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SPECIAL ISSUE

Risk assessment of Genetically Modified Organisms (GMOs)

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ABSTRACT

EFSA's remit in the risk assessment of GMOs is very broad encompassing genetically modified plants, microorganisms and animals and assessing their safety for humans, animals and the environment. The legal frame for GMOs is set by Directive 2001/18/EC on their release into the environment, and Regulation (EC) No 1829/2003 on GM food and feed. The main focus of EFSA's GMO Panel and GMO Unit lies in the evaluation of the scientific risk assessment of new applications for market authorisation of GMOs, and in the development of corresponding guidelines for the applicants. The EFSA GMO Panel has elaborated comprehensive guidance documents on GM plants, GM microorganisms and GM animals, as well as on specific aspects of risk assessment such as the selection of comparators. EFSA also provides special scientific advice upon request of the European Commission; examples are post-market environmental monitoring of GMOs, and consideration of potential risks of new plant breeding techniques. The GMO Panel regularly reviews its guidance documents in the light of experience gained with the evaluation of applications, technological progress in breeding technologies and scientific developments in the diverse areas of risk assessment.

KEY WORDS

GM plants, GM animals, GM microorganisms, post market environmental monitoring, comparative risk assessment

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INTRODUCTION

EFSA's remit in the risk assessment of GMOs is very broad encompassing genetically modified (GM) plants, microorganisms and animals and assessing their safety for humans, animals and the environment. Within this remit, the main focus of EFSA's GMO Panel and GMO Unit lies in the evaluation of the scientific risk assessment of new applications for market authorisation of GMOs, and in the development of corresponding guidance for the applicants.

So far, EFSA has received about 150 applications for market authorization, primarily for GM plants and to a lesser extent for GM microorganisms. Crop plants that are being genetically modified include maize, soybean, cotton, and - to a lesser extent - oilseed rape, potato, sugar beet and rice. The traits introduced into crop plants are predominantly resistance to insect pests and tolerance towards certain herbicides, although applications for plants with modified composition (e.g. changed fatty acid profile) or tolerance to drought have also been received. About half of the applications concern single events whereas the other half of the applications concerns so called "stacked" events in which two or more single events have been combined by conventional breeding, in order to introduce several traits into one crop plant.

In addition, EFSA also provides special scientific advice upon request of e.g. the European Commission; examples are post-market environmental monitoring (PMEM) of GMOs, and consideration of potential risks of new plant breeding techniques. The legal frame for GMOs is set by Directive 2001/18/EC[3] on their release into the environment, and Regulation (EC) No 1829/2003[4] on GM food and feed. Directive 2001/18/EC details the scientific elements and major strategies to be used in the risk assessment of GMOs. A central strategy is the comparative approach, a scientific concept that aims to identify biologically relevant differences by comparing the GMO with a non-GM counterpart.

In the following, the main milestones in GMO risk assessment are highlighted by discussing some of the guidance documents (GD) that have been developed by the GMO Panel, and are used in the evaluation of risk assessments provided by the applicants for market authorisation of their products.

EFSA GUIDANCE DOCUMENTS MARK MILESTONES IN GMO RISK ASSESSMENT

1. Specific Guidance on selection of comparators for GM plants

The selection of appropriate comparators is central to the comparative approach in the risk assessment of GMOs. The identification and production of such comparators is becoming increasingly challenging due to the increasing complexity of breeding schemes and the GM plants themselves, e.g. those developed by combining (stacking) events through conventional breeding, or those in which compositional changes are targeted. EFSA's recent guidance on the selection of comparators (EFSA Panel on Genetically Modified Organisms (GMO), 2011a) develops options for a more flexible and workable framework. It recognises that whilst a non-GM conventional counterpart should always be used to assess new single events, both single events and stacked events, which have already been risk-assessed, could be used as the key comparators. Similarly, negative segregants derived from crosses between events already risk-assessed and which are all present in the GM plant under assessment can be used. For the environmental risk assessment (ERA) different comparators may be appropriate when a conventional counterpart is not available. This will depend on the issue under consideration.

