multiple ingredients. With the probe absent, a perfect specificity is even harder to achieve, which also showed as slight crossreactiveness in one of Plexor<sup>™</sup> designs. Plexor<sup>™</sup> chemistry however performed well when considering LOD. In addition it was the most robust against inhibitory substances of all the chemistries tested and proved practical for routine use. We believe that with additional effort put in design of specific primers Plexor<sup>™</sup> technology provides an appropriate and affordable approach for qualitative analysis.

Our results suggest that probe based TaqMan<sup>®</sup> and LNA<sup>®</sup> technologies are best for quantitative analysis. Primer based Plexor<sup>™</sup> on the other hand could be the method of choice for qualitative analysis if appropriately designed to assure specificity of the method.

### Acknowledgements:

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.

## **P5. GMO analysis: towards assuring confidence in a result**

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The enforcement of current EU legislation for the correct labelling of food materials containing GM derived ingredients, dictates that all stakeholders need access to accurate technologies and methods in order to determine the correct level of GM ingredients present in food samples.

The advent of modern molecular techniques, including high throughput real-time PCR and array based methods, have meant that a number of different and highly sensitive techniques are becoming increasingly available to conduct such GM analysis. However, whilst the technology behind GM detection has become increasingly advanced, there is a need to standardise metrological aspects associated with method validation and experimental design, if confidence is to be attributed to results arising from these new technologies.

The area of standardisation of data analysis and interpretation, is often an overlooked area of the analytical approach, and can cause significant measurement uncertainty associated with analysis of GM. If results are to be interpreted correctly at both national and international level, confidence must be afforded to the values given, and a standardised way to evaluating data from GM analysis can be regarded as a critical step in helping facilitate this. The work presented here outlines two such published approaches, designed to help afford better confidence in results associated with GM analyses.

### **Limit of Detection**

The Limit of Detection (LOD) is a critical performance characteristic of an assay that requires careful evaluation during method validation. One accepted definition for the LOD of an assay is based on the mean value of blank determinations, plus a derivation of their standard deviation. However, this formal calculation for the LOD does not take into account atypical data sets that are generated from real-time PCR techniques, which can be non-normally distributed, truncated, and heteroscedastic.

The LOD can also be defined as the lowest amount of analyte that can be distinguished from a background response, on 95% of occasions. Experimental data for the quantitation of Genetically Modified (GM) material were produced using real-time PCR, in order to model the LOD. A bootstrapping computer simulation calculated the probabilities of detecting PCR positive test results from these data sets, and computer modelling defined a function from the resulting probability plots. The LOD was modelled in response to changes in sample replication level and cycle threshold values.

The bootstrapping and data modelling approach was shown to accurately predict the LOD associated with real-time PCR analyses, and the approach's broad applicability should be of general interest to laboratories conducting trace-level detection.

### **Sample replication**

The level of sample replication within any assay is a fundamental aspect that needs to be considered when producing results with high confidence. A novel approach was used to evaluate the optimum number of sample replicates to use in GM analysis, using real-time PCR as a model system. The work modelled the change in precision associated with the estimation of GM content of sample unknowns, in response to changes in the level of replication associated with both calibrants and sample unknowns. Using an experimentally derived data set, it was shown that it was possible to reduce the sample level of replication from six to three PCR replicates, without a significant change

in the mean value or variability of the expressed result. The use of such an approach can facilitate the use of the minimum number of replicates in order to produce an accurate result, thus saving on important resources involved in quantitation assays.

## Conclusion

Method validation, and the implementation of appropriate experimental designs to support such validation, are two fundamental principles used to provide objective evidence for the “fitness for purpose” of a method or result. The LOD is a critical performance characteristic to evaluate during method validation. A novel approach to evaluating the LOD has been published, that overcomes some of the limitations of traditional definitions for LOD which PCR does not conform to.

In experimental design, a perpetual question is what level of replication should be implemented. The answer is often based on a balance between affording confidence in a result, and being cost efficient with sample throughput. A modelling approach has been published that shows a reduction in the level of sample replication does not necessarily cause a significant reduction in accuracy associated with a result.

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## Acknowledgements:

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.

## P6. Detection of *Bacillus thuringiensis* by real time PCR

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### Co-Extra project WP5 workpackage

Among the genetic modifications introduced in plants, insecticidal protection is a common trait. This is made possible thanks to the integration of a *cry* gene (isolated from *Bacillus thuringiensis* strains). Following the origin of the *cry* gene, this protection can specifically be oriented against lepidopterans, coleopterans or mosquitos and simulies.

*Bacillus* strains and their spores are largely widespread in the environment. They can be found in soil, plants, food and animals. Some species can be present in all kinds of food and cause food poisoning and toxi-infections (some species are extremely pathogens).

The aim of this study is to develop a Real Time PCR test for *Bacillus thuringiensis* in order to detect a false positive screening result due to the possible presence of the bacterium.

Before the setting up of the tests, it was important to take into account that the unique criterium to distinguish the *Bacillus thuringiensis* strains from other *Bacillus* strains is the presence of a parasporal crystal inclusion (protein) that appears during sporulation . Some sequences of *Bacillus thuringiensis* can also be closer to other *Bacillus* strains than inside the *Bacillus thuringiensis* cluster. Moreover, the conjugation between *Bacillus* strains is not limited to the subspecies but is possible between species.

Eight couples of primers and probes were designed for detection of *Bacillus thuringiensis*:

- One system targeting the *rpoC* gene. This was based on alignment of sequences from *Bacillus thuringiensis* sp. *kurstaki*, sp. *tolworthi*, sp. *thuringiensis*, sv *israelensis*, *alvei* and *mycoïdes* (sequences of *rpoC* genes provided by INRA, France);
- Two systems based on *gyrB* gene and established on sequences of Yamada *et al* (1999);
- Five systems based on *cry* genes. The information available on *cryIA(a)*, *cryIA(b)* and *cryIA(c)* was collected and different primers and probes were selected in regions supposed as not introduced in transgenic plants

Tests were done on different strains of *Bacillus* (*Bacillus cereus*, *Bacillus mycoïdes*, *Bacillus weihenstephaniensis*, *Bacillus thuringiensis aizawai*, *Bacillus thuringiensis israelensis*, *Bacillus thuringiensis kurstaki* and *Bacillus subtilis*), on commercial insecticides containing *Bacillus thuringiensis aizawai*, *Bacillus thuringiensis israelensis* or *Bacillus thuringiensis kurstaki* and on different food and feed products of the market.

Results show that *rpoC* is not the best target, *gyrB* target could be useful to distinguish the *Bacillus thuringiensis israelensis* strains and that the best choice for detection of the *Bacillus thuringiensis kurstaki* strains is among the *cry* genes. The selection among the different targets will be possible once tests will be done on additional strains and contaminated samples (laboratory-made samples).

Concerning the tests done on food and feed samples to determine if there was a constant signal observed due to the possible contamination of food and feed products by *Bacillus* strains, we observed that most food and feed samples are negative. Signals are late for ambiguous or positive samples. The most positive sample (hamburger) was a bad conserved sample. This indicates that the risk of positive sample is more located in bad conserved products (bacterial proliferation).



Tests were also done to know if some existing *cry* targets (for detection of GM plants) give a positive signal with the *Bacillus* strains. The patented primers of Eppendorf Array Technology (EAT, Namur, Belgium) used for *cry* detection by microarrays and of ISP (Brussels, Belgium) used for *cry* detection by Real Time PCR with SYBRGreen, the primers developed by Matsuoka *et al.* for classical PCR and the primers of CRA-W for detection in Real Time PCR with TaqMan® probes (developed within the Belgian GMODetec project) were tested on *Bacillus* strains and commercial Bt insecticides. No signal was observed. This indicates that existing “transgenic” primers are doing a good distinction between natural and integrated sequences.

**Acknowledgements:**

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.

## **P7. Development of a new probe for qualitative identification and quantification of Bt 11 maize**

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In this study, a new system consisting of a set of new primers, a Taq Man probe has been developed for identification and quantification of Bt 11 maize. Additionally, this system including TaqMan® probe was compared with that of including SYBR Green™ I.

## P8. Development of construct-specific TaqMan real-time PCR for detection and quantification of transgenic Bt11 maize (*Zea mays*)

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Qualitative and quantitative Polymerase Chain Reaction (PCR) techniques are utilized for detection and quantification of genetically modified crops in foods. Many countries have issued regulations to label foods that include genetically modified crops. PCR Methods used in detection of GM products are required to amplify a specific target gene. Bt11 maize is a genetically modified corn and is resistant against *lepidopteran* insects. The Bt11 transformation event has been produced by using plasmid pZO1502 containing a truncated synthetic *cry 1A(b)* gene encoding *Btk* endotoxin. It also contains a synthetic *pat* gene to allow transformant selection on glufosinate ammonium. 35S CaMV is the promoter, nos termination sequences are included and introns IVS 2 or IVS 6 are incorporated to enhance expression.

The objectives of this study is to screen GM maize in samples in Turkey, compare methods used for the quantification of Bt11 maize from few GM positive samples from the markets and to initialize quantification studies in Turkey by developing TaqMan probes, to decide cost effective, reliable method (analyzing higher numbers of samples) to quantify GM maize starting from Bt 11 maize.

In this study 100 corn samples were collected from markets though Turkey from 2006 to 2008. The samples were screened for genetic modifications using 35S, nos, PAT, Bar. Then, end point PCR identifications for Bt11 were carried out by validated primers and Bt11 Certified Reference Materials (CRMs) with GMO contents of 0%, 0.1%, 0.5%, 1%, 2% and 5% were obtained from JRC-IRMM. Bt11 maize was detected in 4 samples.

