The Theory and Practice of European Traceability Regulations for GM Food and Feed

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In the United States, genetically modified (GM) crops are normally considered as being substantially equivalent to the crop from which they are derived. In contrast, in Europe, GM crops are considered as being produced by a new process, with each GM plant requiring a new assessment and authorization procedure. In Europe, the safety of all food and feed is assured by the European Food Safety Authority (EFSA). As part of its duties, EFSA assesses the safety of genetically modified organisms (GMOs) so that all GMOs appearing on the market carry a positive EFSA recommendation and have the approval of the European Commission (EC). Despite these guarantees, the European population remains skeptical or hostile to GMOs. Consequently, the EU has developed the strictest GMO regulations in the world. Under regulations (EC) 1829/2003 and 1830/2003, GM food and feed needs to be traced and labeled above a threshold of 0.9% of adventitious presence of EU-approved GMOs. The official reason for labeling is to provide consumer information and to enable free choice. Labeling is not a food safety issue, since food deemed unsafe by EFSA does not appear on the market. This review considers the difficulties in interpretation, application, and compliance with regulation (EC) 1829/2003 and other GMO regulations, as well as the possible consequences for EU trade with GMO producing countries. It also points out some of the benefits of GMO traceability and detection research and of the coexistence studies on GMO and non-GMO supply chains as carried out under the EC's Co-Extra project.

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- Europe, along with some other countries like Japan, Korea, and Russia, has taken a different path for dealing with GMOs based upon the belief that GMOs require special attention since they are produced by a new and different process.
- Traceability serves to enable the customer to have an informed choice as to what he is eating and may facilitate product withdrawal in case of problems.
- Labeling provides information for consumers and users of the product and allows them to make an informed choice.
- Due to the low pressure level of GMOs in Europe, the current traceability and controls systems have not increased retail prices, but that may change due to the expensive infrastructures and methods necessary for compliance.
- Another important factor affecting food costs is the asynchronous approvals and trace botanical presence of GMOs that are authorized elsewhere, but not in Europe. Such presence may have the expensive consequence of the shipment being returned to the country of origin.

Introduction

The cultivation of GM plants began in the United States more than 10 years ago, and it has since grown enormously in size and economic importance (6,60). It is estimated that, in 2007, about 12 million farmers in 23 countries planted 114 million hectares of biotech crops. Most of these GM crops were grown in the United States, Canada, Argentina, and Brazil, though cultivation is presently rapidly increasing in China, India, South Africa, the Philippines, and Australia (10). Presently cultivated GM crops are mostly limited to herbicide tolerance and insect resistance, though the number of commercialized traits will increase dramatically in the next few years. Similarly, relatively few types of GM crops are presently cultivated (corn, soybean, canola, alfalfa, and cotton), but the future will see the commercial planting of GM rice, wheat, potatoes, tomatoes, sorghum, sugar beet, and many others (23,37,64).

During these 10 years of cultivation and consumption of GM plants, no incidences of negative health effects as a consequence of eating GM food or feed have been reported. In contrast, a variety of positive effects have been documented, such as reduced workload, reduced exposure to dangerous farm chemicals and easier planning for the farmer, facilitated pest control, minimized environmental damage due to the use of low-tilling, reduced herbicide and pesticide applications, and consequently reduced tractor fuel consumption (53). Insect resistant maize shows reduced levels of mycotoxins (fumonisins) as a consequence of a lower level of fungal infection corresponding with the reduced insect damage (61,66). Future GM plants may provide advantages for the consumer in the form of enhanced nutritional value in the form of increased vitamins (vitamin A, vitamin E, and folate) (28), trace elements (zinc, iron), beneficial fatty acids (omega-3), and essential amino acids (lysine [59] or enhanced production of a balanced protein [9]), and reduced allergens (soybean, wheat, and peanuts). A great deal of research is devoted to the construction of GM crops that may provide tolerance to drought and salinity. Transgenic crops may produce human pharmaceuticals in the form of antigens, antibodies, human proteins, and industrial enzymes, though naturally these will require rigorous segregation from crops destined for human consumption (38). Transgenic trees may provide low-lignin raw material resulting in a less polluting paper industry (3), while other transgenic plants may provide for phytoremediation (13) of contaminated soils and ground waters. Transgenic plants in a variety of species are being developed for tailor-made biofuel production. As most of the taxa used to develop such new GMOs are also used for food and feed pur-

poses, these will naturally require rigorous segregation from crops destined for human consumption. A recent U.S. Department of Agriculture (USDA) review, created to consider the future of U.S. agriculture, gives various scenarios as predictions for the future of GMO technology (62).

Curiously, Europeans have developed mistrust in several sectors of science and innovation and have resisted the worldwide trend in GM cultivation (5). Instead, Europeans have become involved in serious, and even violent, polemic about the hypothetical dangers of GM crops, not only in human food, but also for feed for livestock. These include the potential risks of the consumption of meat derived from animals that have eaten GM feed, the potential of horizontal gene flow to related species (e.g., sugar beet and wild beets in Europe), the possibility of the transfer of antibiotic resistance from GM plants to soil bacteria (and even humans), the possibility of risks to non–target insects (bees, butterflies), negative growth effects on GMO-fed rats and worms, and the development of herbicide tolerant volunteers. Most of these potential problems have been shown to have no scientific basis (e.g., Monarch butterflies) or to be avoidable by good farming practice (e.g., horizontal gene flow). Nonetheless, there have been numerous instances of admixing of GMO and non-GMO food and feed, indicating that good management practices are not always followed.