In cases where appropriate comparators are not available (e.g. where significant compositional changes have been targeted) a comprehensive safety/nutritional assessment on the GM plant *per se* is advocated.

[3] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official Journal L106, 1-39.

[4] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Official Journal of the European Union L 268 1-23.

2. Guidance for food/feed risk assessment of GM plants

The first EFSA GD was adopted in 2004, updated in 2005, and published in 2006 (EFSA, 2006a). In 2008, initiatives were taken by the EFSA GMO Panel to further update the GD and to incorporate the scientific outputs of various EFSA GMO Panel Working Groups regarding 1) the risk assessment of stacked events; 2) selection of comparators for the risk assessment of GM plants and derived food and feed; 3) appropriate field trial designs and statistical approaches for analysis of compositional, agronomic and phenotypic characteristics of GM plants; 4) the role of animal feeding trials in the safety and nutritional assessment of GM plants and derived food and feed; and 5) the assessment of allergenicity of GM plants and derived food and feed.

The updated food/feed GD (EFSA Panel on Genetically Modified Organisms (GMO), 2011b) outlines the principles of the risk assessment of GM plants and derived food and feed, providing a detailed description of the comparative approach and definitions of the different steps and objectives of the risk assessment process. Reference is made to internationally agreed protocols for the toxicological assessment of newly expressed proteins and other new constituents, and of natural compounds the levels of which may have been altered through the genetic modification. Furthermore, attention has been paid to the testing of whole GM food/feed, which may be considered on a case-by-case basis, and approaches for allergenicity assessment of food/feed derived from GM plants have been further updated (EFSA Panel on Genetically Modified Organisms (GMO), 2010b).

Special attention was paid to the design and statistical analysis of field experiments. The updated food/feed GD provides requirements for minimum replication of trials over sites and years. It also quantifies the distinction between statistical significance and biological relevance (EFSA Scientific Committee (SC), 2011) through a 'bioequivalence' approach. This uses an equivalence test, for which the usual scientific null hypothesis of equality is replaced by one of "non-equivalence". Advantages are: future experiments should be well-replicated with sufficient statistical power and the error of most concern to the consumer may be set explicitly. The new approach has led to a scientific opinion of the GMO Panel (EFSA Panel on Genetically Modified Organisms (GMO), 2010a).

This document, adopted on 14 April 2011, is currently used by the European Commission and the Member States as a basis for the preparation of the Regulation on "*Implementing rules concerning applications for authorization of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and the Council"* (in progress).

3. Guidance on environmental risk assessment of GM plants

Assessment of the environmental effects of cultivation of GM plants has remained controversial for the entire ten years of EFSA's existence. Two working groups of the Panel have updated guidance for environmental risk assessment (ERA) in 2008. The focus was on four areas: potential effects on non-target organisms (NTOs); new criteria for design and analysis of field trials; characterization of different relevant receiving environments within the European Union (EU) where GM plants may have environmental effects; techniques to assess potential long-term effects. The work on NTOs resulted in a stand-alone scientific opinion (EFSA Panel on Genetically Modified Organisms (GMO), 2010c) adopted simultaneously with the new guidance in October 2010 (EFSA Panel on Genetically Modified Organisms (GMO), 2010d). Specific requirements were given for data to evaluate possible effects on NTOs: *in planta* studies are essential to study plant-environment interactions; field-generated data related to NTO species and their functionality must be provided in the majority of cases. The guidance requires a prospective statistical power analysis, based on a clear statement of the magnitude of the environmental effects that the experiment is designed to detect, these effects themselves being related explicitly to protection goals relevant to particular receiving environments. Long-term effects, arising either from delayed responses or from increases in spatio-temporal complexities, were recommended to be identified by modelling and meta-analyses. The structure of the ERA was developed around the concept of a Comparative Safety Assessment, based on the principles outlined in Directive 2001/18/EC. This central part of the ERA process starts with the crucial first step of problem formulation which facilitates a structured approach to identifying potential

risks and scientific uncertainties. This is followed by five further steps in which all other relevant issues are addressed. The six-step procedure is applied to each of the nine areas of risk listed in the Directive.