For quantification studies, corn kernels were grinded by electric blender and according to CRL validation report CTAB method was applied for DNA extraction from test samples (100 mg). DNA was extracted by CTAB solution and after purification with chloroform it was precipitated out with isopropanol. DNA concentrations were set to 40 ng/ul after UV spectrophotometry. PCR tests were conducted by primers specific for the maize *zein* gene to verify specificity and quality of DNA. Specific primers and corresponding probe labeled with 5'-FAM and 3'-TAMRA were designed for amplification of a 93 bp fragment of the IVS6/*Cry1A(b)* junction region in the Bt11 gene construct. Considering the required conditions for designing of specific primers and probe for TaqMan real-time PCR assay, online Primer3 software was used for designing primers and probe. The DNA and reaction reagent mixture with a total volume of 25 µl were incubated in PCR thermocycler (ABI 7500) under the following program: initial denaturation in 95°C for 10 min followed by 40 cycles of amplification, each consisting of 95°C for 15 sec, 60°C for 30 sec and 72°C for 32 sec. Analyses of amplification curves were used for calculating of. Standard curves were set up on the bases of Threshold cycle values of Bt11 Certified Reference Materials. GMO quantification of Bt 11 positive samples was calculated by a limit of quantification of less than 0.1 % (w/w). To confirm the results, one more DNA extraction was performed for each Bt11 positive samples and three parallel qPCR applications were carried out for each extract. The results of parallel samples showed correlation with each other.

Furthermore, SYBR Green™ I real-time assays were used to verify the result obtained in this study. SYBR GreenI real-time PCR techniques were also utilized to confirm the quantification results. In this study, It was also confirmed that the Bt 11 samples were not Bt 10. Comparing the overall results, it

was concluded that primer and TaqMan<sup>®</sup> probe set developed in this study can be used as a functional method for detection and quantification of Bt11 maize line.

## **P9. State of the art on sample preparation and assessing the validity of procedures deriving test portion from laboratory samples**

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One of the main objectives of this work is to provide “real world” and empirical data concerning sample preparation and procedures deriving test portions from laboratory samples on one hand, and confronting these data to standards and guidelines generally followed in the GMO detection field on the other hand. This work could help to implement these standards or guidelines but should also provide information to be taken into consideration in any decision support system used in the context of GMO detection, or any modular analytical scheme using DNA targets as analytes.

The first part of this abstract presents a brief overview of the state of the art concerning the sample preparation (especially test portion). The state of the art on sample preparation has been carried out using a questionnaire distributed to Co-Extra partners and ENGL members. The questionnaire was also extended to the Italian network of GMO laboratories and one Turkish laboratory (neither Co-Extra partners nor ENGL members). Thirty-six laboratories answered the questionnaire. Fourteen are Co-Extra partners, twenty-four are ENGL members. Twelve of them are Co-Extra partners and ENGL members. Ten laboratories are neither Co-Extra partners nor ENGL members. The present state of the art focus on the test portions properties : size, number of replicates, final particles size. This abstract concerns only grains (seeds and kernels).

Concerning the test portions number, ISO Standards on GMO detection require 2 tests portions to perform two independent DNA extracts per sample. The study shows that this requirement is generally followed by laboratories (one laboratory performs three tests portions). For the test portion size, DNA extraction protocols described in ISO standards annexes are usually written for 200-300 mg tests portions sizes. ISO standards recommend not to exceed 2g test portion. Most of laboratories perform 200 mg to 2g tests portions Eight laboratories perform 100 mg tests portions. Only one laboratory performs 10 g test portions. Concerning final particles sizes, ISO standards don't have any precise requirement but AFNOR XP-V-03 recommends to use a < 0.5 mm final particle size (as much as possible). This study shows that the final particle size is not always known. Thirteen laboratories reported an unknown final particles size. Nineteen laboratories reported a value (between 0.08 and 4.0 mm). Thirteen laboratories reported particles size  $\leq 0.5$  mm. One lab reported 0.75 mm, one lab < 1.0 mm and one lab 4.0 mm. Other labs reported variable values (between 0.3 and 1.0 mm). Three labs didn't report any value. One lab doesn't perform analysis on grains.

These results shows that, when data are available, ISO and/or AFNOR standards on GMO detection are quite well followed. However, the differences observed between some modalities of sample preparation modules (or sub-modules) could lead to different analytical performances (if it's not demonstrated that the combination of different modalities of two or more modules are equivalent in terms of performance criteria).

The second part of this work consists in assessing the validity of procedures deriving test portions from laboratory samples, taking into consideration the main conclusions coming from the above state of the art, especially concerning the test portion size. The aim of this part is from a modular point of view, to answer to the question : “is a test portion representative –from a quantitative point of view- of a laboratory sample ?”. In other words “does the test portion size influence quantitative PCR response and then, the measurement uncertainty ?”.

To carry out this task, soybeans (grains) have been chosen as a model matrix. Samples (containing ~1000 grains each) have been built by spiking non GM soybeans with RR soybeans. Seven levels (from 0.1 to 1.8 % in weight) were set up. For each level, five test portion sizes (from 50 to 800 mg, taking into consideration ISO standards guidelines and the above state of the art) have been analysed.

First analysis of results shows that, for the respective ranges of both GM contents and test portion sizes, the influence of the GM content seems more important than the test portion size, especially in terms of variability on the GM content measurement. The major preliminary conclusion of this work is that the different combinations “GM %-test portion size” are fit for purpose (together with the several analytical modules used) in the context of the present GMO regulation framework.

**Acknowledgements:**

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.

## P10. Designing the PCR markers *Agrobacterium tumefaciens* gall-forming strains.

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**Abbreviations.** DIR-PCR – *D*iverged *I*nterspersed *R*epeats PCR, SCAR-PCR - *S*equence *C*haracterized *A*mplified *R*egion - PCR

Agrobacterial plant transformation is one of the common methods in modern plant biotechnology. There are several problems associated with bacterial strains involved in transformation process. First of all, the efficacy of any new plant species transformation strongly depends upon the agrobacterial strain used and the search of new highly effective bacterial strain from natural habitats is an instant process. Fast and direct detection of crown gall forming agrobacterium in natural specimen could be substantially facilitated with simple and reliable PCR assay. Second, there are special biosafety requirements during field trials of biotech plants aimed to prevent introgression of bacterial strains used for transformation into natural habitats. The sensitive and reliable PCR assay on the presence of transformationally active *Agrobacterium tumefaciens* (At) in the plant material is quite necessary for such testing. The aim of this study was designing the PCR-marker specific for the *tumefaciens* (At) strains, testing its specificity within *Rhizobium/Agrobacterium* group and designing the combined duplex PCR assays for detection of crown gall forming *A.tumefaciens* strains in bacterial and plant samples.

The aim of this study was designing the PCR-marker specific for the *A. tumefaciens* strains belonging to the biovar 1 according to classification of Keane et al [1] and testing its specificity within *Rhizobium/Agrobacterium* group. All laboratory strains commonly used for bacterial plant transformation belong to this biovar. Four laboratory strains (EHA105, AGLO, LBA4044, GV3101) and 16 field isolates from *Rhizobium/Agrobacterium* group were studied.

The fingerprints of DNAs of studied bacterial strains were obtained using DIR-PCR method [2]. Several primers were tested on their ability to produce the valuable spectra of PCR fragments.

The DNA band from DIR-PCR profiling obtained with KRPN 2 primer which was common for all laboratory strains was chosen for further purification, cloning and sequencing. The consensus clone sequence corresponded to the region of circular chromosome of *A. tumefaciens* strain C58. This region includes two oppositely oriented ORFs with undetermined functions and intergenic spacer. Designing the SCAR primer system, we placed forward primers within the first ORF and reverse primers inside the second ORF. The designed primers (6 primer combinations) were tested on DNAs of all studied strains. In the result of PCR the expected fragments for each primer pair were obtained only for agrobacterial strains belonging to biovar 1. Primer pair N2f2r2 was chosen for the further work due to its highest specificity.

After optimization of PCR reaction conditions, primers N2f2r2 were shown to allow the specific detection of *A. tumefaciens* strains belonging to biovar 1 and thus are proposed to be the SCAR primers.

The specificity of SCAR primers N2f2r2 was tested in PCR on 10 various plant DNA matrices (soya, maize, sugar beet, potato, tomato, cabbage, pea, durum wheat, barley, cucumber). The convenience of the plant DNAs for PCR was tested in the reaction with 18S rDNA-specific primers. In all cases the corresponding PCR-products were obtained. No amplification was observed with N2f2r2 primers.

Then PCR with N2f2r2 primers were performed on mixed DNA matrices, when every between 10 tested plant DNAs was mixed with *A. tumefaciens* DNA in ratio 95 ng of plant DNA to 5 ng of bacterial one per reaction. The presence of the target PCR bands and the absence of non-specific PCR products in these reactions proved the specificity of N2f2r2 primers in reactions on complex mixed matrices.

The duplex reactions with N2f2r2 – 18Sfr2 primers were performed on mixed plant/bacterial DNAs and corresponding pure plant and bacterial DNAs as a positive controls. A single bands of the correct sizes were detected in reactions with pure DNAs. Two bands of correct sizes were detected for reactions with mixed plant-*Agrobacterium* DNAs.

Proposed duplex PCR approach with newly developed primer system allows to substantially accelerate and increase the accuracy of primary plant transformants screening.

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#### Acknowledgements:

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.



## **P11. A rapid & simple point-of-use diagnostic for GMO detection in plants**

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In this investigation, the utility of two commonly used nucleic acid amplification technologies (iNAATs) were judged for their ability to detect common genetically modified elements *in-planta*. An isothermal amplification technology (Loop-mediated amplification; LAMP) coupled to a real-time bioluminescent reporter (BART) was compared to real-time PCR (qPCR). The rate of amplification, detection limit, and robustness of each technique were assessed against a variety of samples obtained using conventional or novel DNA extraction procedures. Both amplification techniques were sensitive enough to routinely detect 0.1% GM within a blended maize seed reference samples. Unlike qPCR, LAMP-BART was also robust, tolerating the usual PCR inhibitors found in unprocessed extracts, making this technology a more suitable high throughput technique. Moreover, the isothermal nature of LAMP-BART, together with the simplistic instrumentation and extraction procedure, allowed the analysis to be performed within a field environment.

