

Review article

Contributions from scientific research to the risk assessment of GMOs. Lessons learned from a symposium held in Brussels, Belgium, 21–22 October 2010

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INTRODUCTION

In many domains, risk assessment is a defined methodology aimed at determining and quantifying risks associated with a specific activity, including the identification and characterization of potential hazards and their probability of occurrence. It relies on the gathering and interpretation of scientific and technical data and must be carried out in a scientifically sound and transparent manner. With regard to the use of genetically modified organisms (GMOs), risk assessment relies primarily on the results of research and studies aiming at evaluating the potential impacts of GMOs on the environment and/or human health.

Given the increasing number of GMOs tested or commercialized worldwide and the development of new types of GMOs, new and up-to-date scientific information generated by basic or applied research in various fields can strengthen the risk assessment and/or provide answers to potential knowledge gaps identified during risk assessment.

The gathering of data obtained from scientific research relevant to the risk assessment, as well as the implementation of new research activities resulting from the identification of potential knowledge gaps, would be facilitated by regular exchanges of views between experts involved in risk assessment/evaluation and scientists involved in basic or applied research (from public institutions or companies). As this interaction could be of mutual benefit and could also serve to inform competent authorities and policy-makers, platforms bringing together these communities are considered of relevance.

Within this context, a symposium on “Contributions from Scientific Research to the Risk Assessment of GMOs” was held on 21–22 October 2010 in Brussels, Belgium. The objective was to gather people that are interested or involved in biosafety-related issues and whose expertise may contribute to a better evaluation of the potential risks associated with the use of GMOs. Several speakers were invited to provide some recent results in different domains of research and share their views on how scientific research and developments can give new insights or contribute to the risk assessment of GMOs. Within two days, the meeting brought together more than a hundred participants from academia, advisory bodies and biotech companies from fourteen different countries. By hosting an audience with this particular variety of scientific background, expertise and perspectives, a joint and multidisciplinary forum was created, enabling a fruitful exchange of information, thoughts and views. The program of the symposium consisted of five sessions of three to four oral presentations, highlighting the role of scientific contributions to risk assessment of GMOs (session 1) and emphasizing more particularly on research conducted with GMMs used as vectors for gene delivery (session 2), the relevance of epigenetic changes and effects in alterations in gene expression (session 3), the use of GMO field studies in environmental risk assessment (session 4) and the usefulness of generating profiling data (session 5).

This paper highlights some of the main points raised throughout the presentations and discussions. The analyses of the oral presentations do not necessarily reflect the views of the invited speakers of the symposium. Abstracts of the presentations are available at the following URL: <http://www.biosafety.be/ERA2010/abstracts.html>.

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SUMMARY AND ANALYSIS FROM THE DISCUSSIONS

Scientific contributions to the risk assessment of GMOs

Risk assessment of GMOs aims at identifying and evaluating their potential adverse effects on human health or the environment. It relies on internationally accepted basic principles and concepts and is performed on a case-by-case basis according to a step-by-step approach (NIH, 1976; OECD, 1986; SCBD, 2000). Risk assessment is also performed on the basis of a methodology including the following steps: hazard identification (which is part of the problem formulation in the context of environmental risk assessment), hazard characterization, exposure assessment (likelihood of occurrence), risk characterization, identification of risk management measures followed by an overall risk evaluation to assess whether the overall impact of the use of the GMO on human health and environment is acceptable taking into account the management strategies applied. These principles, concepts and methodology are key to the GMM and GMO regulatory framework adopted in the European Union (EU) (EC, 2001, 2009).

In many EU Member States, the implementation of the GMO regulatory framework involves advisory bodies advising competent authorities about the safety of activities involving GMOs. These advisory bodies are important actors to build bridges between scientific research and risk assessment. The first session of the symposium provided examples of how advisory bodies in Belgium (the Biosafety Advisory Council – BAC – and the Biosafety and Biotechnology Unit – SBB), in France (the High Council for Biotechnologies – HCB) and in the Netherlands (the Commission on Genetic Modification – COGEM) have organized their interaction and partnership with the scientific community. These are composed (partly or fully) of scientists covering different fields of expertise. Moreover, in order to cover relevant issues raised during the evaluation of biosafety dossiers and to complement internal expertise, they have adopted a similar approach which seeks expertise from the scientific community at large through collaboration with external experts. In addition, the organisation of events or expert meetings addressing specific or transversal topics associated with GMO risk assessment is exploited as a mean to gather expertise in various fields.