A recent (2006) survey of the Eurobarometer, involving 25,000 citizens from all member states, showed massive rejection of GM food (26). Europe has implemented the most restrictive GM plant legislation in the world, including regulations on GM safety; case-by-case GMO authorizations for import, processing, and planting; GM traceability of GM food and feed; and GMO labeling above a given (arbitrarily determined) threshold for the adventitious presence of GM products. EU regulations even require the labeling of highly processed foodstuffs, in which transgenic DNA or proteins can no longer be detected (e.g., corn-oil, refined sugar, and lecithin). New national regulations, with EC guidance, will treat the coexistence of GM and non-GM food and feed chains and permitted levels for fortuitous GM presence in seeds for planting. The practical implementation of the separation of GM and non-GM food and feed supply chains and of traceability and detection of low levels of GM materials, including legal and socioeconomic aspects, are addressed in the EC integrated project Co-Extra (www.coextra.eu/) (GM and non-GM supply chains: their CO-EXistence and TRAceability), of which one of the authors of this paper, Yves Bertheau, is the coordinator. The purpose of the present communication is to address the difficulties in interpretation, implementation, compliance, and consequences of the EC GM-plant regulations, and also to address possible cost reductions and hidden benefits for the stakeholders.

European GMO Regulations

European GMO regulations have recently been reviewed (14). A summary of these, including the legal texts and a summary of their contents, may be found at the Belgian Biosafety server (www.biosafety.be/) and at Europa (www.biosafety.be/ and http://europa.eu/scadplus/leg/en/s89500.htm). The first European GMO directives were 90/219 and 90/220, for the confined use and the deliberate release in the environment of GMOs. This trend was reinforced in 1997 with "Novel food and novel ingredients" (EC 258/97 regulation), which imposed labeling on any new product including phospholipids from egg yolk, bacterial dextran, and plant sterols to irradiated products or GMOs. After a difficult period for GMOs approvals, the EU tried to fill the gaps between the existing regulations and directives and to develop a set of "one door, one key" regulations and directives.

Directive (EC) 2001/18

The main aim of this directive is to make the procedure for granting consent for the deliberate release and placing on the market of GMOs more efficient and more transparent, to limit such consent to a period of 10 years (renewable), and to introduce compulsory monitoring after GMOs have been placed on the market. This directive provides a general frame for coexistence in fields, such as isolation distances and buffer zones, and often serves as a publicly accessible register of GM crops, etc. The difference between an EC regulation and an EC directive is that the latter needs to be transposed into national legislation. Some member states have still not done this for regulation (EC) 2001/18, and, as a result, they may be subjected to heavy fines. Very recently (June 2008), the French government finally succeeded in transposing directive (EC) 178/2002 into French law.

Regulation (EC) 178/2002

Regulation (EC) 178/2002 (also called the "general food law") concerns the traceability of any product in the EU. It also resulted in the creation of the EFSA and established procedures for food safety. EFSA is responsible for the safety and risk assessment, including environmental effects, of all food (food additives, plant and animal welfare, chemical and biological contaminants, mycotoxins, allergens, etc.), including GM food, feed, seed, and derivatives. Traceability, detection, and labeling of GMOs, which are the main subject of this review, are not part of EFSA's remit. Instead, they are the responsibility of the national enforcement laboratories and of the Community Reference Laboratory for Genetically Modified Food and Feed (CRL-GMFF) with the support of the European Network of GMO Laboratories (ENGL).

Regulation (EC) 1829/2003

Regulation (EC) 1829/2003 (19) requires the labeling of GMO food and feed, including food and feed produced from or containing ingredients produced from GMOs. The labeling requirements do not apply to foods containing less than 0.9% of fortuitous presence of GMOs, provided that this presence is adventitious or technically unavoidable. If the GMO presence is intentional, then the product must be labeled irrespective of the percentage. Processed food requires labeling if it is produced from GMOs, even though it may have been processed to a point where no GM component can be detected (maize oil, lecithin, starch derived products, and refined sugar). In this case, documentary traceability replaces analytical proof, which should decrease the prices of traceability, but could lead to fraud by unscrupulous operators. At this moment (March 2008), animal derived products (e.g., meat eggs and milk) do not require labeling, even if the animals have been fed on GM feed. Some member states, such as Finland, would like to remove this exception, while Germany has legislated a new label, "fed without gene technology." Labels claiming "free of GMOs" are not usually permitted and may, in any case, be risky for the fabricant, since the exact provenance of supplies may not be known, thus exposing him to charges of fraud.

Due to the initial insights from the Fifth Framework Program (FP5) research projects QPCRGMOFood and GMOchips, regulation 1829/2003 has made it mandatory for applicants to provide the CRL-GMFF with quantitative identification methods and control samples for the validation of the detection methods (discussed below).

Regulation (EC) 1830/2003

Regulation (EC) 1830/2003 (20) amends directive 2001/18 and concerns the traceability and labeling of GMOs and the traceability of food and feed products produced from GMOs. It facilitates the monitoring

and checking of the nutritional claims made on labels, the surveillance of the potential effects on human health or the environment, and the withdrawal of products if an unforeseen risk to human health or the environment is identified. This regulation harmonizes the traceability measures with directive 2001/18. However, at this moment, post-market monitoring is quite theoretical.

Regulation (EC) 1946/2003

Regulation (EC) 1946/2003 (21) covers the transboundary movement and relevant documentation for living modified organisms (LMOs) destined for deliberate release or for food, feed, or immediate processing under the terms of the Cartagena Protocol on Biosafety, of which the EU is a signatory. The Cartagena Protocol does not use the EC system of GMO thresholds, but is presently putting in place a system dependent on the separation of GM and non-GM crops by the segregation process known as identity preservation (IP) (35). In the meantime, GMO products are labeled "contains GMOs" if the IP system is already functional or "may contain GMOs" if it is not yet functional. The Cartagena Protocol also resulted in the creation of the Biosafety Clearing-House for biosafety information. Globally, this EC regulation imposes the same food and feed quality requirements for domestic markets and exports.