### **Acknowledgements:**

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.

## **P12. Development of an integrated platform for the detection of materials derived from genetically modified crops in food and feed products**

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The production of Biotech Crop Products (BCP) has become an important global business for food and feed products. A common-ground of understanding of the GM-nature of the sold/bought products is considered essential. Indeed, a sufficient level of information on the GM-content present in the product must be available to comply not only with legal and safety obligations but must also be in line with both seller's/buyer's risks and benefits.

To date, the key technology applied in GMO detection, identification and quantification in the European Community is the "Polymerase Chain reaction" (PCR). In view of the recent recommendation by the EC to apply the "Haploid Genome Copy number" (HGC) as the standard DNA detection unit, all routine screening and identification methods are focused on DNA technologies (e.g. RT-PCR, micro-arrays, (bio) chips, etc).

The marginal cost of GMO analysis could be significantly reduced if 1°) cheaper denominator reference materials to be used in a qualitative/quantitative GMO detection (e.g. mono-specific target plasmids (MTPs)) would be available (M. Van den Bulcke et al, 2006), and 2°) a cost-effective, technically simple but robust GM-event screening approach, allowing to optimize the identification of all EU-authorized GMO events present in the sample, could be developed.

The Scientific Institute of Public Health (further abbreviated as ISP) has developed a 96-well plate matrix-based decision model applying a standard SYBR®GREEN Q-PCR amplification strategy, using MTPs as target denominators. A minimal set of screening elements has been identified, taking the EC-authorized GMO Universe as starting source (date: June 2005). MTPs for the different elements have been developed and have been submitted under safe deposit at the BCCM (Ghent, Belgium). The potential application of the combination of subsets of these MTPs as a reference material for GMO screening in maize and soybean, has recently been demonstrated in a 96-well plate liquid phase format (A. Lievens et al., 2007).

Here, we report the description of the basic elements of the developed GMO detection platform and present some data on the results obtained with this new GMO screening system; designated as "CoSYPS" ( for "**C**ombinatory **S**ybrgreen **q**PCR **S**creening").

### **Acknowledgments:**

This work was partially performed within the Work Package 5 (Co-ordination K. Gruden, NIB, Slovenia) of the EC granted project Co-Extra (contract 007158). Co-sponsors of this work are the Belgian Federal Ministry of Public Health and the Environment (GMOdetec project) and the EC-JRC Institute of Health and Consumer Protection (Ispra, Italy).

### **P13. Use of pJANUS™-02-001 as Calibrator Plasmid for GTS 40-3-2 (Roundup Ready Soybean) Detection: An Inter-Laboratory Trial Assessment**

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This study aims to evaluate the use of dual-target plasmids as calibrators in the quantification of GM-materials in food and feed products. As a model system, Roundup ready soy GTS40-3-2 (RRS), the major GM-material on the market, was chosen.

Two dual target plasmids, designated as pJanus™-02-001 and pJanus™-Monsanto, comprising part of a junction region of the GM soy Event GTS-40-3-2 and the endogenous taxon-specific lectin gene were evaluated for use as reference calibrator. The efficiency of both plasmids as DNA template was tested for using various RT-PCR methods applying both SYBR®GREEN PCR-methods and Taqman chemistries. Based on this limited evaluation, both plasmids are shown to perform equally well. The pJanus™-02-001 plasmid was the preferred choice for further study as this plasmid offers a broader versatility in choice of PCR methods to be used in the quantification.

The pJanus™-02-001 plasmid was passed down an inter-laboratory trial for performance comparison to genomic DNA of leaf tissue from RRS. In total 11 laboratories participated in the study. The data generated in the inter-laboratory trial have been analyzed both by conventional statistic & fuzzy logic approaches.

Data analysis confirmed that both the plasmid and the genomic DNA perform equally well as calibrator in a stable and homogenous way throughout the different laboratories involved in the trial and throughout the different quantities of tested GM% (0,2%, 1,8% and 4,4%).

These results indicate that plasmid calibrators may represent a cost-efficient valuable substitute for genomic DNA or reference powders as calibrators in the detection and quantification of RRS in products.

#### **Acknowledgments:**

This study was performed within the Work Package 4 (Co-ordinator: R. Onori (ISS, Rome, Italy) of the EC granted project Co-Extra (contract 007158).

## **P14. Testing the “Modular Approach”: an example with Round-Up Ready Soybean**

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The concept of “modularity” for the analytical procedures and validation of methods in GMO analysis lies on the basic idea that GMO analysis consists of a limited set of distinct steps that represent a certain elementary unit with the process, called “module”. Modularity implies independency and flexibility of combining modules on the one hand, and uniformity and harmonisation on the other hand. If modular validation is to be applied, fit for purpose procedures and general acceptance of minimum requirements for each module are needed in order to evaluate the uncertainties associated with each module.

If the real-time PCR measurement is not influenced by the type of extraction method applied, the validation of the real-time PCR measurement can be performed on DNA extracted with any method and from any type of matrix, but scientific evidence for this approach has still to be provided. Indeed, different extraction methods can influence the DNA quantification in food products through real-time PCR. As well, the quantification of GMO can also be affected by the degree of processing of the matrix from which genomic DNA is extracted. In this study, an experiment was performed to test the independence between modules. A balanced experimental plan was designed where three methods based on different mechanisms of DNA extraction and purification (CTAB, Dellaporta, and Wizard) and three matrices of different nature (feed, biscuits, CRM 1%) were fully combined. DNA yields were estimated by spectrophotometric and fluorometric determinations. Criteria were applied to assess DNA quality for the possible presence of inhibitory compounds. The % values of GM DNA were processed via statistical and fuzzy-based approaches. The findings of this study generally support the independence between modules when appropriate performance criteria are met by DNA extracts so that they can be fit for the purpose of the analytical exercise, independently from the previous matrix-DNA extraction combination. However, independence cannot be confirmed when highly processed material (biscuits, in this study) is coupled with a magnetic beads system for DNA extraction.

### **Acknowledgements:**

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.

## **P15. NAIMA: a fast quantitative method for high-throughput GMO diagnostics in food and feedstuffs**

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In the future, GMO testing will become economically difficult to cope with increasing numbers of approved GM lines. Several multiplexing approaches are therefore in development to provide a cost-efficient solution. Despite its high sensitivity and specificity, PCR technology has some limitations, including the lack of true multiplexing solutions. Furthermore, the accuracy of quantification using qPCR has its limits due to its exponential amplification nature.

In order to alleviate some of these inconveniences associated with PCR technology, we have investigated the ability of NASBA technology to be used for GMO diagnostics.

Therefore, we have developed a novel fast multiplex quantitative DNA-based amplification method suitable with detection on microarray. This new method named NASBA Integrated Multiplex Amplification (NAIMA) allows fast amplification of target DNA (15 to 60min) in a multiplex fashion. Sensitivity (Abs LOD is 2-20 copies, all GM targets detected between 0.1% and 100%) and specificity are suitable with requirements for GMO detection. The method allows quantification with same precision as for singleplex qPCR in the tested GM range (0.1%- 20%) on food and feed samples. In combination with microarray detection, NAIMA allows fast identification and quantification of GMOs in food and feed samples. The concept could easily be extended to domains where diagnostics rely on DNA based sequence detection.

### **Acknowledgements:**

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.

## P16. GMO versus mycotoxins sampling plan: a pragmatic approach

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The application of Recommendation 787/2004 for the GMOs sampling in the food and feed chain raised some difficulty and sometime the worthlessness / unfeasibility of its implementation. It should be considered that the control plan is an action to be undertaken with a global perspective implying the implementation of control procedures suitable for several analytes. In addition, it would be desirable to make available a reliable, easy, and low-cost sampling methodologies.

This study aims at verifying if the current sampling methodologies for mycotoxins (the most heterogeneously distributed analytes in a lot) could fulfil the requisite of a representative sampling also for GMOs and derived products. This should minimize the costs for the control plans for mycotoxins/GMOs and could provide a template for the harmonization of sampling procedures also in accordance with trends raised during recent "ISO/IWA on Bulk Commodity Grain Sampling" meeting held in Seattle May 2008.

The work implies the pragmatic simultaneous implementation of three sampling methodologies for soybeans and flour consignments: mycotoxins sampling plan according to Regulation 401/2006, the GMO sampling according to Recommendation 787/2004 and a more intensive systematic sampling that can evaluate as closest as possible the "true" GMO content of the lot (mean value) and can allow the evaluation of the GMO distribution in the lot.

The results of mycotoxins and GMOs sampling will be compared with the mean concentration of the intensive sampling, through appropriate statistical test (t test), and through re-sampling techniques. The sampling procedures were carried out with manual dynamic sampling on shipments at low GMO level: 4 lots (5000 tons each) of soybean grains and 2 lots (5000 tons each) of soybean flour. For mycotoxins and GMOs sampling plans, duplicate sampling were also performed for evaluating sampling precision .

The incremental and the bulk samples were homogenized by dry milling (Romer labs. Inc. Ras mill) and a representative portion was further homogenised by dry milling (Retsch ZM 200 centrifugal mill, sieve 0.5 mm) to obtain the analytical sample. Genomic DNA was extracted from 200 mg of sample by Qiagen plant DNA extraction minikit, according to producer instructions. The concentration of the extracted DNA was determined by NanoDrop™ ND-1100 (Euroclone s.p.a., Milan, Italy). The extracted material was then diluted to a final concentration of 80 ng/ul for the following Real Time PCR analysis.

Quantification of Roundup Ready Soybean (RRS) was performed by real-time PCR with TaqMan chemistry (ABI PRISM 7700 Sequence Detection System 1.9.1) by means of a construct-specific method (ISO 21570: 2005 Annex C.4). According to the limit of quantification of the method ( 20 genomic copies for RRS) the practical LOQ was close to 0.008% RRS.