The presentations of the first session fueled broader discussions on some challenges associated with expertise in the frame of GMO risk assessment and possible ways forward to improve the interface between this expertise and scientific research.

As regards the challenges, one of the speakers noted that scientific expertise in general is facing an increased

distrust from some political decision-makers and sectors of civil society. Citizens no longer trust scientists as in the past. In the field of GMOs, it was mentioned for instance that expertise has to cope with an increased awareness of societal responses of a non-scientific nature (*e.g.* fear for the unknown) and an increasing resistance against the monopolizing position of multinationals. To gain public confidence, some speakers suggested that advisory committees should ensure that their functioning is supported by appropriate rules or procedures aiming at avoiding conflicting interests of members, ensuring integrity in serving the higher purpose of the committee's mission, providing explicitly all used arguments including minority opinions when needed, and allowing a periodic evaluation of their functioning. The work of the advisory committees should also be based on a multidisciplinary expertise and be as transparent as possible for the public.

Some participants also noted that an increasing number of voices call for taking into consideration in decision-making processes the ethical, economic or societal implications of GMO applications. A broader risk analysis framework could therefore be envisaged that would consider risk-benefit analysis of the use of GMOs. One of the presentations showed how such a broader framework has been implemented recently in France. Indeed, a particular feature of the HCB is that it consists of two independent committees: a scientific committee (SC) – composed of scientists with diverse expertise – and an economic, ethical and social committee (EESC) – composed of representatives of the civil society and various stakeholders relevant to the HCB's mandate. Both, the SC and the EESC provide case-by-case scientific opinions and recommendations to the French competent authorities, respectively on the potential risks to the environment and public health associated with biotechnology and on the impacts of biotechnology with respect to social, economic and ethical issues. In the Netherlands also, the COGEM provides advice not only on risk assessment but also on ethical and societal issues related to genetic modification.

As regards the interaction between expertise in risk assessment and scientific research, it was recognized that risk assessment could benefit from the growing accumulation of knowledge in many disciplines, such as epigenetics and “omics”, or studies on the functioning and interaction of biological systems, both at the molecular and at the level of systems biology. It was also mentioned that risk assessment is a collective learning process and that new data or developments could be used to reconsider or refine existing regulatory procedures and risk assessment guidelines. However, although the generation, gathering and evaluation of scientific data within the context of GMO risk assessment could contribute to fill knowledge

gaps and to conduct assessment in a more comprehensive way, several participants noticed that generating new scientific data would not always reduce uncertainties. In that context, the need to consider the relevance of such data to the risk assessment of GMOs and to make a difference between what is “need to know” and “nice to know” was highlighted by several speakers.

Another aspect that was addressed is related to the fact that risk assessment of GMOs is currently mainly based on a qualitative approach, thereby taking into account the “weight of evidence” approach and using qualitative estimates (“high”, “moderate”, “low” or “negligible”). According to one speaker, risk assessment could benefit from an improved quantification of uncertainties and from experimental data with an increased statistical power. However, increasing the quantitative dimension of risk assessment would probably necessitate the gathering of more scientific data to build appropriate baseline information, *i.e.* information related to the non-GM counterpart. Risk assessment of GMOs is based indeed on a comparative approach where properties of a GMO are compared to those of an appropriate comparator, taking into account genotypic- and environmentally-mediated variation.

Finally, the similarities and differences between basic research and expertise in risk assessment of GMOs were discussed. Although they could be considered as two approaches with different objectives, both are made by scientists and are based on the testing of hypotheses. They are both iterative and have to deal with uncertainties as some of the scientific data may be of conflicting nature and ambiguities or contradictions may remain on some aspects. Therefore, similar to basic research, it is important to keep expertise in risk assessment science-based and open to scientific controversy.

Genetically modified micro-organisms, vectors for gene delivery

The genetic engineering of micro-organisms (viruses and other biological agents) provides a tool for both basic research and therapeutic applications as new gene transfer vehicles can be developed to produce new vaccines or gene therapy products with improved efficacy, specificity and safety. In the context of clinical trials and from a regulatory point of view, patient safety considerations associated with the administration of genetically modified micro-organisms (GMMs) are assessed by ethical committees and by the European Medicines Agency (EMA) when the medicinal product comes at the stage of application for its commercialization. The manipulation or administration of these GMMs during pre-clinical laboratory work or clinical trials also triggers the need for an assessment of the risks to human health and the environ-

ment (biosafety), in particular as regards their potential spread.