4. EFSA's involvement in post market environmental monitoring

Since 2007, EFSA has been asked to play an increasing role in recommendations for risk management for GM plants. For example, in 2011 EFSA assumed responsibility for the assessment of yearly reports on the post-market environmental monitoring (PMEM) for all cultivated GM plants, designed to detect and limit possible adverse environmental effects, including those that are long-term. In 2011, the GMO Panel issued the new GD on PMEM (EFSA Panel on Genetically Modified Organisms (GMO), 2011d). The PMEM GD made recommendations for both, general surveillance to detect unanticipated adverse effects, as well as case-specific monitoring when the ERA identifies a particular potential risk or uncertainty that can be mitigated during cultivation. Updated tools were given for general surveillance, including the design and analysis of farmer questionnaires, recommendations on the use of existing biodiversity monitoring networks and proposals for the establishment of reporting centres at the Member State level. New methodology, proposed for case-specific monitoring, included requirements for experimental design and analysis. The GD stressed how information from both these forms of monitoring may feed back into and strengthen the original ERA and emphasised the need for further definition of protection goals and their linkage to PMEM.

5. Guidance on GM microorganisms

The guidance on the risk assessment of genetically modified microorganisms was updated in 2011 (EFSA Panel on Genetically Modified Organisms (GMO), 2011c) based on the feedback and experience gained with the 2006 version (EFSA, 2006b). Another motivation for the update was a recent change in the legislation, as e.g. food enzymes became regulated products that require pre-market safety evaluation. Previously the main focus of the GMO Panel had been in genetically modified microorganisms used to produce feed additives. The 2011 guidance was a joint effort of a working group consisting of members of the GMO, CEF (Food Contact Materials, Enzymes, Flavourings and Processing Aids), NDA (Dietetic Products, Nutrition and Allergies) and BIOHAZ (Biological Hazards) Panels as well as independent experts. The aim was to simplify and clarify the guidance, but at the same time provide more details where felt necessary. As the guidance covers a range of products besides enzymes, a major effort was made to develop sensible and realistic categories to reflect the extent of data needed to provide a reasonable evidence for safety. Synthetic biology was excluded from the 2011 guidance but as an emerging field it needs more attention in the future.

6. Guidance for non-food/feed risk assessment

EFSAs mission also includes products other than food and feed relating to genetically modified organisms as defined by Directive 2001/18/EC, which excludes medicinal products for human and veterinary use. Applications would include e.g. plants used for the production of non-food enzymes or biofuels. As the safety evaluation has somewhat distinct character from that for food and feed, a scientific opinion was developed in 2009 on guidance for the risk assessment of genetically modified plants used for non-food or non-feed purposes (EFSA, 2009). Until now the GMO Panel has not given scientific opinions on plants that would be definitely excluded from human consumption. Examples of borderline cases are potato developed for the production of industrial starch with specific properties, and carnations with modified flower colours intended for cut flower business but not excluding the use of petals for salad decoration.

7. Future challenges

7.1. The role of "omics" technologies in GMO risk assessment

The molecular characterisation of a GM event forms an important baseline for the risk assessment strategy. Advances in genomics technologies and bioinformatics have been acknowledged in the development of EU GDs where the analysis of sequencing data has become increasingly sophisticated and detailed. Further developments in high-throughput, Next Generation Sequencing are offering unprecedented possibilities for detailed comparisons of genomes. Parallel advances have been made in high-throughput analysis of gene expression (transcriptomics), protein expression (proteomics) and metabolite composition (metabolomics) but the application of "omics" technologies has not been emphasised in EU GDs. EFSA notes that "omics" may be useful in specific cases e.g. where specific metabolic pathways have been modified, leading to enhanced nutritional profiles and could be developed to help minimise animal experiments. The strengths and weaknesses of "omics" in food safety assessment have been previously discussed (e.g. Davies HV, 2009).