Furthermore, samples were analyzed by qualitative method (lateral flow strip, LOD 0.1% RRS) according to sub sampling strategies deriving from cost based statistical tool OPACSA (OPTimal ACceptanceSampling by Attribute of grains) based on OCC (Operation Characteristics Curves).

The poster illustrates the methodologies applied and summarizes the results of the first sampling performed at Ravenna harbour on a GMO free soybean lot of 5,000 tons.

**Acknowledgements:**

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.

## **P17. Approaches to monitor the adventitious presence of transgenes in *ex situ* collections of national gene banks**

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With the dramatic increase in the commercial cultivation of transgenic crops, the concerns regarding their potential impacts on environment and human health are required to be addressed in proper perspective. These issues have more relevance to developing countries, particularly India being rich in biodiversity and centre of origin of many crops. National Bureau of Plant Genetic Resources (NBPGR) is the nodal institute at the national level for management of indigenous and exotic plant genetic resources for food and agriculture and to carry out related research. National Gene Bank at NBPGR conserves more than 3,68,584 germplasm accessions of field and horticultural crops and their wild relatives. With the commercial release of five events of Bt cotton in India and their cultivation on an area of 7.6 million hectares, an appropriate approach to study the probability of adventitious presence of transgenes into *ex situ* collections of the Gene Banks needs to be established. The main aim of Gene Banks is to collect, conserve and make genetic resources available to the breeders, so the maintenance of the purity of genetic identity of the accessions is of critical importance. Therefore, all possible efforts need to be made to prevent the adventitious presence of transgenes in the accessions conserved in the Gene Banks for the posterity. The different approaches to monitor the adventitious presence of transgenes in *ex situ* collections need to be developed aiming to minimize the gene flow of the transgenes. The major area of adventitious presence of transgenes is the collection and acquisition stage as genetic resources may have been exposed to gene flow outside the control of the Gene Bank. So the strategies should aim to minimize the gene flow of the transgenes at these stages. As a part of risk analysis, while collecting or acquiring new accessions by other means, the Gene Banks should consider before testing, whether transgenic events (both the commercial as well as under research) in the relevant taxa are likely to be present in the area of exploration/collection. To randomly tested the accessions from the Gene Bank, the reliable, sensitive and cost-effective qualitative as well as quantitative methods for detecting the transgenes are needed to ensure seed quality and to monitor for its adventitious presence in non-GM seed lots. Conservation of *ex situ* collections with minimal adventitious presence of transgenes in the National Gene Banks would be urgently required to sustain the biodiversity while fully harnessing the benefits of transgenic crops.



## P18. Monitoring the adventitious presence of transgenes in *ex situ* cotton collections of the National Gene Bank

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Gene flow through seed/pollen dispersal from genetically modified (GM) crops to non-GM crops or to their wild and weedy relatives is one of the major concerns related to ecological risks pertaining to GM crops. The introductions of transgenic DNA constructs into the agricultural field provide unique markers to measure the introgression of transgene in *ex situ* collections of Gene Banks. Bt cotton expressing insect resistance *cry* gene is the first biotech crop that has been commercialized in India in 2002 and five events of Bt cotton, viz., MON531, MON15985, Event1, GFM-cry1A and BN-Bt are currently planted on an area of 7.6 million hectares. National Gene Bank at National Bureau of Plant Genetic Resources, New Delhi conserves more than 3,68,584 germplasm accessions of field and horticultural crops and their wild relatives including 5,443 of cotton germplasm. The qualitative methods for detecting GM cotton are needed to ensure seed quality and to monitor for its adventitious presence in non-GM seed lots. The present study reports on the monitoring of adventitious presence of Bt cotton in the *ex situ* collection comprising 50 accessions of cotton collected from the different cotton growing regions of India that have been conserved in National Gene Bank. Hundred seeds of each accession were taken and homogenized thoroughly to form the seed powder. The efficacy of all the homogenized samples for amplification by polymerase chain reaction (PCR) was determined using the primer pair specific for cotton-specific endogenous *fs-ACP* gene encoding fiber-specific acyl carrier protein and all the samples amplified the product of 112 bp for *fs-ACP* gene. The polymerase chain reaction (PCR) based assays for detection of *Cauliflower Mosaic Virus* 35S promoter, *nptII* marker gene and *cry1Ac* gene were performed and the desired amplicons of 112 bp for 35S promoter sequence, 215 bp for *nptII* gene and 203 bp for *cry1Ac* gene were detected in the Bt cotton events used as positive amplification control, whereas no amplification was observed in non-GM cotton and water control used as negative controls. All the 50 test samples were screened and no amplicons for *nos* terminator, *nptII* marker gene and *cry1Ac* gene were detected. Furthermore, detection of different Bt cotton events was undertaken to check the transgenicity of *ex situ* cotton accessions, which confirms the absence of transgene constructs in *ex situ* seed lots of all the 50 accessions tested for adventitious presence of transgene, indicating transgenic DNA has not been introgressed in any of cotton accession collected from the regions where GM cotton has been commercialized.

## **P19. Molecular diagnosis of commercialized or unapproved Bt crops of India using qualitative and quantitative PCR assays**

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To date, five events of Bt cotton, viz., MON531 with *cry1Ac* gene, MON15985 with *cry1Ac* and *cry2Ab* genes, Event1 with *cry1Ac* gene, GFM-cry1A with fused *cry1A* gene and BN-Bt with truncated *cry1Ac* gene have been commercialized in India and other Bt crops such as Bt brinjal, Bt cauliflower, Bt okra and Bt rice are currently under different stages of testing under limited and large scale field trials. Globally, area under cultivation of genetically modified (GM) crops has dramatically increased from 1.7 million hectares in 1996 to 125 million hectares in 2008. Despite the potential benefits of GM crops, perceived environmental and health-related issues associated with GM crops have to be addressed in proper perspective. To enforce an effective monitoring and traceability system for GM crops, it is necessary to develop sensitive and reliable GM detection methods. Polymerase chain reaction (PCR) methods are being routinely and widely used for GM detection. The present study aimed at the development of PCR based qualitative and quantitative diagnostic assays for detection of Bt cotton events, Bt brinjal with *cry1Ac* or modified *cry1Ab* gene, Bt cauliflower and Bt rice and Bt okra with *cry1Ac* gene. The multiplex PCR assays for simultaneous detection of specific *cry* gene, *Cauliflower Mosaic Virus* 35S promoter, *nos* terminator and selectable marker gene, viz., *nptII* (*neomycin phosphotransferase*) or *aadA* (*aminoglycoside-3'-adenyltransferase*) along with the respective endogenous reference genes in different Bt crops such as *fs-ACP* gene encoding fiber-specific acyl carrier protein in cotton, *SRK* (*S-receptor kinase*) gene in cauliflower,  *$\beta$ -fructosidase* gene in brinjal and *TubA* ( *$\alpha$ -tubulin*) gene in rice were developed. The quantitative real time PCR assays have also been reported for estimation of copies of specific transgene integrated in the different Bt crops. The assays for detection of specific GM trait/transgene upto 0.01% have also been developed and validated that meet the Supreme Court of India's stipulations for developing a protocol for testing contamination to a detection limit of 0.01%. The developed qualitative and quantitative PCR assays provide a robust, cost-effective and sensitive method for diagnosis of different Bt crops.

## P20. Multiplexing of SIMQUANT

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### Aim

The idea is to develop the SIMQUANT approach (Berdal et al., 2008) for GMO quantification into a multiplex quantification or multiplex threshold quantification scheme. This approach may be used as a screening strategy to identify whether the total percentage of several GMOs of one species is above a threshold. The strategy is similar to SIMQUANT, but rather than scoring the absence or presence of one GMO analyte in a PCR, the score is made using a mixture able to detect several GMOs at a single molecule level. This is achieved by mixing several primers and probes together. The result will not be able to decide which GMOs that is present, but rather the total concentration of the multiplexed GMOs.

### Materials and Methodology

The multiplexed methods are all singleplex validated as part of the European procedure for GMO authorization, or validated according to other collaborative projects and used in our laboratory for accredited GMO detection. In this study we have mixed 8 different GM-maize methods: Bt176, Mon810, NK603, Mon863, TC 1507, DAS 59122-7, T25 and GA21.

First we wanted to compare the sensitivity of the multiplex method with the sensitivity of validated singleplex PCRs for all the GMOs at a concentration of one target DNA molecule using certified reference materials (IRMM, Belgium) material for each maize event. For the singleplex real-time PCR we used TaqMan Universal PCR MasterMix (Applied Biosystems), with primer and probes concentrations and PCR cycling conditions according to validated singleplex methods. For multiplex real-time PCR we used QuantiTect Multiplex PCR MasterMix (Qiagen). We tested both the same concentrations of primer and probes as singleplex (A), or made a simple modification of the concentrations of the primers and probes to accommodate a multiplex situation (B). The real-time PCR cycling conditions was run according to QuantiTect Multiplex PCR MasterMix's protocol.

Finally we quantified several GeMMA proficiency tests and several CRMs with the modified multiplex approach (B), and compared these results with the results from traditional real-time PCR quantifications and GeMMA's assigned values. The PCR forming unit (PFU) number for the reference gene *Adh1* was determined *a priori*, and the samples were successively diluted to a concentration where the presence of (on average) one GM-target PFU per PCR corresponded to 0.9% GMO.

### Results and discussion

To compare the sensitivity of Multiplex SIMQUANT with Singleplex SIMQUANT we used the same batch of one PFU DNA per test portion for each GM-maize. The number of positive PCRs out of 10 parallels was compared. Multiplex A (no primer and probe modifications) showed similar sensitivity as the singleplex for all maize GMOs, except GA21, while multiplex B had similar sensitivity as the singleplex for all maize GMOs.

The accuracy of multiplex SIMQUANT method was acceptable when tested on several samples of known GMO concentrations (GeMMA and CRMs). This work, intended as a proof-of-principle, shows that multiplexing of SIMQUANT is possible with no or minor optimization.