Research in this field is often driven by functional qualities (*e.g.* productivity and transduction efficiency), therapeutic efficacy and patient or animal safety considerations, rather than by biosafety considerations. However, experimental results and developments in this domain could also directly or indirectly benefit the risk assessment of these GMMs.

One example that was discussed during the symposium relates to safety improvements that have been achieved in designing recombinant adeno-associated viruses (rAAV) (Samulski et al., 1982). It is recognized that the accumulated knowledge on wild-type (wt) AAV (a small single stranded, DNA virus that requires co-infection with a helper virus to establish a productive infection) cannot be fully extrapolated to their recombinant derivatives. For example, integration specificity of rAAV is different and needs to be evaluated by conducting experiments using recombinant vectors. It has also been shown that the serotype of wt AAV from which the recombinant vectors are derived as well as the choice of the promoter greatly influences the biodistribution of transgene expression (Bockstael et al., 2008; Tenenbaum et al., 2000). The delivery method should also be evaluated as it may alter the vectors capacity to disseminate into different tissues and, if cells of excretory systems are transduced, potentially change the shedding route of the recombinant viruses. For example, an altered biodistribution has been observed in a study where rAAV vectors have been delivered to the brain through several modes of administration (Duque et al., 2009; Hadaczek et al., 2009).

Data on biodistribution, shedding, transmission potential and capability to infect other cells are key information for the risk assessment of recombinant vectors used in gene therapy and vaccine development. Human adenovirus type 5 (HAdV-5) is another example of a non-replicating viral vector frequently exploited for this purpose. While large variation in data sets hampered the development of a quantitative kinetic model, qualitative models exist that may help to determine the possible biodistribution and shedding in function of the route of the administration of the viral vector (Brandon et al., 2010; Tiesjema et al., 2010). These models show that the conclusions drawn for replication deficient HAdV-5 can not be extrapolated to other serotypes or viral vectors, thereby underlining the necessity of a case-by-case approach, taking into account the transmission potential and the capability of the vector to infect non-target cells.

Other approaches, such as those using genetically modified food-grade bacteria, provide an alternative means to deliver therapeutic proteins. For example *Lactococcus lactis* has been genetically engineered to

allow secretion and topical delivery of human Interleukin 10 (hIL-10) in the gastrointestinal tract (Steidler et al., 2000). In this case, scientific information related to the metabolism of the micro-organism, and its interaction and behavior in defined environments has been exploited to contribute to the development of efficient biological containment strategies. Concerns as regards the survivability and propagation of the bacteria have been met by replacing the thymidylate synthase gene, which is essential for growth of *L. lactis*, with the expression cassette for hIL-10 (Steidler et al., 2003). Thymidine growth dependence differs from most other auxotrophies as absence of the essential component is bactericidal instead of bacteriostatic. Furthermore, owing to deficient conjugative transposition and impaired phage replication in thyA-deficient *L. lactis*, several mechanisms for lateral transfer do not function, improving safety features of this approach.

One of the main conclusions from this session is that biodistribution of recombinant micro-organisms in the body of the treated patient or animal, and its possible subsequent shedding and spreading, depends on several factors thereby necessitating a case-specific approach. It should also be kept in mind that shedding and spreading of the recombinant vector is not an adverse event *per se* as it depends on its survival outside the body and its infectivity and again needs a case-by-case risk assessment.

Epigenetic effects

In most cases, genetic engineering aims at modifying DNA sequences in order to obtain desired phenotypic traits. However, the result of changes in DNA sequences in terms of phenotypic outcome is not always predictable. Likewise, some heritable phenotypic variations can not be assigned to alterations in DNA sequences only. “Epigenetics” refers to the study of changes in the genome functions that occur without a change in DNA sequences. Epigenetic research is interested in the study of molecular mechanisms and interactions underlying these changes and may be relevant to the risk assessment of GMOs as it may improve our understanding of potential pleiotropic and indirect effects associated with genetic modification. The symposium reflected on some of the current insights of the epigenetic machinery and how this knowledge has triggered the establishment of quantitative models to assess the relative contribution of genetic modification and epigenetic variation in the phenotypic outcome of an organism on the one hand and to potential applications in terms of development of novel organisms on the other.