Regulation (EC) 65/2004

Regulation (EC) 65/2004 establishes a system for the development and assignment of unique identifiers for GMOs in line with the Office of Economic Cooperation and Development (OECD) rules.

Regulation (EC) 641/2004

Regulation (EC) 641/2004 (48) lays down implementing rules for the authorization of GM food and feed under regulation 1829/2003. It clarifies what information and data have to be provided to support applications for the authorization of new GM food and feed and the notification of existing products.

The World Trade Organization Dispute

Since 1999, Europe has practiced a de facto moratorium on GMO, where no approvals for new GMOs were granted. Though this ban has recently been lifted, and some GMOs recently approved, there remain problems within the EC and with member countries (see below). The United States, Canada, and Argentina viewed this moratorium as being without a scientific basis and as being a simple trade barrier based on economic reasons. The case was presented to the World Trade Organization, who largely judged in favor of the plaintiffs (58,65). EC is presently (March 2008) beginning its second extension of the deadline for compliance. The effect of these decisions on transatlantic trade is difficult to predict at this moment.

The European GMO Risk Assessment and Authorization Procedure

EFSA is responsible for all food safety and risk assessments, including GM food and feed. GM food and feed must have a positive safety EFSA assessment before it can be imported, cultivated, processed as food or feed, or marketed (the one-key, one-door policy). In Europe, the company that has developed the GMO plant and intends to cultivate it or use it as food, feed, or in processing must first submit its application to the national competent authority, who must then pass the documents to EFSA. EFSA then has six months in which to produce a report, which it then submits to the EC and member states. Within three months of receiving the recommendation, the EC must prepare a decision for accepting or refusing the report (refusal must be justified). The EC then submits its recommendation to the Standing Committee on the Food Chain and Animal Health and eventually to the Council of Ministers, which has 90 days to reach a decision. In reality, no decision by a qualified majority is ever reached by this body and so the final approval reverts, by default, to the EC, which then invariably approves the product in agreement with the EFSA recommendation. The approved product is then written on the community register of GM products established in accord with regulation (EC) 1829/2003. In parallel, the detection method has to be provided to the CRL-GMFF, as discussed below under GMO detection.

Approvals of GM food and feed are published on the EFSA website (www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_home.htm) and the EC Rapid Alert System for Food and Feed (RASFF) bulletin (http://ec.europa.eu/food/food/rapidalert/index_en.htm) permits stakeholders to retrieve information on GMO legislation and food safety issues.

The authorization procedure, described above, is slow and cumbersome and highly subjected to the political whims of the day. At present (March 2008) the authorization process is in complete political disarray. Thus, while EU Environment Commissioner Stavros Dimas has recently stated that he will not grant certain (Bt11 of Syngenta and TC1507 of Dow/Pioneer) GMO authorizations, German Minister of Agriculture Horst Seehofer has called for an absence of political interference in the authorization process (as is already the case with pharmaceutical products), while, curiously, calling for a (temporary?) ban on new authorizations. This latter position was in turn criticized by EU Agriculture Commissioner Mariann Fischer Boel, who warned against a new moratorium on authorizations and called for EFSA recommendations for authorization of new GMOs to be accelerated. The antiscience attitude of NGOs, retail companies, and many politicians, as well as its effect on European competitivity, was severely criticized by the EU Trade Commissioner Peter Mandelson (40).

In the meantime, some European member states, such as Austria, Hungary, Poland, Italy, Greece, and recently France, have independently declared a moratorium on the planting of GMOs, invoking the so-called safeguard clause (46). Commissioner Mariann Fischer Boel has pointed out that this is illegal in the absence of new scientific evidence regarding the health or environmental safety. She added, "We cannot simply ban all GM crops from an entire region because of hostility to GM products per se. Where a product has been shown not to be harmful, the rules of the free internal EU market apply." The Polish GMO ban has recently been pronounced illegal by the commission, which may impose heavy fines for noncompliance.

Recently, France dismantled the science-based Commission du Génie Biomoléculaire (Biomolecular Engineering), which was in charge of the environmental deliberate release of GMOs, the Comité du Génie géntique (Genetic Engineering), in charge of the confined use of GMOs, and the temporary Comité de Biovigilance, in charge of postmarket monitoring, and replaced them all with a temporary group named the Comité de Préfiguration d'une Haute Autorité Sur les OGM, whose duties were to determine the compositions and rules of the future committee and to study the particular case of GM maize MON810. At a press conference, the senator chairing the committee claimed that there were "serious doubts" about the safety of GM-maize MON810 (the only GM crop cultivated commercially in France), and the French government thereby invoked the safeguard clause banning its cultivation. This conclusion and decision have been strongly criticized as being political and without scientific basis, both by the French Association for Scientific Information (AFIS) and by 14 of the members involved in the Comité (whose report does not contain the words "serious doubts"). The French action has

also been condemned by the French National Farmer's Union, FNSEA, and the European Association of BioIndustries (Europabio). EFSA has upheld its assessment that MON810 poses no risk. France had been warned in advance, by EU Agriculture Commissioner Mariann Fischer Boel, of the consequences of its actions, which would be illegal under EC regulations. Clearly, such mixed messages, at high levels, do not help restore consumers' confidence in GMO technology. Following the recent transposition of regulation (EC) 2001/18, the French government has elected a Haut Conseil (High Council), which seems to have the same composition and the same duties as the previous disbanded committees.