**References:**

Berdal, K.G., C.B. Andersen, T. Tengs and A. Holst-Jensen. (2008) Improving hundred-fold the limit of quantification (LOQ) of GMO analyses by qualitative single molecule quantification (SIMQUANT-PCR). *European Food Research and Technology*, 227: 1149-1157.

**Acknowledgements:**

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.

## **P21. Use of computational subtraction to search for unknown genetic modifications**

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When generating a genetically modified organism (GMO), the primary goal is to introduce with gene technology one or several novel traits to the target organism. A GMO will differ from its non-GMO parent in that its pool of transcripts is altered. Currently, there are no methods that reliably can determine if an organism has been genetically modified if the nature of the modification is unknown. We have used computational subtraction and high-throughput cDNA sequencing to determine if an organism is genetically modified as well as to define the nature of the modification. We believe that this approach will represent a powerful new strategy where fewer assumptions will have to be made compared to methods currently in use.

### **Acknowledgements:**

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.

## **P22. Effect of different storage conditions on PCR amplificability of genomic DNA extracted from pellets containing maize MON 810 maize**

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In 2009, the European Commission has published the second report on the coexistence of GM crops with conventional and organic farming, outlining the activities undertaken in response to the Council's request and providing an update of the state of implementation of national coexistence measures. In view of further enhancing the efficiency of national coexistence measures, the European Coexistence Bureau (ECoB) is developing suitable codes of good practices to be adopted by the stakeholders involved in the production, harvesting, storage, processing and marketing of GM. Within such a framework of enforcement purposes and liability issues, GM traceability along the supply food and feed chains is an essential prerequisite. GMO detection, based on DNA technologies, plays a key role in this process often implying the storage of samples for up to 6 months (Recommendation EC/787/2004, Regulation EC/172/2002).

Within the Co-Extra Project, the University of Parma and the Scientific Institute of Public Health, have explored the effect of time and different storage conditions on DNA extraction and Q-PCR amplification efficiency in feed samples. Fodder pellets were prepared from a mixed flour (composed of 48% maize, 25% bran, 10% barley, 9% soybean and 8% sunflower flours in weight)) containing three (0.1%, 1% and 5%) different percentages of maize flour MON810.

In the UPAR experiments, the pellets have been stored at -20 C °, 4 C° and RT and extractions have been performed at T0, T1 (3 weeks) and T2 (6 months). using a CTAB protocol (ISO 21571:2005) previously validated by JRC-IHCP. The quality of DNA extraction efficiency has been monitored in terms of yield (ng/micro litre), 260/230 and 260/280 ratios. The evaluation of PCR amplification efficiency has been carried out through both end point PCR and RT-PCR analysis. The results from these analyses demonstrated that gDNA recovery and amplificability remains constant over time in all experimental storage conditions. A slight decrease in the quality of extracted DNA was however observed.

In the IPH experiments, the mixed flour (as dry powder and admixed with liquid soup) and the fodders were stored for up to 6 months at +4°C (with and without silica gel), -20°C and -80°C. The assessment of the conservation of the different matrices was carried out at different time points: day 0, week 3, month 2, month 4 and month 6. gDNA was extracted by a validated CTAB protocol. The SYBR®Green QPCR methods applied in the Cosyps screening system involving 11 markers (kingdom, species, generic recombinant, trait targets) and appropriate TaqMan® identification qPCR analysis for the present GM-events were used for determining the influence of the respective storage conditions on GMO detection. The results of this study show that flour and fodder are well conserved for up to 6 month in all conditions but that in liquid samples freezing at -20°C and -80°C is recommended.

### **References:**

EC report of 2 April 2009 on national strategies to ensure coexistence of genetically modified crops with conventional and organic farming.. Please see: [http://ec.europa.eu/agriculture/coexistence/index\\_en.htm](http://ec.europa.eu/agriculture/coexistence/index_en.htm)

Commission Recommendation EC/787/2004 of 4 October 2004 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"

Regulation EC/178/2002 of the European Parliament and of the Council of 28 January 2002 laying down "the general principles and requirements of food law, establishing the European Food"

**Acknowledgements:**

This work was performed within the framework of Work Package 4 of the Co-Extra Project (Co-coordinator: R. Onori (ISS, Rome, Italy), contract 007158, under the 6th Framework Programme, priority 5, food quality and safety.

## **P23. Multiplex DNA Detection System For Identification Of Genetically Modified Organisms (GMOs) In Food And Feed Chains; Co-Extra WP6 results**

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The adoption of GM crops has continuously increased over the last decade with 125 million hectares of these crops grown in 2008 worldwide. There is a great demand from both the general public and the organic farming community for the possibility to choose from genetic modification (GM) free or GM-containing foodstuffs and for the ability to grow GM-free crops. Coexistence is a way of allowing farmers to choose between conventional, organic and GM crop production and demands a traceability system. In Europe, such traceability is legally mandated for food and feed originating from or containing GMOs (EU directive 1829/2003). In order to monitor and enforce compliance with coexistence regulations, authorities require the ability to trace, detect and identify GMOs.

Conventional real time PCR reaches a 0.1% detection level for most targets. However, the numbers of different approved and unapproved GM plants make detection and identification of GM material in food a time-consuming and expensive puzzle in cases when many subsequent real time PCR reactions have to be performed for a final identification. There is a clear need for a method that can identify many DNA targets within a limited set of experiments and at a sensitive level.

Padlock probe (PLP), ligation-based multiplex detection provides a promising method to meet all the demands of GM detection. This method is based on the detection of a unique DNA sequence by a PLP in isolated plant DNA. Only when both ends of the PLP hybridize juxtaposed to their specific complementary target sequence, ligation can occur and will result in a circular molecule. Universal primer sites in the PLP then enable amplification of only the circularized probes. Only amplified probes will yield a signal when the pool of PCR products is hybridized to a microarray.

PLPs have been developed for 29 targets already, including GM plant species and several GM events, elements and constructs. We have detected positive targets in mixtures up to 13 DNA targets. During the PLP experiments 0.1% detection level has been reached so far in case of elements, and 1% in case of events. Similar results were reached during transfer of the method to another institute. Further aims are to design padlock probes for more targets and to lower the detection level. An alternative, so called rolling circle amplification method is also being explored. The latest results will be presented.

### **Acknowledgements:**

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.



## **P24. The Co-Extra website, a key tool in the Co-Extra external communication strategy**

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Communicating and interacting with the public about research is of vital importance. Science that is communicated poorly remains unrecognized. Participants in EU-funded projects are encouraged by the European Commission to promote more and better communication on science and research, by paying particular attention to the "public communication" dimension of their work.

Disseminating and facilitating access to science-based information has been therefore one of the major objectives of Co-Extra, a European-funded project addressing co-existence of genetically modified organisms and non-genetically modified organisms in Europe and their traceability. To this end, a dynamic and interactive website (<http://www.coextra.eu>) has been developed as the core element of the Co-Extra external communication strategy. This website has been designed to make it attractive and accessible to a large audience in a very simple and practical manner, building on practical experiences gained in the development of other websites related to biotechnology and genetically modified organisms.

The website was developed taking into account that "the public" is not a homogeneous population; rather it encompasses numerous sub-groups, each of them constituting a distinct audience seeking information that answers their questions and concerns with an appropriate level of detail. Accordingly, The Co-Extra website has been structured to allow 3 main readership levels: Level 1 corresponding to the most accessible pages and providing general and popularized information (such as news and reportages); Level 2 offering information for non-specialists about the dozens of research projects within Co-Extra; Level 3 providing for the more expert readers the detailed scientific data from the running projects including the most recent results, reports and publications, and the list of partners/institutions involved.

Another important aspect of the website is that it supplies background information on progress in the implementation of coexistence and traceability measures in various European countries ("country sections"). This part of the website is available in several European languages to overcome potential barriers of the users by allowing access to local information in their native language.

Last but not least, the website also provides for various permanent tools allowing multidirectional interaction with its visitors (electronic newsletter, online discussion forum...).

Content is displayed using a web-based platform, based on a sophisticated Content Management System. In order to maintain consistent management policy in content edition, an Editorial Office (responsible for the public information layer) and an Editorial Board (responsible for the review and endorsement of certain types of documents before they are published) have been established.

The frequency and profile of the use of the Co-Extra website have been monitored on a regular basis all along the duration of the project through the use of various indicators. Data indicate that the "popularity" of the Co-Extra website as well as its ranking in major search engines for relevant keywords have gradually increased over time. The interest of the public goes mainly on local information ("country sections") and on content written in journalistic style (news, reportages), while scientific results attract fewer visitors, most probably due to the low amount of detailed scientific data currently available on the website.

Broadly speaking, one can consider that the website met its objectives: providing balanced and fact-based communication and contributing to improve awareness and understanding about co-existence aspects of GMO. Technically speaking, the website represents also a powerful web-based communication platform that will remain active for the next 5 years.

**Acknowledgements:**

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.

## P25. Influence of the (non-GM) soybean price on compound feed price

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The added cost for the production of non-GM feed compared to GM feed is mainly related to its composition. The amount of (non-GM) soy used, which depends on the target animal, will highly influence the final price of the compound feed. Data show that during the period January 2006 till December 2008 the evolution of the soybean market price has a similar pattern as the compound feed price. However, by the end of 2007, the GM and non-GM soybean prices increased significantly. As a result, benefits for agricultural companies have increased and gain margins of compound feed manufacturers were reduced. Many believe that this increase, which also affected the GM and non-GM soybean prices, was due to the size of the South American acreage, the continued strong Chinese demand for soybeans and the decreased US supply. However, the US acreage used for biodiesel production is still relatively small. Moreover, all commodity prices increased in that period and could therefore be explained by speculations on the global market. Anno 2009, the commodity prices went more or less back to their status of 2006.