From the several molecular mechanisms underlying epigenetic effects, three different mechanisms are closely interconnected *i.e.* (i) *DNA (de)methylation*, (ii) gene regulation by *non-coding RNA* whereby various forms of

small RNA (miRNA, siRNA, shRNA etc.) are involved and (iii) *chromatin modification* encompassing various modifications of histone proteins, such as acetylation and methylation, which create a “histone code” for gene regulation. Structural characteristics of the chromatin itself also play a role in the accessibility for transcription (for review, see Nap and van Kessel, 2006). All these cellular mechanisms are at the origin of epigenetic effects and add some layers of complexity to the regulation of gene expression on top of DNA sequence-based mechanisms.

While some of these mechanisms of regulation are transient in nature and basically provide an organism a rapid way to adjust its gene expression as a response to environmental signals, there is evidence that alterations in chromatin states can be transmitted across mitosis and meiosis in mammals and plants, and can determine the heritable phenotype of an organism (Richards et al., 2006; Henderson and Jacoben, 2007). These heritable alterations (termed epigenetic inheritance) in which the DNA sequence remains unchanged, imply that alterations in DNA sequence are not the only source of heritable phenotypes. As the latter is a resultant of several components, a better understanding of epigenetics may help to estimate the impact of modifications in the DNA primary structure on the phenotypic outcome of an organism relative to the impact of other sources of phenotypic variation. Within this respect and based on the assumption that heritable phenotypic variation is a resultant of a component originating from DNA sequence variation, epigenetic variation and a residual variation, model systems have been developed to delineate phenotypic variation into its several components. For example, a system using two *Arabidopsis thaliana* parents with identical DNA sequences but drastically divergent methylation profiles as a result of differential epigenomic perturbation (Johannes et al., 2009; Reinders et al., 2009) has been employed to this end.

A better understanding of the epigenetic machinery and its impact may also pave the way towards epigenetic engineering. A novel plant breeding technique, termed reverse breeding, is a concrete example that allows breeders to produce a new F1 hybrid in a much shorter timeframe compared with conventional plant breeding techniques (Dirks et al., 2003, 2009). The approach is based on the RNA interference (RNAi) mediated down-regulation of genes involved in meiotic recombination of heterozygous lines that have been selected for their elite qualities. This leads to the formation of haploid microspores from which the genome will be subsequently doubled (using doubled haploid technology) to give rise to homozygous plants. Selection of homozygous plants that do not contain the RNAi constructs ensures that the obtained F1-hybrids are non-GM. Hence, reverse breeding has the potential to create non-GM organisms with distinct desirable traits

that have been obtained by means of genetic modification techniques during one of the breeding steps. This approach is an illustration of how epigenetic engineering may broaden the perspective to develop organisms and/or products by targeting changes in the epigenetic information or the epigenetic state of a gene instead of modifying the DNA primary structure. These approaches lead to a different concept of alteration of gene function that was not readily envisaged at the time that the European GMO regulatory framework was established. The issue of whether organisms developed by means of epigenetic engineering would fall under the scope of the EU GMO legislation is still a matter of debate at the European level as it challenges a different interpretation of the GMO definition and raises questions about potential safety concerns associated with these organisms. It also poses the question of whether the current risk assessment methodology would fit with the assessment of organisms obtained through epigenetic engineering.

Epigenetics is an example of a new field of research for which one can address the question whether data generated in this domain can actually contribute to a better risk assessment of GMOs. A better understanding of the epigenetic machinery may ultimately lead to a better prediction of pleiotropic effects (*i.e.* one of the sources of unintended effects that may be observed upon classical breeding, mutagenesis, transgenesis or epigenesis) thereby potentially reducing some uncertainties resulting from the safety assessment of GMOs. For example, it would be beneficial for the design and assessment of integrative recombinant vectors as epigenetic alterations and the capacity of integration of foreign DNA into the host genome (*e.g.* integration of recombinant viral vectors in mammalian cells or integration of T-DNA into the plant) rely on the dynamic interplay between chromatin, small RNAs, regulatory enzymes and DNA sequence. However, an improved understanding of the layers of epigenetic regulation could be less relevant to the risk assessment of GM plants as safety concerns associated with the use of these organisms could be addressed by looking solely at the outcome of the genetic and epigenetic alterations, *i.e.* the phenotype, without addressing the relative contribution of some of its several components.

