GM and non GM supply chains: Their CO-EXistence and TRAceability

Outcomes of Co-Extra

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

Yves Bertheau (Coordinator of Co-Extra)

INRA, Versailles, France

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), 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[1] 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 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 HGE2. 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.

[1] A seed threshold has not yet been set up at the EU level,

[2] Haploid Genome Equivalent

In conclusion, according to the results of SIGMEA[3] 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 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 1/3rd to 1/10th 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[4] 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).

[3] Sustainable Introduction of Genetically Modified Crops into European Agriculture, FP6 research program.

[4] Identity Preservation

- 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);
- 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 are 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 22005:2007) 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[5]").
  - 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.

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.

[5] Identity Preservation (meaning management of 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[6].

[6] 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[7] (IRMM and IHCP institutes) and of numerous ENGL[8] 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 with suitable reference materials, 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[9]). 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 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%.

[7] Joint Research Center of the European Commission (Geel, Belgium and Ispra, Italy)

[8] European Network of GMO Laboratories.

[9] http://bgmo.jrc.ec.europa.eu/home/sampling_KeLDA.htm

- 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 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 sample to the final measurement outcome is to be validated as a whole and
  - the "modular approach", in which an analytical method is considered as a combination of separate "modules": one for analyte extraction/purification, one or more for the analysis (e.g. PCR). and where each module 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 increase in the number of approved and non-approved GMO result in an obvious need for screening tools[10] for simultaneous detection of different GMOs in a sample in one step. The DualChip® GMO microarray is one of several novel multiplex screening methods developoed within the Co-Extra for the detection and identification of GMO. The DualChip GMO assay is based on the use of several multiplex PCRs followed by hybridization of the amplified DNA to a microarray for the identification of the amplified targets. The validation of this assay was performed within the framework of Co-Extra, according to the ISO 5725 standard. Furthermore due to the complex nature of microarray experiments with many potential sources of variability, a fuzzy-logic validation based approach was successfully applied to the analysis and data interpretation in connection with the assay validation exercise. This type of assay can be used for GMO screening and identification but also, in combination with suitable software building on the "matrix approach", to assess if a sample is likely to contain unauthorised GMO.

The current legal frame resulted in the establishment of the CRL[11] which is responsible for the validation of specific quantitative identification methods provided by the notifiers in connection with applications for authorisation of new GMOs. The validation studies are performed in collaboration with the ENGL[12]. Unfortunately, the current mandate of the CRL is restricted and does not cover validation of other methods applied by the routine laboratories such as screening and multiplex methods needed to decrease the analytical costs and timespan.

[10] Detection targets present in several GMOs.

[11] Community Reference Laboratory, Joint Research Center, Ispra, Italy

[12] European Network of GMO Laboratories, http://engl.jrc.ec.europa.eu/

Most of the analytical controls are made on raw and low processed products while documentary traceability is more predominant for the remainder of the supply chains. The analytical traceability may impact the costs and time required to control products for GM presence; - development costs, lack of appropriate methods, the need for methods to be validated and established for routine use, discrepancies between laboratories applying different methods, different implementation of the methods in accredited laboratories and unclear communication between stakeholders in relation to test reports and interpretation. 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[13] 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 discriminate false positive results in screens, methods to detect sequence donor organisms were developed. One example targets the Figwort mosaic virus, i.e. the donor of the P-FMV which has been introduced into several commercial GMOs.
- 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 products amplified in PCR were also developed; such as assays exploiting capillary gel electrophoresis (CGE-SC) for the separation and identification of PCR products based on length and fluorescent tag (colour). Two real-time PCR multiplex systems are already available as commercial kits and a pentaplex[14] PCR-CGE method for identification of 4 of the most common GM maize lines is subjected to collaboriative trial validation 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 combined 'most-probable-number[15]' statistics and real-time PCR approach, was developed. This approach can yield a 100-fold improvement in the 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[16] identification methods to be used as references for the relative quantification of GMO. The reliability of the relative GMO quantification (expressed in % GMO relative to the reference) was addressed through detailed evaluation of characteristics of reference gene methods. Besides strong relationships with EuropaBio[17] 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 the production and secure supply of independent, cheap and reliable reference materials were searched for and found in the form of plasmids and genomic DNA. To improve the reliability of GMO quantification a guidance document on different options for systematic DNA quality control was prepared. Different real-time PCR technologies (with and without probes etc.), 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. These comparisons did not reveal any significant differences in performance reliability 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 alternatives tested, loop-mediated isothermal amplification (LAMP) combined with a Bioluminescent Assay in Real-Time (BART) detection system is promising. The

[13] GMO identification method

[14] Five (5) PCR in a tube

[15] Statistics based technique used in microbiology

[16] Generally a species like corn. Can be a lower taxonomic level as for instance sugar-beet.

[17] European association of biotech companies.

sensitivity and quantification of this system is similar to that of PCR, but LAMP-BART is less sensitive to inhibitors and cheaper. A 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[18]" 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[19] 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[20] 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 approach usable in routine for detecting the unapproved GMOs, the differential quantitative PCR, was also developed. The attractiveness of this approach is that primarily requires statistical re-evaluation of data that may anyway be available from routine analyses (data from quantitative screening versus identification). This approach is currently subject to collaborative trial validation following the ISO 5725 standard. These two approaches for detection of several unauthorised GMOs can now be implemented in routine laboratories. However, other unauthorised GMOs and in particular those that may be classified as unknown GMOs may require more sophisticated technologies. Such technologies were also explored within Co-Extra, e.g. high throughput mRNA sequencing or analysis of total genomic DNA on high density microarrays without selective amplification.
- 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 authorised in the EU while their stacked counterpart may be unauthorised). 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

[18] For instance: 1 kg of GM soybean in a 40 000 metric tons shipment of non-GM corn.

[19] http://www.bats.ch/gmochips/contact/index.html

[20] Several PCR carried out in the same tube, as opposed to uniplex PCR (1 PCR per tube).

solutions to cope with the challenges posed by gene stacking were also presented in the review. A statistics based detection approach is currently proposed. However, this approach is likely to increase the analytical costs.

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 MD amplification may introduce some bias (i.e. alter the relative copy number ratio of various DNA sequence motifs). Consequently, it is not recommended to use MD amplification for the preparation of (reference) materials for quantitative analyses.
- NASBA[21] 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.
- Direct analysis of genomic DNA via microarray 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. The strategy may therefore be used for detection and characterisation of unauthorised (including unknown) 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 treatments 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.

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

[21] http://en.wikipedia.org/wiki/NASBA_(molecular_biology)

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.

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[22].

[22] Decision Support System

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?
- 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 a 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[23] and GMOchips[24], 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.

[23] http://www.vetinst.no/eng/Research/EU-projects/QPCRGMOFOOD

[24] http://www.bats.ch/gmochips/