At the same time, the price premium for 1 ton of non-GM soy increased from 12 € in January 2006 to 77 € in January 2008 due to the decreased availability and uncertainty regarding Brazil's potential to produce non-GM soy. This is the major factor which sets the price for non-GM soy. Again, this increased premium further decreased the margin gains of compound feed producers using conventional non-GM soy.

Two major factors should therefore be taken into account in order to maintain the co-existence of GM and non GM soy for its use in compound feed products in the future: (1) a sufficiently high premium or incentive for farmers producing non-GM and (2) food products derived from animals fed with GM should be labelled.

### Acknowledgements:

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.

## **P26. The cost-effectiveness of the coexistence of GMHT35 oilseed rape in Ireland: an analysis of crop management strategies.**

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The coexistence of Genetically Modified (GM) and non-GM crops specifically refers to the ability of farmers to make a practical choice between conventional, organic and GM crop production, in compliance with the legal obligations for labelling and/or purity standards. In 2005, the Irish Government published a set of crop-specific recommendations to facilitate the establishment of an Irish coexistence framework (McGill *et al.*, 2005). However, this report omitted specific guidelines for the coexistence of GM and non-GM oilseed rape due to the scarcity of Irish-specific research and the nature of the crop with regard to gene flow.

Following from this knowledge gap research was initiated to develop production measures for the cultivation of GMHT oilseed rape in Ireland that were both agronomically sustainable and economically viable. The objective of this paper is to outline the cost-effectiveness of different methods by which coexistence between GM and non-GM oilseed rape can be achieved. Typical methods include isolation distances, pollen barriers and rotation intervals together with specific crop management techniques. The GeneSys spatial model was used to generate computer simulations that were agronomically sustainable in an Irish context whereby gene flow was minimised and coexistence thresholds were not exceeded. Each of these simulations were then analysed using the Annualized Net Present Value (ANPV) method. Therefore each simulation was assessed from both an agronomic and economic viewpoint.

The traditional crop rotation for cultivating oilseed rape in Ireland is to grow oilseed rape followed by three rotations of winter wheat. This was regarded as the baseline model for the purpose of the analysis. Specifically four different crop management regimes were analysed:

- Impact of introducing a spring crop in the rotation, specifically spring barley. The baseline model is compared with a rotation of oilseed rape, winter wheat and two rotations of spring barley. This scenario also examines the impact of lengthening the rotation cycle from four to six years.
- Increasing weed control with management use by increasing the mortality rate to 99% for volunteer control in cereal crops.
- Introducing field border management regimes and changing management times by conducting hedge-cutting in November rather than in May.
- Impact of reduced seed loss at harvest through use of better machinery and lower combine settings at harvest time.

The Net Present Value (NPV) method is a standard approach for evaluating investment opportunities. It is built on the principle of the time value of money which states that a pound/euro today is worth more than a pound/euro tomorrow. Projected future cash flows are discounted, using an appropriate discount rate, so as to estimate their present value. Each of the present values are then summed together and should the NPV be positive then the investment is deemed to be worthwhile. However, use of this approach becomes problematic in evaluating projects which have different lengths of years. For example, a 6 year investment in a project generating a positive NPV can be assumed to be more favourable over a 4 year investment in the same project. To properly differentiate and distinguish between investing in projects with different lengths an Annualized Net

Present Value (ANPV) is computed. The ANPV is computed using the same formula as the NPV. However, once the NPV is computed this figure is discounted by using the corresponding annuity factor so as to arrive at the ANPV of the investment. The investment which has the highest ANPV is considered the most rational to choose by a profit maximising investor. Toivonen and Tahvanainen (1998) stated that the required interest from investment in agriculture is usually in the 3-5% range and assumed a 5% discount rate in their analysis. A number of recent studies using the NPV method to evaluate investment in various crops have also used a 5% discount rate (Goor *et al.*, 2000; Styles *et al.*, 2008). Therefore a 5% discount rate was also adopted in this analysis similar to that used in other recent literature.

While the results presented above show that the ANPV of the baseline model is larger than the rotation which includes spring barley both ANPVs are greater than zero.

In each of *Scenario 2* and *3* the ANPV for the baseline model was significantly higher than that associated with a model with greater weed control (*Scenario 2*) and a model with additional hedge-cutting expenditure (*Scenario 3*). *Scenario 3* is likely to be prohibitive with regard to Ireland as changing cutting patterns to months such as November is contrary to environmental legislation. By contrast a mortality rate of 99% for volunteer control can be expected to have significant agronomic benefits in reducing the incidence of volunteers in subsequent years of the rotation interval. However, in the absence of additional Irish agronomic studies, or field trials, on this subject it is difficult at present to recommend *Scenario 2* ahead of any of the others due to cost issues.

In relation to *Scenario 4* the results indicate that a minimum yield gain greater than or equal to 3% would be required for the ANPV for scenario 4 to be greater than the baseline model. Additional agronomic analysis such as an extension of this study to field trials may help determine whether a yield gain of the levels discussed in this paper will arise from adopting newer technology to harvest GMHT oilseed rape. Regardless any reduction in the level of seed loss at harvest time can be assumed to lower the likely incidence of volunteers in subsequent years of the rotation interval.

As the yield and price associated with spring barley are lower than that of winter wheat, rotations using winter wheat as the sole break crop will always report a higher ANPV than those which also include spring barley. This is demonstrated in *Scenario 1* as the baseline model appears to be the rational investment for the profit-maximising producer.

These results can be regarded as a considered first step in assessing the effectiveness of alternative co-existence strategies in helping to ensure that coexistence tolerance thresholds are not exceeded. The analysis also has highlighted how economic and agronomic costs and benefits must be examined together so as to obtain a more complete picture from adoption of a new technology such as GM oilseed rape.

## P27. Modelling coexistence between GM and non-GM supply chains

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### Introduction

Coexistence is an approach allowing farmers to choose between conventional, organic and genetically modified (GM) crops and allowing consumers to choose between different food products subject to obligations regarding labelling and purity. Coexistence between GM and non-GM supply chains is a complex issue, because adventitious mixing of GM material with non-GM product can occur at any one of the stages of production and anywhere along the supply chain, from the field where the crop is grown to its handling and processing. Another major facet of GM and non-GM coexistence is the fact that the GM content of a product is not a visible attribute. Means to bridge the gap in information do exist (product testing, using model), but they are subject to error. In this paper, we present a simulation model of the coexistence between GM and non-GM products along supply chains. More specifically, the framework of the model is inspired by the starch maize supply chain. The aim of this model is to assess the ability of the supply chain to provide final non-GM product compliant with a required threshold (0.9% labelling threshold for example) and to discuss the impact of the means to bridge the information gap on this probability of compliance.

### Materials and Methods

The model simulates GM and non-GM flows, and takes into account admixture and dilution functions between GM and non-GM batches along the supply chain. Inspired on the example of the starch maize supply chain, three key stages of the supply chain are considered: grain production at field level, grain collection (including drying), and processing. Firstly, the MAPOD gene-flow model (Angevin *et al.*, 2008) is used to simulate GM adventitious presence in non-GM harvests due to cross-pollination between GM and non-GM maize. Within the downstream supply chain, there is only one dryer and one processing plant. Hence, GM and non-GM material are successively handled in the same equipments. On the contrary, storage capacities are considered non-limiting in the model and admixture due to storage equipments is considered negligible. At the maize collection level, the model simulates on the one hand admixture between several batches blended in a same bin, and on the other hand admixture between succeeding batches during drying process. Finally, the model simulates admixture between succeeding batches at processing. We have adopted a compartmental modelling approach of the process to quantify risks of admixture.

Stakeholders define the frequency at which GM and non-GM flow alternate at drying and processing levels (scheduling parameters). GM and non-GM batches are then randomly ordered according to these variables.

Once sequences of batches have been scheduled, uncertainty remains about the GM content of the batches, all the more that it is not a visible attribute. Three kinds of control system might be set up in the model:

1. Simple traceability: this system allows stakeholders to identify whether the batches comes from either GM or non-GM varieties.
2. Automatic downgrading: the simple traceability system is supplemented by rules on automatic downgrading of non-GM batches dried and/or processed after GM batches.
3. PCR Testing: in addition to the simple traceability system, testing is used to gather information on the non-GM batches. The model takes into account the fact that testing can be inaccurate (Starbird, 2007). We assumed a proportional error by simulating measurement uncertainty with a lognormal distribution. Testing can be carried out before and/or after processing.

Two contrasted sets of admixture parameters (at drying and processing levels) were taken into account for the simulations, corresponding to low and high level of admixture between succeeding lots. In addition, previous studies have highlighted that the distribution of GM adventitious presence in non-GM harvests is quite variable among regions (Le Bail *et al.*, submitted). Thus, three contrasted distributions of the GM adventitious presence in non-GM harvest were taken into account, in order to assess the effect of the input purity rate on the output purity rate. As far as the scheduling scenarios were concerned, two values of the scheduling parameters were taken into account: 10 and 100.

For scenarios 2 (automatic downgrading) and 3 (PCR testing), the model identifies the strategy that maximises the profit. Profit depends on the number of batches of each type (GM and non-GM), on the testing cost and, on the probability that non-GM batches are compliant with the required threshold, according to clients testing. Client testing is performed several times and the mean value is considered for the profit calculation.

### Results and discussion

Work on the simulation model is still on-going. Nonetheless, first simulation results show that chain organization, from the upstream producers to the downstream stakeholders, plays a crucial role in maintaining or improving the non-GM product compliance with the labelling threshold. In addition, model should allow comparing various strategies.

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### Acknowledgements:

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.