These considerations opened a more general questioning during the symposium on the nature and amount of data gathered within the context of risk assessment. The collection of more data does not necessarily reduce uncertainty as regards safety issues. It is important to recognize that safety will never be proven no matter how many data are collected. In line with these considerations, several speakers and participants underlined the importance to distinguish data that are relevant to risk assessment and also questioned the need to have or generate larger amounts of data in the context of the use of GMOs

compared to data required for comparable non-GM organisms or products.

Design of field studies contributing to the environmental risk assessment of GM plants

GMOs released into the environment for experimental purposes or as commercial products are subject to an environmental risk assessment (EC, 2001) to determine their potential impact on human/animal health and the environment relative to non-GMOs. The required information for this assessment may vary depending on the type of the GM plant and trait(s) concerned, their intended use(s), and the receiving environment(s). Field trials with the purpose to test possible environmental impacts constitutes one of the ways to collect information and may provide risk assessors with data concerning potential adverse effects of a GM plant in a specific receiving environment. Before setting up field trials to test potential environmental impacts, it should be considered carefully whether the data retrieved could contribute to an improvement in the overall estimation of risk and facilitate decision-making. In this respect, applicants noted that they sometimes have the impression they need to provide field data for review irrespective of the relevance for decision-making. Similarly, people involved in ecological scientific research have observed that public research associated with the release of GM plants into the environment is sometimes supported by some governments more to address public concerns than to generate scientific data relevant for environmental risk assessment. An example, where there may be no need for tests in the field to address potential adverse effects on non-target organisms interacting with the GM plant, is the case where no harmful effects on insects not targeted by an insect resistant GM plant have been observed under worst-case exposure studies in the laboratory. This is because under these laboratory conditions, concentrations of test material are used which are much higher than those where insects may be exposed to in the field (Raybould, 2006; Romeis et al., 2008). On the other hand, with respect to herbicide tolerant plants, one could argue that it would be difficult to solely rely on laboratory studies to test possible harmful effects on insects, because the potential impact of herbicide treatment on the food web cannot be studied in the laboratory (Albajes et al., 2009).

An aspect that appears to be crucial is the approach of setting up field trials for environmental risk assessment. To exemplify this, differences between environmental risk assessment and ecological scientific research were highlighted. The identification and selection of relevant problems for ecological scientific research is driven by observations (results) and predictions, thereby advancing fundamental and/or applied knowledge. In environmental

risk assessment, one needs to take into account that the purpose of risk assessment is to help decision-making, which is not necessarily equal to increasing scientific knowledge *per se*. Therefore, selecting problems needs to be related to the aim to protect valued entities from being harmed. This necessitates a definition of what is valuable and implies that the selection of problems needs to reflect protection goals (Raybould, 2007; Wolt et al., 2010).

Like any scientific study, field studies done in the context of environmental risk assessment gain value if the data generated are obtained on the basis of clear and defined experimental design (*e.g.* replication, sample size, appropriate controls) ensuring sufficient statistical power. Regarding statistics, the advantages of the “equivalence test” for environmental risk assessment as compared to the traditional “difference test” were elaborated (Perry et al., 2009). Contrary to the test of difference, the test of equivalence employs the null hypothesis of inequality implying that the applicant has to actively disprove this inequality in order to conclude that the GMO is equivalent to its appropriate comparators. In order to reject the null hypothesis, the differences between the GMO and its comparator must fall entirely within the limits of concern, which are the minimum ecological effect deemed of significant magnitude to cause harm. The setting up of these limits, the size of effects the experiment is designed to detect, as well as a power analysis, were considered of utmost importance. The minimum statistical requirements for the experimental design of field trials are further detailed in the updated guidelines of the European Food Safety Authority (EFSA, 2010).

That the setting-up of an appropriate design for field trials is not always evident, was illustrated by 3-year studies conducted with maize events MON 810 and MON 88017, respectively expressing the insecticidal protein Cry1Ab against the European corn borer and Cry3Bb1 against the Western corn rootworm (Rauschen et al., 2008, 2009). The aim of these studies was to assess the potential effects on field abundance of insects not targeted by the Cry proteins. It was shown that a statistically sound assessment of the potential impact on insects with naturally low densities was hampered by relatively large confidence intervals. Theoretically, these intervals could be narrowed by an increase in the number of replications, sampling over years and experimental locations in order to assess the equivalence between the maize events and their appropriate comparators (Rauschen et al., 2010). However, taking into account the workload and monetary expenses that would be necessary to obtain narrowed confidence intervals in these cases, it could be questioned whether these efforts would be proportional compared to their contribution to the overall estimation of potential impact of these maize events on non-target insects.