Labeling of GM Food and Feed in Europe

Under regulation 258/97, any novel food or novel ingredients have to be labelled. Similarly, under regulation (EC) 1829/2002, food containing greater than 0.9% of adventitious presence of GMOs must be labeled as containing GMO with the phrase "This product contains genetically modified organisms." There is a common, but intuitive, misconception that this label serves as a warning with respect to food safety. This is false, since, as explained above, food safety is assessed by EFSA and food does not appear on the market without a positive EFSA food safety evaluation. Thus, unsafe food and feed does not require labeling since it cannot be imported, cultivated, or sold in Europe. Indeed, GMO labeling, GMO detection, and GMO traceability are not part of EFSA's duties. The official EC explanation for the enigma is that the regulation on labeling is not concerned with food safety, but instead provides freedom of choice to the consumer as to which kind of food he or she wishes to eat. "Labeling provides information for consumers and users of the product and allows them to make an informed choice" (17,25). Globally, the traceability requirements decrease the recalls of products (not only GMOs), facilitate their withdrawal off the market, and improve the quality and safety of marketed products. Naturally, however, the message that reaches the consumer via the highly active anti-GMO organizations is that labeling is a warning of unsafe food (which they term "Frankenfood"). In reality, the European retailers refuse to sell GM food, so that virtually no products labeled GMO can be found; and thus there is no possibility of "informed choice."

Regulation (EC) 1829/2003 refers to the labeling threshold of 0.9% for adventitious presence of GMOs in food or feed. It should be noted that the producer must demonstrate each time that the presence of GMOs is really accidental and that all precautions were made to avoid admixing. Recently, this was also applied to organic food, which is no longer considered organic above the level of 0.9%.

The 0.9% threshold is simply a number devised for political reasons (economic costs of GMO detection and psychology of consumer acceptance) and has no scientific basis (45). In other countries with a GMO threshold the number may be different (5% in Japan, 3% in Korea, and recently 0.9% in Russia). The U.S. Food and Drug Administration (FDA) takes a different attitude to that of the Europeans, judging GM food according to whether it is (or is not) substantially equivalent to that of conventional food or feed. If this is determined to be the case, then no further action is needed other than the overall safety requirement, and it can be rapidly deregulated. Thus, GM food and feed is not labeled (or only on a voluntary basis) in the United States and many other countries. In contrast, Europe begins with the premise that GM and non-GM food and feed are made by a different process and thus must be distinguished by legislation.

Regulation 1829/2003 also specifies that the threshold 0.9% is per ingredient so that, for example, a non-GMO batch of maize mixed with a tiny quantity of 100% authorized GM-soybean would require labeling. This point is discussed below in the section on mixtures of GMOs.

European regulations take into consideration the documentary traceability as a support for control. This means that the economic burden of analytical controls can greatly be decreased by using traceability documentation, permitting analytical controls to be applied to critical points of the supply chains.

Detection of GM Food and Feed in Europe

Under Regulation (EC) 1829/2003, the applicant company that requests the authorization of a GM plant for cultivation, food, feed, or processing must provide, in addition to the EFSA application document, a method of detection of their GM plant as well as control samples (reference material for validating the method). Reference material for the Institute for Reference Materials and Measurements (IRMM) is needed for preparing international certified reference materials (CRM). This regulation relieved the burden on regulators, detection laboratories, and research institutes (e.g., in the EC projects QPCRGMO-Food (www.vetinst.no/eng/forskning/eu_prosjekter/qpcrgmofood) and GMOchips (www.bats.ch/gmochips/), which were previously required to elaborate effective detection methods without aid from the applicant company). The control samples and the detection methods are verified (compliance to performance criteria and appropriate prevalidation data) and validated by CRL-GMFF (which charges the applicant company for this task) in collaboration with ENGL. Validation of the detection method is through collaborative ring trials within the network of enforcement laboratories and is a necessary requirement for GMO approval. Once authorized, the detection methods are then published on the CRL-GMFF website (http://gmo-crl.jrc.it/statusofdoss.htm). From all of this, it follows that the GMO authorization procedure is slow, cumbersome, and expensive, such that only the largest international biotechnology companies are able to afford it.

While protein-based detection methods are useful for rapid screening, they do not have the sensitivity necessary to comply with the quantitative requirements of the European labeling regulations. In addition, since they are based upon antibody-based detection, they require the presence of the protein antigen and do not provide information on the nature of the genetic cassette in the GMO (for example, protein based methods are unable to distinguish the EU authorized Bt11 maize from the unauthorized Bt10).

In contrast, qualitative and quantitative real-time polymerase chain reaction (QRT-PCR) provides a highly sensitive quantitative method for determining low levels of DNA due to the adventitious presence of GMO in a sample (30,43). Furthermore, since it directly detects DNA, QRT-PCR functions equally well with nontranscribed regions such as promoters and terminators, thus satisfying the requirements of specificity. QRT-PCR has the further ability to identify individual genetic constructs by targeting the site of insertion of the transgene in the genome; this being unique for each transformation event. In the EC, all validated methods of detection are based upon QRT-PCR, with which the absolute limit of detection is in the order of five copies and the absolute limit of quantification is about 100 copies. The relative limit of quantification is almost 0.1%.

The percentage of the GM-DNA sequence in a sample can be rapidly determined using QRT-PCR by simultaneously amplifying a standard reference gene known to be present in that taxon. For example, for maize, the gene Adh, developed by INRA, is often used. However, the classical taxonomic classifications do not always correlate with the reference genes.

For instance, using reference genes, it is currently impossible to distinguish sugar-beet from fodder-beet and other subspecies. Similarly, rapeseed (*Brassica napus*) cannot be distinguished from *Brassica rapa* and *Brassica oleracea*. There is, thus, a clear need of harmonization of the reference genes for improving the reliability of the reference systems and for reducing the costs and duration of methods development, validation, and accreditation. Within the Co-Extra project, a task force, including all stakeholders (EuropaBio, ISTA, and research scientists), has been launched with the aim of developing an open "core collection" of crops with appropriate varieties (inbred and commercial varieties) and related taxa used for introgression programs as well as related species (e.g., fodder beet, chard, etc., versus sugar-beet).