7.2. New techniques in plant breeding question the boundaries between GMOs and conventional breeding

Plant breeding approaches continue to evolve through technological advances linked to contemporary genetics and genomics and in some instances it is unclear whether the technologies used give rise to GMOs as defined by current EU legislation (established in 1990). Some of these breeding and genetic modification techniques have been subject to field trials in the EU and a number of them are now approaching commercialisation. Following a request of the European Commission the EFSA GMO Panel is currently addressing two questions with regard to new techniques: The first is to determine whether there is a need for new guidance or whether the existing guidance on risk assessment should be updated or further elaborated in advance of such products entering the marketplace. The second is to assess the risks in terms of impact on humans, animals and the environment that the techniques could pose, irrespective of whether or not they fall under the GMO legislation. The techniques proposed for assessment are (1) zinc finger nuclease technology; (2) oligonucleotide-directed mutagenesis; (3) cisgenesis (comprising cisgenesis and intragenesis); (4) RNA-dependent DNA methylation via RNAi/siRNA; (5) grafting; (6) reverse breeding; (7) agro-infiltration; (8) synthetic biology. EFSA has already published its scientific opinion on cisgenics and intragenics (EFSA Panel on Genetically Modified Organisms (GMO), 2012), and is now focused on developing a scientific opinion on zinc finger nuclease type 3.

7.3. Will GM animals be the future? Development of GD for GM animals

One of the major challenges for the medium-term future is likely to be the ERA of applications for the release into the environment of genetically modified animals (GMAs). Future applications for the commercial use of GMAs may include not only food-feed applications but also companion animals. The traits involved may be related to disease resistance, growth enhancement, sterility, population suppression, cold tolerance, dietary performance and ornamental uses. The GMO Panel, together with the EFSA Animal Health and Welfare Panel, has developed guidance on the food and feed risk assessment of GMAs, including health and welfare aspects (EFSA Panel on Genetically Modified Organisms (GMO) and EFSA Panel on Animal Health and Welfare (AHAW), 2012). Since mid-2010 three working groups developed a GD for ERA of GMAs. The major challenges have centred around the superior mobility of animals over plants and the consequent potential for greater risks due to persistence and invasiveness in receiving environments. In addition, for certain traits such as cold tolerance, it is expected that the GM animal will enter receiving environments in which there is no conventional counterpart non-GM animal with which it may be compared. The GD develops the use of non-GM surrogates with similar characteristics and the need for containment in the experimental environment. The GD will contain separate sections relevant to fish, insects and terrestrial mammals & birds, with case studies.

CONCLUSIONS

The establishment of EFSA and of the EFSA GMO Panel in 2003 has been an important landmark in the history of the European Union. The EFSA GMO Panel has provided and will continue to do so, mere scientific assessments of the safety of GMO's and derived food/feed for humans/animals and the environment of GMOs, guaranteeing the separation of risk assessment from risk management.

Risk assessment strategies for GMO's as developed by the EFSA GMO Panel and outlined in the various EFSA GDs are not unanimously accepted by Member States and certain NGO's. Therefore dialogues with interested parties on principles of risk assessment of GMO's should continue, using accessible and transparent ways of communication.

EFSA should play an important role at the European and international level to further develop and harmonize risk assessment strategies for GMO's. In this respect, EFSA's presence at and contribution to relevant international fora should be further expanded. Validation and use of new genomic and chemical profiling technologies for risk assessment purposes remains an important challenge.

Given the fast development of new breeding/production technologies applied to organisms, which may need a revision of current regulatory definitions of genetic modification, EFSA is prepared to investigate risk assessment strategies for modified organisms, based on the characteristics of obtained products rather than based on the applied breeding/production technology.

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