The examples above illustrate that there are certain limitations to field studies at the experimental level. Besides these constraints, political and regulatory requirements were also considered to hamper the development of field trials with GM plants in Europe relevant to risk assessment. The conduct of field trials in geographical regions representative for where the GM plant will be grown, may be hindered by the fact that GM field trials are not allowed in those regions (only a restricted number of EU Member States allow field trials) or by relatively high isolation distances imposed between the field trial and surrounding agricultural areas with non-GM crops. Also the destruction of field trials by anti-GMO activists was mentioned as a major constraint.

In cases where field observations are challenging, such as for the reasons mentioned above, modeling exercises may still enable an assessment. This was exemplified at the symposium with a mathematical approach analyzing the exposure of certain non-target Lepidoptera to Cry1Ab insecticidal protein pollen from maize MON810 and estimating their mortality (Perry et al., 2010). Whilst recognizing that any modeling exercise is subject to uncertainties and that the availability of sufficient data is crucial for refining the estimates, it was argued that modeling could nevertheless provide a structured framework to risk assessment and that in some cases it may offer an alternative to field observations.

To conclude, while laboratory data could offer conclusive results to support the environmental risk assessment of GM plants, field trials sometimes represent an additional source of information provided valuable results are retrieved. Several considerations during this session highlighted the importance of defining the context in which field trials are planned to be conducted and the challenges of setting up field trials with high quality design in order to retrieve results relevant for evaluating the potential impacts of GM plants on the environment.

“Omics”

The term “Omics” refers to studies involving different profiling techniques such as genomics (the quantitative study of genes, regulatory and non-coding sequences), transcriptomics (RNA and gene expression), proteomics (protein expression) and metabolomics (metabolites and metabolic networks). The technical aspects in collecting “Omics” data are continuously improving and profiling techniques, which have been initially developed in bacteria, yeast and animal cells, have now served several distinct purposes, including those in plants, such as the study of epigenetic phenomena, the study of plant environmental interactions or the impacts of agricultural practices. Within the context of the risk assessment of GMOs, “Omics” techniques could contribute to the detection of potential unintended effects, *i.e.* effects which

go beyond that of the original genetic modification. They could also provide additional tools to compare and study potential differences at the compositional level between GMOs and their comparators (*e.g.* detecting differences in levels of nutrients, anti-nutrients, endogenous toxicants or allergens).

Metabolomics is a first example of a profiling technique that was presented during the symposium. It can be used to identify significant sources of variation in the composition of crops. For instance, capillary gas chromatography-based metabolite profiling methodology enables the generation of data aiming at investigating alterations in a broad spectrum of metabolites and providing information that could be considered complementary to the information obtained by transcriptomics or proteomics (Davies et al., 2010). This metabolite profiling approach could be applied to characterize alterations induced by both mutation breeding and genetic engineering. It could also be used to distinguish between natural variability and variability induced by genetic modification in crops grown at different locations and/or growing seasons (Frank et al., 2009; Röhlig and Engel, 2010). Interestingly, as shown during the symposium, a comparison of several GM maize lines with their near isogenic lines for several growing locations over several years indicated that the impact of the environmental factors on the metabolomic profile was more pronounced than that of the genetic background (Barros et al., 2010).

Transcriptomics is another profiling approach which consists in generating profiles of mRNA or gene transcripts by means of micro-arrays. While this approach has the advantage to provide relatively reproducible data, differences revealed by transcriptome analysis may not be equally reflected in differences in the proteome and/or metabolome and as such in the plant's physiology. Therefore, this technique has also been used as part of a more holistic approach involving several profiling techniques in parallel. During the symposium, it was shown how transcript profiling, metabolome profiling and metabolic fingerprinting have been used in parallel in order to assess the substantial equivalence of transgenic barley plants relative to the natural variation between cultivars, and to have a better understanding of the effect of plant interaction with mycorrhizal fungi (Kogel et al., 2010). Principle component analysis of the differentially regulated genes (microarray data) and a metabolite profiling indicated that, in contrast to the transcriptome, alterations in metabolites were observed. The results also indicate the higher impact of environmental factors (mycorrhization) in terms of metabolite differences compared to the effect of the introduction of transgenes or the effect of introgression of genes by classical breeding techniques.