The results of GMO detection analyses are currently given according to the recommendation (EC) 2004/787, which proposes that GM copy numbers should be expressed as the percentage, in relation to taxon-specific gene target DNA copy numbers, calculated in terms of haploid genome equivalent (49). However, this definition does not have total consensus among member states and ENGL members and does not satisfy the seed producers, where it will have considerable impact on the seeds' threshold, particularly in the case of stacked genes.

In Europe, some detection methods now use the modular approach, whereby analytical modules (DNA extraction procedures, PCR reactions, reference genes, etc.) are developed, standardized, and verified independently (31). This results in greater homogeneity and transportability of the results and time/cost savings, even though it is still the subject of controversy.

Statistical methods help in reducing measurement uncertainty, by, e.g., detecting the inhibiting effects of some samples and by taking into account all the data of calibration curves (4,15). As part of the deliverables of the EC Co-Extra integrated project, a statistical program called OPACSA is now freely available. This is a robust and optimized statistics-based approach of multiple control plans by attributes for improving accuracy and time/cost effectiveness of qualitative methods (11). The new fuzzy logic statistics packages that have been produced for the analysis of the DualChip (discussed below) screening method can be used for the validation of other multi-step detection methods. A current trend in analytical methods development is the growing number of qualitative methods such as micro-array hybridization and SNPlex (described below). We can, thus, expect that control plans with multiple attributes, a very powerful statistics based strategy, will be increasingly used to complement quantitative methods.

Difficulties in Interpretation and Implementation of Regulation (EC) 1829/2003

Regulation (EC) 1829/2003 seems to have been written with little forethought or attention to the limits of scientific techniques or cost/benefit analysis. It, thus, raises numerous issues that have been the subject of discussions at standardization committees and ENGL.

Sampling from a Large Batch

The purpose of sampling is to obtain samples that are representative of the lot from which they are derived (51,52). Maize or soybean shipments arriving in Europe from North or South America contain mixed grain from many different farms, transported in trucks and rail-cars and ships. Consequently, there are multiple potential sources of admixture with GM grain, and this mixture may contain several different GMOs of different origins, in different proportions, and heterogeneously distributed in the cargo. When the cargo is very large (40,000 tons may take 2–3 days to off-load from the ship), representative sampling for GMO analyses becomes a major problem. The EC GMO recommendation 2004/787/EC (50) on sampling issues is scientifically sound but poorly implemented due to its costs and complexity. This problem is largely unresolved, particularly since good sampling plans can be very expensive, while the consequences of using poor sampling plans may be legal disputes and commercial losses. Sampling plans are still being discussed within Co-Extra, ENGL and the Codex Alimentarius. Similarly, there is no consensus plan for field sampling, even though some have been proposed (42).

GMO Labeling Threshold

Regulation (EC) 1829/2003 does not define the meaning (units) of the 0.9% threshold. Following an EC recommendation, detection laboratories work in DNA copy numbers (which is measured by the QRT-PCR reaction defined above), while seed producers and farmers work in mass. A major difficulty arises in the conversion of DNA ratios to mass ratios, and this has been frequently discussed in ENGL and in the scientific literature (12,22,32,63). There is no evident relationship between the DNA content and the mass of a plant. Identical plants growing under different environmental conditions and in different stages of growth and maturation exhibit different DNA/mass contents. Different varieties of the same plant species exhibit different DNA/mass ratios. Different parts of a plant may differ in genome content; e.g., in maize kernels, the endosperm is triploid (having 2n from the ovule donor and 1n from pollen donor), the embryo is diploid (with equal contribution from each parent), while the integument is diploid (with both genomes derived from the female). Thus, the DNA/mass ratios are different according to the direction of fertilization; whether the GM plant is the pollen donor or the recipient. Recommendations to improve the coherence of GMO legislation are currently under discussion at ENGL. As stated above, the haploid genome equivalent (HGE) definition of DNA copies recommended by the (EC) 787/2004 (50) will drastically impact the future seed threshold, particularly as more stacked genes will be commercialized.

Certified Reference Material

A partial, but inexact solution to the problem of conversion of units of measure is provided by the use of CRM. CRM is produced by the IRRM/JRC and is a mixture of GM and non-GM material in certified proportions of mass (36). In the absence of the difficulties discussed in the previous section, the use of CRM in the QRT-PCR reaction permits the calculation of a standard curve, to which the DNA estimations may be applied, resulting in indirect mass/mass ratio determinations. The CRM are usually in the form of ground powder and may pose problems of high cost and instability. There may also be problems of availability since certain GM crops (e.g., Bt176 and Starlink) are no longer grown or commercialized, yet may continue to be present at low levels in shipments. Although the ΔΔCt method allows quantifying the relative content of a GMO without the need of calibrants, CRM are still necessary, at least as positive and negative controls.

A probable end result will be to replace plant-based CRM by plasmid-based CRM carrying a variety of transgene DNA inserts. These would have the advantage of low price and continuity, but may increase problems of PCR contamination. Such plasmids have already been produced in Europe, Japan, Korea, and Taiwan, and conversion factors giving mass/mass ratios have been elaborated (33,34,39,41,54,55). Accordingly, the IRMM/JRC started, over the last few years, the validation of plasmid-based CRM to ascertain the commutability of reference materials and recently released its first commercial plasmid-based CRM.

Screening for GMOs

Regulation (EC) 1829/2003 and (EC)

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appeared here in the printed version of the journal.