## **P28. Supply chain description and analysis for maize, potatoes and fresh tomatoes in Slovenia**

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Slovenia is characterised by large geographical diversity which results in the distribution and size of farmland, which is in most parts characterized by a very small size of less than two ha. Due to expected move of GM plant production in to the Slovenian farming, there is a need to construct the scientific and technological basis for GM production and to organize the agricultural production in a way, that will enable the co-existence between conventional, organic and GM production. The overall objective of our work is to facilitate co-existence along the feed and food chains by characterising the organisational schemes of supply chain product management from the farm to the shelf. Consumers need to have information whether they are going to choose GM or non GM food or feed. That's why coexistence cannot be restricted only on production field but as well to the whole supply chain of the specific crop or product. There is a need to establish a system that allows separation of different production systems, that feed afterwards to the supply chains and with that minimizes the risk of mixing. Three case studies are trying to determine generic results.

Maize is the most widespread field crop in Slovenia. Present range of maize production is a reflection of specialization and concentration in agriculture. In the case of Slovenia, field crop production supports well-formed animal husbandry with a comprehensive part of voluminous and concentrated feed. Since 1985 its share in crop rotation has been over 40%. Majority of approx. 73.000 ha of maize is grown for grain (58%), the rest is grown for silage (42%). Almost all the production is intended for animal feeding and less than 3% is used for human consumption. Maize does appear as market good in a small share (up to 20% of total grain production). There is a critical point related to seeds in the case there is admixture of Gm in the seed purchased. This would also have an impact on sowing and harvesting machinery as well as transport facilities particularly if machinery is used jointly with other farmers. From the two supply chains described we can conclude, that for maize silage supply chain in Slovenia there is a very low risk of contamination and admixture due to the fact that no silage is being imported and thus there is no risk of mixing the silage when the commodity arrives to Slovenia. However, some farmers have on farm storage from where they may sell silage to other farmers. This constitutes a critical point in terms of admixture.

Potato is as well an important field crop, which has a long tradition in Slovenia. Due to problems with diseases and pests we can expect interest of producers to use resistant genetically modified varieties. The harvested potatoes are sold directly to the consumer or to the retailers or wholesalers and further distributed towards the final consumer. The sale to the industry and the processing of potatoes is of minor importance in Slovenia. Only small proportion of seed potato is produced in Slovenia. Most of it is imported from northern and western European countries. Potato production in the last 15 years dropped from 13.000 ha in 1992 to 5.400 ha in 2007. We believe that we have reached the dew-point in the Slovenian potato production. The acreage will probably stay at the present level, but the total number of potato producing farms will still decrease. The quantity of imported ware and seed potato is directly connected to the quantity of ware potato produced in a previous year. The largest import (194000 tons) was noticed in a year 2004. The potato horizontal supply chain is not well defined. The market is not well organised, therefore there are a lot of supply chains which can't be traced. Practically it is impossible to establish the importance of each supply chain. Sufficient traceability from the producer to the consumer is prescribed by the legislation and is obligatory. The problems might occur because of large number of small farmers and other chains



which can't be adequately controlled. The easiest way to apply traceability system would be for the integrated management system, which is on the other hand not obligatory for all farmers.

In Slovenia an average production of tomatoes in the last five year period from was around 4000 t. Generally, seeds (category: standard) are imported by the representatives of different seed companies and further sold to producers of transplants or directly to farmers and gardeners who produce the transplants themselves. The transplants produced by specialized plants are sold to the retailers of transplants or directly to the growers and gardeners. Harvested tomatoes are sold directly to the consumer or to the groceries and canteens or to the wholesalers and farmers cooperatives who afterwards sell to the groceries and canteens. The sale of industry and the processing tomato is of minor importance in Slovenia. The import of fresh tomatoes in Slovenia is between 10 and 13 thousand tons per year, which is more than two times higher than the volume of the domestic production. The production of tomatoes in Slovenia appears to be segmented and through that without overall control. In Slovenia there is one existing certified quality system that addresses the Integrated Production of Vegetables only. The traceability of the production process, from seed to the sale of tomatoes is in Slovenia not well defined.

**Acknowledgements:**

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.

## **P29. Preference heterogeneity among German consumers regarding GM rapeseed-oil**

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Consumer acceptance is a determining factor for the profitability and the development potential of GM food. Although tests in the USA have not found safety problems deriving from GMOs and GM ingredients in food products the acceptance towards the application of genetic engineering in the agro-food sector is still low in the EU - especially among German consumers. In the opinion of most European consumers there is nothing to gain by GMOs, but instead serious disadvantages could occur (Evenson and Santaniello, 2004; Gaskell et al., 2006).

Using the example of rapeseed-oil this poster investigates the acceptance of German consumers towards GM food. In order to analyze this question 319 German consumers were interviewed in spring 2007 regarding their knowledge/trust in GM technology, their attitudes towards organic and GM products as well as regarding their socio-demographic characteristics. In addition respondents had to make choices between several alternative rapeseed-oil products within a Discrete-Choice-Experiment. The products were composed of different levels of the attributes *price* and *origin*. The third attribute taken into account was *production technology* comprising the levels conventional, organic, GM with associated health benefits and GM with associated environmental benefits.

By means of latent class analysis three different consumer segments could be identified. The first cluster consists of consumers who set special value on organically produced food products while for the second group of respondents cheap prices are the most decisive purchase criterion. For the third segment of consumers none of the attributes is of outstanding interest, but they prefer indeed GM rapeseed-oil with associated health benefits. All respondents favour locally produced rapeseed-oil, but consumers of the different clusters significantly differ in their risk perception of GM technology as well as in their attitudes towards the feasibility of laws and regulations to protect consumers from risks of GM food, the negative impacts of agriculture for the environment and the assessment of prices of organically produced food. On the basis of the obtained results this poster will additionally give recommendations regarding special marketing activities for the different achieved consumer segments.

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### **Acknowledgements:**

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.

## **P30. Costs of co-existence and traceability systems in the food industry in Germany and Denmark**

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In contrast to the increasing use of GM plants in world-wide agriculture, the acceptance of GM food is still low in the European Union (EU). In order to ensure freedom of choice for consumers and users of GM and non-GM products, GM food and feed products have to be labeled to contain GMOs or GM material in case a tolerance threshold of 0.9 % is exceeded for EU authorized GMOs.

This poster aims to quantify the costs of traceability and co-existence systems for GM food from the seed to the retail level for sugar, wheat starch and rapeseed oil for human consumption in Germany and Denmark respecting the 0.9 % threshold for labelling of GM food. The cost calculation for traceability and co-existence measures are done with a specific model which follows the principle of aggregating all incurred cost for cultivating and transporting crops or processing of the raw material crop on the different levels of the value chain and of increasing the price of the final product at each level. Thus all co-existence and traceability costs emerging in the value chain are transferred to the final end products.

Altogether the measures to ensure co-existence and traceability lead to 5 % to 8 % higher price for GMO-free rapeseed oil in Germany and 8 % to 10 % higher prices in Denmark. In case of GMO-free sugar the price loading amounts to 2 % to 5 % and in case of GMO-free wheat starch to 8 % to 11 % each related to the current price of the respective product in Germany. In Denmark the price loading for non-GM wheat flour for baking will be at the level of 7 % to 8 %. For GMO-free sugar the analog figure lies between 0.3 % and 2 % in Denmark. Finally recommendations for practical implementation and handling of co-existence systems will be given.

### **Acknowledgements:**

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.

## **P31. Analysis of the extra-costs generated on French “Label Rouge” chicken supply chain by non-GM feed policy.**

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The poster presents the results of a study conducted about the extra-costs borne by “Label Rouge” French chicken producers who chose GM-free feed policies. This research estimated these extra costs and studied their distribution along the supply chain through different scenarios. The poster shows an intermediate scenario, where extra-costs on GM-free soy are constants (at the 2008 level) and GM corn is authorized in France. The poster shows, at the middle, a diagram of the supply chain, from the crop production of corn and soy, to chicken sales in supermarkets. The scenario presented in the diagram is detailed through boxes located at its side.

This research has been accomplished between January and July 2008 by the Research Centre on Management of the Université de Pau et des Pays de l'Adour and financed by the NGO Greenpeace France. In addition to secondary data and literature review, the investigations included several surveys and meetings carried out in the sectors of chicken production and crops production and markets. The data concerning the chicken industry are based on information collected from three chicken producers organizations representing 40% of the French chicken “Label Rouge” production.

The producers of chicken “Label Rouge” who have adopted GM free policies are currently paying a premium to purchase non-GM soy. The poster shows the evolution of this premium during the past years. Since the middle of 2008, the value of the premium is stabilised around 25-30€ per ton. The amount of premium chosen in the scenario is 30 € per ton.

As shown by several researches and as it has been experienced on other crops and countries, an authorization and generalization of GM corn in France would also translate into extra costs to purchase GM free corn for the poultry producers. The efforts made by non-GM corn producers to protect and control their production would indeed be translated in market segmentation between GM and non-GM corn. The poster shows the different measures necessary to comply with the threshold of 0,9%. Their cost highly depend on the characteristics of the farm and on the GMO crops share on the region, it can vary from 5 to 37 € per ton. The amount chosen in the scenario is 10€ per ton.

The poster then shows the distribution of these extra costs on the different level of the supply chain, from the feed industries to the slaughterhouses, through the farmers. It shows that with extra-costs of 30 € per ton for soy and 10 € per ton for corn, the extra-costs at the end of the supply chain would be 5,7 cents per kilo of chicken meat.

At the bottom of the diagram, the poster shows what is at stake concerning distribution and labelling. In 2008, retailers didn't accept to pay to producers increases of more than 2 or 3 cents per kilo for this kind of chicken. They argued that they couldn't increase their buying price because, as it is impossible in the current French framework to show through labelling GM-free characteristic to consumers, they couldn't increase their selling price.

Without this possibility to transfer the extra-costs to the consumers, the chicken producers would therefore have to bear them, but it would be economically unsustainable if this extra-costs reach 5 or 6 cents per kilo as shown in the scenario presented in the poster. Without possibility of labelling, the chicken Label Rouge French industry with GM-free feed policy would therefore not sustain the authorization of GM corn in France.

As a conclusion, the poster draws new perspectives of research on this issue, focusing on the concept of externalities. Considering extra-costs as production externalities could indeed lead to implement other public policies than labelling.