The above-mentioned studies show that alterations in the “Omics” profiles do not necessarily indicate poten-

tial harm for human health or the environment. Moreover, taking into account that crops with new traits obtained by “traditional” mutation techniques are also prone to genetic alterations and/or rearrangements, some participants questioned whether it is warranted to focus on unintended effects specifically when addressing the safety of GMOs. In that context, results were presented regarding the assessment of the impact of genetic engineering versus mutagenesis in terms of transcriptome alterations. They reveal that the acquisition of the traits is accompanied by modifications in transcript levels of untargeted stress-related genes, both in transgenic and mutant lines. Moreover, the number of differentially expressed genes appeared to be larger in mutant lines compared to transgenic lines (Batista et al., 2008).

From the discussions that took place during the symposium, it appeared that profiling techniques may provide new information for the safety assessment of GMOs by offering a more holistic comparative approach relying on a broad screening, in a non-selective and unbiased manner. However, apart from the considerations on the costs of this approaches, the abundance of information generated could also raise challenges as regards the extraction of information with discernable biological meaning. From several studies, including those performed on organisms obtained through traditional plant breeding techniques, it becomes clear that transcriptional, proteomic and metabolite alterations may have different causes, including environmental and agronomical elements. Therefore, observed differences between GM plants and their comparators should be assessed in light of the natural plant variability, encompassing variability associated with genetic background (*e.g.* commercial available cultivars) and environmental impacts. It was noted that there is still a lack of information on the natural variation within and between given plant cultivars. For comparative purposes and in order to discern the bandwidth of natural plant variation under selected circumstances, it would be interesting to establish a database with sufficient numbers of samples, to develop standardized methods and to perform multivariate modeling of data (Barros et al., 2010; van Dijk et al., 2010).

At the end of this session, the general feeling was that the “Omics” approach needs to be further developed, standardized and validated before the information can be routinely incorporated and evaluated within the context of comparative risk assessment of GMOs.

CONCLUSIONS

Through numerous examples and discussions, the symposium illustrated how research efforts in diverse disciplines may contribute to the science-based risk assessment of GMOs and highlighted some challenges linked to the use

and interpretation of results generated by basic and applied research. Other challenges are the importance to frame correctly scientific experiments and the need to set up good risk hypothesis relevant to risk assessments to guide the generation, collection and interpretation of data, as well as the difficulty to extrapolate to natural conditions data obtained from laboratory studies. The symposium also showed that uncertainties or knowledge gaps identified during the risk assessment process as well as the emergence of new types of GMOs or new techniques of genetic modification (such as epigenetic engineering) can trigger new scientific research. Despite the fact that further investment in research related to risk assessment is sometimes hampered due to external factors such as political and regulatory constraints, or the difficulty to attract public funding or to generate publications, it was also mentioned that risk assessment could benefit in some cases from the use of new tools and approaches, as discussed during the “Omics” session.

Beside the identification and continuation of research that is beneficial for the risk assessment of GMOs, facilitating access to existing data obtained from basic research (e.g. through large open-access databases of biosafety studies) could also highlight research results relevant to risk assessment. However, an important question that emerged repeatedly during the symposium is how far one should go in generating or collecting new data for the risk assessment of GMOs. As mentioned by Di Castri (1992), “*Knowledge is like a sphere. By increasing its volume, the surface in contact with the unknown increases*”. Having more data does not necessarily mean having more certainty. The need to make a difference between “need to know” and “nice to know” was underlined by several speakers and participants.

The symposium also emphasized the importance of ensuring a reciprocal link between risk assessment and scientific research by providing several examples of how scientists could work in partnership with advisory bodies responsible for evaluating the risk assessment of GMO applications. It was recognized during the symposium that exchanges between experts in risk assessment and scientists involved in scientific research allow the development of relevant interdisciplinary collaborations, potentially resulting in peer-reviewed publications, and ensure scientific reliability in the work of the scientific advisory bodies. One can expect that the more scientists are engaged in risk assessment, the fewer criticisms regarding the unbalanced representation of scientific disciplines in the process will occur.

In conclusion, this symposium offered a valuable multidisciplinary forum showing that GMO risk assessors and the scientific community can benefit from fruitful discussions. In this regard, existing or new scientific networks and fora, as well as the organization of scientific

events could be further exploited to promote awareness and further build on the gathered knowledge and expertise.

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