1830/2003 are concerned with the quantification of the percentage of GMO in a sample. When the GMO content is unknown, this may be a difficult and costly task since each set of PCR primers detects a specific DNA sequence (related to a transgene, a promoter, an edge fragment, etc.), but gives no information of other different GMOs. As the diversity of cultivated GMOs increases, the number of necessary primers and amplifications also increases. One solution, not taken into account by the EC regulations, but developed within the Co-Extra project, is the development of screening methods to obtain a rapid assessment of the kinds of GMO that are likely to be present in a sample. This may be achieved by multiplex qualitative PCR reaction for the simultaneous processing of multiple primers. The products may then be identified according to their size by gel electrophoresis or may be separated by capillary electrophoresis and identified by their size or color, when a colored fluorescent label has been added to the primer (44,57). An alternative approach is based on DNA chip hybridization. Under the Co-Extra project and its predecessor, the GMOchips project, a new method of multiplex screening (called DualChip, Eppendorf Array Technologies, Belgium) has been developed and will be commercialized (29). Multiple specific DNA capture probes, corresponding to GMO elements, border fragments, or species-specific targets or control targets are immobilized on glass slides. The different GMO DNA elements present in a sample are determined by DNA hybridization, followed by a colorimetric reaction, and a user-friendly fuzzy logic software called AMPE, used for detection and identification. The reliability of the method was validated by a collaborative ring trial involving several participants of the Co-Extra consortium and showed that target DNA could be detected to a level of 0.1%. The method has great potential for cost/time efficient screening in private and regulatory laboratories. It has the ability to accommodate a large number of target gene sequences, which will be needed due to the expected increase in GMO traits and new GMO crop species.

All of these qualitative PCR combined (or not) to micro-array hybridization are used in the "matrix approach," whose principle was described in the GMOchips European research project by Yves Bertheau. The matrix approach is a strategy/decision system, which can be used in several methodologies such as PCR, PCR with microarray hybridization, SNPlex, etc. Besides its ability to identify GMOs, the matrix approach could be used, with control plans by multiple attributes, for determining the relative GMO content relative to a prefixed threshold. Finally, the matrix approach can also be used for suspecting the presence of unknown GMOs (see below).

Mixtures of GMOs and Trace Botanical Impurities

Mixtures of GMOs in a non-GM batch are detected by the same QRT-PCR methods described above, and the prior identification of their GMO contents is facilitated by screening methods with taxa reference systems. The threshold for labeling applies to each ingredient (taxon), which individually must not exceed the level of 0.9% adventitious presence. Thus, a cargo of entirely non-GMO maize admixed with a trace (e.g., 0.1%) of 100% GM soybean would require labeling, which would reduce the value of the cargo. As a result, there may be a tendency, particularly in the case of feed, to add non-GM soybean to reduce the percentage GMO, though this is a costly procedure. Since the number and percentage of GM-crops is rapidly increasing and trace botanical impurities are likely to become more frequent, numerous stakeholders are requesting more tolerance for the presence of trace botanical impurities, and this is still being discussed at the Directorate General for Health and Consumer Affairs (DG-Sanco) and the Codex Alimentarius. Indeed, even the non-GM CRM provided by the IRRM may contain very low levels of other GMOs. Co-Extra is addressing how to manage low-level botanical impurities.

It again must be stressed that the 0.9% threshold for labeling applies to EC approved GMOs. GMOs nonapproved in the EC cannot be accepted at any level (which in practice means at the limit of detection of the methods). One recent, and extremely costly, case involved trace admixing of the unapproved long-grain rice by LLrice601 (Bayer CropScience) with the non-GMO rice Cheniere. The USDA has since deregulated rice LL601 and it has lately received EFSA approval. A new issue recently arose with the U.S. cultivation of several thousand hectares of unauthorized event-32 maize of Dow Agroscience.

Stacked Genes

Stacked GMOs are usually produced by natural crossing of two single GMOs; for example, insect resistance and herbicide tolerance (Taverniers, I., personal commun.). Double and triple stacked GMOs are now common and higher combinations (up to eight-stacked genes as part of an agreement between Monsanto and Dow AgroSciences) are possible in the future. From the European regulatory point of view, these stacked genes are considered as new GMOs and thus should be distinguishable from mixtures of unique events. However, stacked GMOs pose a problem for detection since they cannot easily be distinguished in a cost effective manner from a simple mixture of GMOs. Recently, one laboratory succeeded in doing this by the laborious and expensive expedient of analyzing individual maize kernels (1). Stacked genes may also be detected by analysis of seed by pools (subsampling or control plans by multiple attributes) by qualitative PCR, followed by statistical analysis (2, Ancel, V., personal commun.). The CRL-GMFF pragmatically accepts the difficulty of distinguishing stacked GMOs from mixtures and simply requires tests that identify individual GMOs; a solution which may be considered as not complying with the spirit of European regulations.

Unauthorized GMOs

Regulation (EC) 1829/2003 requires that the biotechnology company wishing to commercialize a GMO provide a method of detection that is subsequently validated by the JRC-CRL-GMFF. This method of detection is subsequently used as a basis for GMO detection by private and government regulatory control laboratories and then for labeling. In contrast, unauthorized GMOs are not tolerated at any level, but detection methods for these are unavailable under EC regulations. Furthermore, Europe has no legal grounds for requiring the detection methods for GMOs that are part of the intellectual property portfolio of the biotechnology company, which may have no intention of commercializing them. The illogicality of this situation is that Europe thus possesses GMO detection methods for GMOs that it has authorized as safe (and thus are legal), but not for those it has not authorized (and thus are illegal, with zero tolerance). An ENGL working group is currently developing guidelines on the issue of unknown and unapproved GMOs. In some cases, the EC has been able to obtain such information from the biotechnology company, such as in the case of nonauthorized LLrice601 (Bayer Crop Science) and Bt10 maize (Syngenta), both of which were found in shipments destined for Europe. In some other cases, like the Chinese Xianyou Bt63 rice, reference material and specific detection method are still lacking. The recent admixture of DowAgroscience Event-32 maize also necessitates a new detection method.

Unfortunately, nonauthorized GMOs do accidentally enter the food and feed chains by a variety of different pathways, such as

physical mixing during transport or storage, human error, volunteer crops, and gene-flow via pollen. For example, consider the Starlink corn incident, where GM-corn destined for animal consumption contaminated corn destined for human consumption as a result of physical mixing after harvesting. In 2002, Prodigene corn (a GMO producing a pig vaccine) grew as volunteers in a field of soybean, which was then harvested and sent to the grain elevator. The USDA destroyed 500,000 bushels of soybean and Prodigene was fined $250,000 and ordered to provide a trust fund of $1 million. In 2005, Syngenta mistakenly produced and sold a limited quantity of Bt10 corn (instead of the approved variant Bt11). All Bt10 plants were destroyed and the seed stock quarantined by the Animal and Plant Health Inspection Service (APHIS). The U.S. Environmental Protection Agency (EPA) and USDA nonetheless considered that there was no health or environmental risks associated with Bt10, which is very similar to the authorized Bt11 but has a complicated transgene rearrangement. Despite these precautions, some Bt10 corn found its way to Europe and Japan, where it was refused entry. In 2006, imported noodles bought in Asian stores in Europe were found to test positively for Chinese insect-resistant transgenic rice, Xianyou Bt63. In 2006, Bayer Crop Science found that trace amounts of an experimental variety of GM rice (LLrice601) had contaminated non-GM long-grain Cheniere rice. A further study found traces of another GM rice (LLrice604) in the long-grain rice variety, Clearfield 131. After a long study, the USDA was unable to determine the source of these contaminations. The USDA/APHIS quickly deregulated LLrice601, which contains the glufosinate herbicide tolerance gene already present in other varieties of GM-rice. Similar reports by EFSA, as well as food safety authorities in other countries, suggested the probable food safety of this LLrice601. Nonetheless, the crisis severely compromised U.S. long-grain rice exports to the EU. The USDA has a special web page devoted to the history of cases of noncompliance (www.aphis.usda.gov/biotechnology/compliance_history.shtml).

Unknown GMOs

Similarly to unauthorized GMOs, unknown GMOs are not tolerated at any level in the EU, and yet have no known detection method. However, logically, they must be detected, since they are illegal under EC law. An ad-hoc ENGL working group is currently preparing a document on this subject. Several new approaches to unknown GMO detection are being developed under the Co-Extra project. Quantitative differential PCR quantitatively determines the ratios of different genetic elements common to several GMOs and compares this to the absolute number expected for known GMOs. When this difference differs statistically from zero, then the presence of an unknown GMO is indicated (8). The matrix approach simultaneously tests for the presence of a large number of possible DNA sequences and compares the resulting combinations to a database of known GMOs (Chaouachi, M., personal commun.). The presence of unusual combinations of DNA targets indicates a possible unknown GMO. This approach is used in the DualChip (above). An extension of the matrix approach involves the hybridization of total genomic DNA to high density micro-arrays carrying 25 nucleotide probes (47,56). Although quite expensive, this methodology might be used in specific cases, for instance when safety issues may be raised.

All of these strategies involve some knowledge or guesswork as to which sequences are likely to be present. No method, except perhaps total genome sequencing (which is impractical), can detect GMOs that are constructed of entirely new genetic elements. It has to be kept in mind that these methods take time and are expensive. This may be a problem for enforcement laboratories with a constant budget or being paid for analysis according to a fixed price. A careful cost-benefit analysis has thus to be performed before starting a search for unapproved and unknown GMOs.

The Economics of Asynchronous Authorization

In view of the constant problems of asynchronous authorizations in GMO producing countries, the EC's zero-tolerance policy for non-authorized GMOs may become difficult to sustain. Due to the slow EC authorization process there is always asynchronous approval of GMOs, such that a particular GMO may be legal in one country but illegal in the EC. Asynchronous authorization poses a real economic problem for GMO-producing countries, such as those in North or South America, since shipments containing traces of unauthorized GMOs will be refused entry into Europe. The growing number of GMOs produced in developing countries such as China may rapidly raise worldwide concern. The issue of synchronous approval and low-level presence cannot be resolved by individual countries and the United States is currently proposing discussions under the auspices of the Codex Alimentarius.

Europe depends heavily on imported soybean for animal feedstock and strict application of regulation (EC) 1829/2003 would affect these imports and may result in serious economic damage and food price rises. EU Agriculture Commissioner Mariann Fischer Boel has commented that preventing adventitious contamination is technically very complex and expensive. Europe obtains 85% of its soybean imports and 45% of its maize imports from the United States, Canada, Argentina, and Brazil. European meat production in Europe is dependent on massive feed imports. A recent EC Directorate General for Agriculture (DG-AGRI) report simulated various possible scenarios arising from the presence of low-level quantities of unauthorized GMOs (24). Other reports reached similar conclusions (7). A shortage of feed in Europe could result in a crisis in the meat and poultry sectors, with severe price rises. This situation is likely to be compounded by the present biofuel enthusiasm in the United States, whereby it may be more economical to use surplus maize for ethanol production than to export it.

Future Developments in GMO Regulations

Coexistence of GMO and Non-GM Supply Chains

Europe member states must develop national regulations governing the coexistence of GM and non-GM crops and supply chains, which is one of the main outputs of the EC Co-Extra project. EC guidelines have recently been published for the coexistence of GMOs and non-GMOs in the food chain (18). These guidelines will provide a basis for national regulations by the relevant national competent authorities, though some member states would prefer a harmonized European approach. Similarly, the EC has recently created a "coexistence bureau" specific for coexistence issues, at JRC-IPTS, Seville, Spain. Coexistence is not a safety issue but strictly an economic question for the commercialization of approved crops.

As of March 2008, few European countries have coexistence regulations already in place (16). One of the first was the Netherlands, in which minimum separation distances have been set for potatoes, sugar beet, and maize. For GM fields adjacent to conventional fields, the separation distances are 3 meters for potatoes, 1.5 meters for sugar beet, and 25 meters for maize (27). If the GM field is adjacent to a field with a certified GM-free crop (as is the case for organic farming), the minimum separation distances increase to 10 meters for potatoes, 3 meters for sugar beet, and 250 meters for maize. Germany recently legislated

a 150 meter distance of isolation for conventional crops and a 300 m distance of isolation for organic crops. Spain is one of the few EU countries growing GM crops commercially, and in July 2006, it presented the second version of the draft of the Royal law on coexistence, which recommends the obligation of a minimum isolation distance of 220 meters. This was in contradiction to a scientific report ordered by the Ministry, which had indicated that a distance of 20–25 meters, or 4–6 rows, should be used as a barrier to keep the adventitious presence of GMO below 0.9%. In contrast, parts of Austria have developed a fervent anti-GMO attitude and banned every GMO plant authorized by the EU. The EC considers Upper Austria's coexistence regulations to be against EU policies on GMOs. The case is pending at the European Court of Justice. Recently, France has similarly banned the planting of GM crops in defiance of EU regulations.

It is clear that many coexistence measures (separation distances, planting non-GM buffer strips, cleaning of equipment, varying times of planting, and liability and redress [next section] will result in an additional burden of cost and labor for the farmer. Thus, the decision to plant GMOs depends on whether the benefits of the GMO crop outweigh the additional costs. The future will show whether GMOs or non-GMOs will gain the upper hand, though regions with EC registered food quality labels will still probably refuse the introduction of GMOs. It is interesting to note that, so far, no cost-benefit analyses (environment, economic, social with employment, etc.) have been performed for comparing regions cultivating GMOs versus orthodox crops.

Liability and Redress

Liability and redress, whereby a conventional or organic farmer may claim damages for economic loss from an adjacent GM-farmer, represents a major stumbling block in the coexistence of GM and non-GM supply chains. In Europe, no member state has successfully solved the issues of burden of proof and assurance. In Austria, the burden of proof is upon the GM-farmer to provide evidence that he was not responsible; a case of being guilty until proved innocent. This is, in effect, a way of discouraging GMO planting while still superficially respecting EC regulations. In contrast, some member states established the burden of proof on the shoulders of the non-GM farmers. In Spain, which is the very first European country to extensively cultivate GMO, few cases of liability and redress have been documented. The issue of liability and redress may also arise in cross-border disputes between countries. Outside of Europe, this issue is dealt with under the Cartagena Protocol, though no agreement has yet been reached.

Thresholds for Seeds for Planting

There are presently no agreed legal thresholds for the adventitious presence of GMO in seeds for planting. Several recommendations were made in 2001 by the Plant Scientific Committee, but these recommendations did not take several issues into account, such as sampling and measurement uncertainties or the current practices of stakeholders who are using a value of 0.1% of fortuitous presence in non-GM products. Accordingly, a new set of consultations has recently been started in the EC.

For maize, some coexistence reports considered that 0.5% could be easily maintained without additional effort, while 0.3% would increase costs by about 20% and 0.1% is unfeasible. It is clear, however, that coexistence cannot be achieved without regulations on seed thresholds, since pollen flow is strongly distance dependent and thus is much more efficient within a field than between fields.

Future Changes in EU GMO Regulations

Although the new set of European regulations was designed for establishing a one door one key system, the current legislation is far from simple. As indicated above, there are doubts that EU can sustain its zero tolerance policy for the low-level presence of GM-plants asynchronously authorized in other countries (particularly those in North and South America, but also now from China). There are fears that this policy will result in substantial price rises for meat and poultry (as is already happening). Furthermore, the EU only has detection methods for EC authorized GMO and in any case these methods cannot measure very low quantities of admixture.

However, it is foreseeable that the release of GMOs by emerging countries such as China will induce a change in the worldwide cooperation on detection methods, since countries like the United States and Canada will also face the need of detecting unapproved GMOs. A worldwide agreement on asynchronous approvals and thus on safety assessments should be sought as soon as possible to avoid trade barriers.

Conclusion and Perspectives

Europe, along with some other countries like Japan, Korea, and Russia, has taken a different path for dealing with GMOs and is considered by some observers as having burdened itself with excessive and costly regulations based upon the belief that GMOs require special attention since they are produced by a new and different process. In contrast, the regulations in the United States are simpler and more flexible in that they are based on the concept of substantial equivalence of the GMO with the parent plant.

Part of the traceability costs for GMOs are due to a general traceability requirement (regulation 178/02), which is not specific for GMOs. Several GMO detection methods may be used in other fields of food safety, such as their use for other purposes such as organisms producing mycotoxins or allergens; instead of immunological methods. Similarly, the detection of multiple antibiotic resistant bacteria resembles the detection of stacked genes.

Due to the low pressure level of GMOs in Europe, the current traceability and controls systems did not increase the retail prices, since raw products prices are generally only a small part of the final prices. However, the increase in GMO products may change the situation and result in a price increase for both GM and non-GM consumers due to the expensive infrastructures and methods necessary for compliance. Certainly EC regulations could economically favor countries such as Argentina (to the detriment of the EC farmer), whose vast surfaces of arable land make it possible to produce hard IP crops with an extremely low level of GMO, thus conforming to EU regulations.

However, another more important factor affecting food costs is linked to the asynchronous approvals and trace botanical presence of GMOs that are authorized elsewhere, but not in Europe. There is a worry, expressed by EU Agriculture Commissioner Mariann Fischer Boel, that Europe will refuse GM food and feed that it desperately needs because it is admixed with trace amounts of GMOs that are legal elsewhere. Thus, the problem of asynchronous approval becomes of major importance since a shortage of food and feed could result in large increases in the price of meat and poultry. This problem will likely worsen, as the diversity of cultivated GMOs continues to increase and new actors (such as China) enter the domain.

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