## **P32. Towards an optimal management regime to facilitate the coexistence of GM and non-GM oilseed rape in Ireland**

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Capitalising on the existing knowledge base generated through the development of GENESYS, the objective of this research was to utilise the most recent model version to develop an optimal management strategy for potential early adopters of GMHT oilseed rape in Ireland. The considered landscape was Bridgetown, Co. Wexford, which is situated in the south east of Ireland. A digitised map was created from a suite of aerial photographs (provided by Ordnance Survey Ireland, [www.osi.ie](http://www.osi.ie)) using ArcView v3.2. The map consisted of 1704 plots identified as cropped fields (607 plots), hedgerows (972 plots) or no vegetation areas (roads, farms... 58 plots). The number of fallow (uncropped) fields across the landscape was set at 10% (n = 67). Owing to the distinct field shape in the landscape, the hedgerows of each field were created using the manual tracing tool in ArcView v3.2.

For each simulated cropping system, three regional proportions (5%, 15% and 30%) of GM OSR sown in OSR fields were simulated for. OSR fields were cultivated with either a winter conventional variety or a winter GM herbicide tolerant variety homozygous for a dominant transgene conferring resistance to glyphosate. Crops were randomly allocated on each cropped field based on (i) the regional proportion of GM OSR and (ii) the number of fields with the simulated rotation. For two coexisting cropping systems (GM and non-GM OSR), the allocation of GM to conventional fields for a 5% regional proportion was 577 and 30 respectively, 516 (GM) to 91 (non-GM) for 15% and 425 (GM) to 182 (non-GM) for 30%. For each simulation, the starting crop was randomly derived from the available crops within the managed rotation. Simulations examined the impact on temporal and spatial gene flow across the landscape of:

- Alternative cropping rotations;
- Hedgerow management;
- Improved herbicide efficiency during cereal crops

In brief, GENESYS confirmed that the inclusion of successive winter wheat crops following a GMHT oilseed rape crop (Scenario 1 - OSR/WW/WW/WW) presents a high risk scenario that will negate the potential for efficient coexistence at a regional level. Extending the duration of the rotation to 6 years (Scenario 3 – OSR/WW/WW/WW/WW/WW) provided no significant difference ( $P > 0.05$ ), with 16.46%, 47.82% and 85.48% of fields possessing GM admixture  $> 0.9\%$  for a 5%, 15% and 30% regional adoption respectively. In contrast, the substitution of winter wheat with a spring barley crop in year 3 and 4 of both Scenario 1 and 3 reduced the harvest impurity 4.1-fold for a 4 year rotation (Scenario 2 – OSR/WW/SB/SB) and 1.5-fold for the extended 6 year rotation (Scenario 4 – OSR/WW/SB/SB/WW/WW). The influence of an alternative spring crop to barley was assessed by simulating the impact of potato (Scenario 5 – OSR/WW/POT/SB) or maize (Scenario 6 – OSR/WW/MAIZE/SB) management in the third year of the four year rotation. In both cases, the levels of gene flow across the landscape were comparable to Scenario 2, with neither maize nor potato cultivation decreasing the degree of harvest impurities or the % of fields  $> 0.9\%$  for any of the three adoption levels.

The introduction of field border management (cutting and/or herbicide treatment in May) reduced the level of harvest impurity in neighbouring non-GM oilseed rape crops from 3.5% to approximately 2% (for OSR/WW/WW/WW rotation – 30% GM adoption) but this approach may conflict with existing EU environmental directives via REPS. By increasing the herbicide efficiency in a single application through winter wheat crops at seedling stage, harvest contamination (HC) did not exceed the 0.9% threshold at either the 15% (0.39% HC) or the 30% (0.76% HC) regional adoption level. Including a herbicide treatment (95% efficiency) at adult volunteer stage provided for a comparable decrease in transgene flow relative to the single application with 95% herbicide efficiency. Critically, for % HC (5% regional uptake) there was no difference between adopting a spring crop rotation (Scenario 2, 5 or 6) and improving the efficacy of volunteer control in the standard winter wheat rotation (Scenario 1) via enhanced herbicide efficiency or increasing the number of applications.

The significance of these results, along with datasets from additional simulations will be discussed in light of developing an optimal coexistence-based management strategy for the potential early adopters of GMHT oilseed rape in Ireland.

### **P33. Brazilian GMO Free Areas Experiment and the Release of RR Soybeans**

Roseli Rocha dos Santos, Ana Paula Myszczyk, Frederico Glitz

Universidade Estadual do Parana, Brasil.

This presentation aims to examine how the release of the Roundup Ready soybeans commercialization has been done in Brazil and the resistance of the organized society to this fact. For that, the Brazilian legislation on Biosafety is considered, as the applications process for release and commercialization of GM soy by Monsanto to the National Biosafety Technical Commission (CTNBio). The lawsuits made by the consumers' organizations to prevent such release and the main Court decisions on this case are also examined.

In conclusion it will be verified that, although organized society and various states of the Federation tried to establish a prohibition on the use of GMO or create some GMOs free area, the pressure from farmers and industry and the lack of effective supervision of the Federal Government, has allowed the RR soybean to be released and spread in all country.

#### **Acknowledgements:**

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.



## **P34. A bibliometrics approach on Soybean Research in Brazil**

Roseli Rocha dos Santos, Evelin Lucht

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Brazilian non-GMO soybean production faces challenges, especially with seed. With the big emphasis on GM soybeans in recent years, private and public seed research have focused their breeding efforts on GM varieties. Several actors are complaining about the non GM improvements scarcity in Brazil. Is it true or not?

The aim of this research is to present the findings reached by the research into the scientific and academic Brazilian production about research on soybean genetic plant breeding in the period of 2000 to 2009, and to classify them according to quantity, chronology of publication, author's function, source, kind of study, topic, and key words. The research has been carried out taking a bibliometrics methodology. The works analyzed consisted of all papers about the issue that reached the highest grade in the Capes, or A, and also from a network of institutions that study the issue, like Embrapa, Ocepar/Coodetec, FT, Indusem, Cotia, FECOTRIGO, IPAGRO, EMGOPA, EPAMIG, EMPA, EPABA, EMAPA, EMPASC, EMPAER, IPAGRO.

### **Acknowledgements:**

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.

## **P35. The Agroindustrial Chain of Soybean in Brazil: Brief Notes on the Contract of Sale**

Roseli Rocha dos Santos, Ana Paula Myszczyk, Frederico Glitz

Universidade Estadual do Parana, Brazil.

This presentation aims to do a juridical analysis on the commercialization of soybeans that is performed in Brazil, through networks contracts. Such operations are conducted in a well structured and complex agro-industrial juridical system, with the establishment of contractual relations among several agents of the soybean supply chain. One of the most usual contracts used in this network is the contract of soybeans sale. The form and objectives of these agreements will depend on the economic needs involved. However, it is certain that Brazilian courts have recognized the contractual practices that took place on these topics.

### **Acknowledgements:**

This study was financially supported by the European Commission through the Integrated Project Co-Extra, Contract No. 007158, under the 6th Framework Programme, priority 5, food quality and safety.



**Table 1: Estimate of possible time requirements and financial expenditure for potential coexistence measures in Switzerland**

<b>Measure</b>	<b>Time requirements</b>		<b>Financial expenditure</b>		<b>Comments</b>
	<i>favourable</i>	<i>unfavourable</i>	<i>favourable</i>	<i>unfavourable</i>	
<b>Isolation distance</b> *	<i>15 min/field</i>	<i>80 min/field</i>	<i>5 CHF/field</i>	<i>20 CHF/field</i>	<i>50m</i>
	<i>41 min/field</i>	<i>295 min/field</i>			<i>300m</i>
<b>Special machine cleaning</b> **	<i>9 min/year</i>	<i>84 min/year</i>	<i>7 CHF/year</i>	<i>294 CHF/year</i>	<i>sower</i>
	<i>35 min/year</i>	<i>210 min/year</i>	<i>23 CHF/year</i>	<i>1790 CHF/year</i>	<i>combine harvester</i>
<b>Monitoring of volunteers</b> *	<i>13 min/field</i>	<i>36 min/field</i>	<i>none</i>		<i>Per check</i>
<b>Permission</b>	<i>6 min/year</i>	<i>9 min/year</i>	<i>0.50 CHF/year</i>	<i>2.00 CHF/year</i>	<i>Online registration</i>
	<i>34 min/year</i>	<i>46 min/year</i>			<i>Written Permission</i>
<b>Notification of neighbours</b>	<i>50 min/year</i>	<i>83 min/year</i>	<i>5 CHF/year</i>	<i>10 CHF/year</i>	<i>Written notification of 5 neighbours</i>
<b>Information and training</b>	<i>½ day</i>	<i>2 days</i>	<i>8 CHF/year</i>	<i>28 CHF/year</i>	<i>Training once every 10 years only</i>
<b>Documentation</b>	<i>25 min/year</i>	<i>38 min/year</i>	<i>3 CHF/year</i>	<i>15 CHF/year</i>	

\*Depending on statutory regulation, crop and agricultural structure

\*\*Depending on technical circumstances (cultivation technique, crop and mechanisation (own machinery, hired machinery, agricultural contractor)) and the coexistence regulations in force

The cultivation of herbicide-tolerant maize and oilseed rape has an economic advantage in labour productivity for medium and large Swiss farms. Restrictions are that the weed pressure is low or moderate (no additional special herbicides except for total herbicides are used) and if the cultivation system is changed from ploughing to non-plough tillage. Otherwise the labour and financial savings in herbicide use do not compensate the expenditures for coexistence. At all events, large Swiss farms (< 35 ha arable land) with rounded off fields and large-scaled agricultural structure could have the same gross margin and better labour returns in ploughing systems both for conventional and herbicide-tolerant cultures and if the seed price premium does not exceed 30 %.





Thank you to the following sponsors for their generous support of the Co-Extra Final Conference:

