Transgenic Res (2014) 23:1–25
DOI 10.1007/s11248-013-9741-4

REVIEW

EFSA's scientific activities and achievements on the risk assessment of genetically modified organisms (GMOs) during its first decade of existence: looking back and ahead

Yann Devos · Jaime Aguilera · Zoltán Diveki · Ana Gomes · Yi Liu · Claudia Paoletti · Patrick du Jardin · Lieve Herman · Joe N. Perry · Elisabeth Waigmann

Received: 19 June 2013 / Accepted: 14 August 2013 / Published online: 21 August 2013

Abstract Genetically modified organisms (GMOs) and derived food and feed products are subject to a risk analysis and regulatory approval before they can enter the market in the European Union (EU). In this risk analysis process, the role of the European Food Safety Authority (EFSA), which was created in 2002 in response to multiple food crises, is to independently assess and provide scientific advice to risk managers on any possible risks that the use of GMOs may pose to human and animal health and the environment. EFSA's scientific advice is elaborated by its GMO Panel with the scientific support of several working groups and EFSA's GMO Unit. This review presents EFSA's scientific activities and highlights its achievements on the risk assessment of GMOs for the first 10 years of its existence. Since 2002, EFSA has issued 69 scientific opinions on genetically modified (GM) plant market registration applications, of which 62 for import and processing for food and feed uses, six for cultivation and one for the use of pollen (as or in food), and 19 scientific opinions on applications for marketing products made with GM microorganisms. Several guidelines for the risk assessment of GM plants, GM microorganisms and GM animals, as well as on specific issues such as post-market environmental monitoring (PMEM) were elaborated. EFSA also provided scientific advice upon request of the European Commission on safeguard clause and emergency measures invoked by EU Member States, annual PMEM reports, the potential risks of new biotechnology-based plant breeding techniques, evaluations of previously assessed GMOs in the light of new

Disclaimer: The present paper is published under the sole responsibility of the authors and may not be considered as a scientific output by the European Food Safety Authority (EFSA). The positions and opinions presented in this publication are those of the authors alone and do not necessarily represent the views of EFSA.

Electronic supplementary material The online version of this article (doi:10.1007/s11248-013-9741-4) contains supplementary material, which is available to authorized users.

Y. Devos (✉) · J. Aguilera · Z. Diveki · A. Gomes · Y. Liu · C. Paoletti · E. Waigmann
GMO Unit, European Food Safety Authority (EFSA), Via Carlo Magno 1, 43126 Parma, Italy
e-mail: Yann.Devos@efsa.europa.eu

P. du Jardin
Unité de Biologie végétale, Gembloux Agro-Bio Tech, University of Liège (ULg), Passage des Déportés 2, 5030 Gembloux, Belgium

L. Herman
Unit Technology and Food Sciences, Institute of Agricultural and Fisheries Research (ILVO), Brusselsesteenweg 370, 9090 Melle, Belgium

J. N. Perry
Oaklands Barn, Lug's Lane, Broome, Norfolk NR35 2HT, UK

scientific publications, and the use of antibiotic resistance marker genes in GM plants. Future challenges relevant to the risk assessment of GMOs are discussed. EFSA's risk assessments of GMO applications ensure that data are analysed and presented in a way that facilitates scientifically sound decisions that protect human and animal health and the environment.

Keywords Food and feed safety · GM animals · GM plants · GM trees · Monitoring · New biotechnology-based plant breeding techniques · Protection goals · Safeguard clauses · Safety

EFSA's remit pertaining to genetically modified organisms

Genetically modified organisms (GMOs) and derived food and feed products are subject to a risk analysis and regulatory approval before entering the market in the European Union (EU). In this process, the role of the European Food Safety Authority (EFSA) is to independently assess and provide scientific advice to risk managers on any possible risks that the consumption or cultivation of a GMO may pose to human and animal health and the environment. EFSA was created in 2002 in response to multiple food crises that caused considerable public concern in Europe about food safety and the ability of regulatory authorities to fully protect consumers. EFSA's remit in the risk assessment of GMOs encompasses genetically modified (GM) plants, GM microorganisms and GM animals, and involves the assessment of their safety for humans, animals and the environment (Waigmann et al. 2012). Besides ensuring a high level of protection of human and animal health and the environment, EFSA's responsibilities also include communicating its independent scientific advice to its principal partners, stakeholders and the public at large in a timely, clear, accurate and meaningful way. By communicating risks in an open and transparent manner, EFSA aims to continue to bridge gaps between science and the consumer, and to build consumer and public confidence in risk assessment and the safety of the EU food chain (Deluyker and Silano 2012).

The decision on whether a certain risk is acceptable and whether a GMO or a derived product can be placed on the EU market is not part of the risk assessment itself, but part of the wider risk analysis. Such decisions are taken by risk managers, such as the European Commission and EU Member States, as they involve political, socio-ethical and economic considerations. Natural sciences in risk assessment lend credibility to regulatory decisions, but they do not dictate decisions because decision-making is not based solely on scientific evidence. While recognising that natural sciences are not the only relevant factor in regulatory decision-making (Johnson et al. 2007; Raybould 2012), it is important that both the assumptions underlying risk assessments and the nature and magnitude of scientific uncertainties associated with the characterised risks are made explicit in a transparent manner, so that the results of the risk assessment can appropriately be taken into account (EFSA 2012e).

EFSA's scientific advice on the risk assessment of GMOs is given through its scientific Panel on GMOs (referred to hereafter as EFSA GMO Panel), which adopts scientific outputs by qualified majority. Currently (August 2013), the EFSA GMO Panel consists of 19 scientific experts who come from EU research institutes, universities or risk assessment bodies, and is supported scientifically by several working groups and EFSA's GMO Unit. These experts are selected based on their scientific competence and expertise after an open call for interest from the scientific community. Besides members of the EFSA GMO Panel, additional experts, who are invited on an ad hoc basis, compose each working group. All experts sign amongst others a commitment to act independently, and declare any interests in EFSA's remit. EFSA then decides in line with its policy on declaration of interests (http://www.efsa.europa.eu/en/efsawho/doi.htm) whether a declared interest constitutes a conflict and requires taking specific measures on a case-by-case basis. With this pool of independent experts, EFSA relies on a broad range of expertises, covering agronomy, allergenicity, biology, biotechnology, breeding, crop protection, ecology, environmental sciences, genetics, immunology, microbiology, modelling, molecular biology, nutrition, physiology, statistics, toxicology and related sciences, for the risk assessment of GMOs.

This review presents the scientific activities and highlights the achievements of the EFSA GMO Panel, its working groups and the GMO Unit on the risk assessment of GMOs for the first 10 years of EFSA's existence. Some of the GMO-related scientific outputs

issued during the last decade are summarised and described below. Future challenges relevant to the risk assessment of GMOs are also discussed.

Achievements of EFSA in the field of GMO risk assessment

The main focus of EFSA in the field of GMOs lies in the evaluation of GMO market registration applications (referred to hereafter as GMO applications) and in the development of risk assessment and monitoring guidelines. EFSA also provides scientific advice upon request of the European Commission on specific issues. EFSA's scientific advice is made available to all in the EFSA Journal, or on its webpage (http://www.efsa.europa.eu/). In specific cases, the EFSA GMO Panel has also published deliberations on scientific publications or research findings in the minutes of its plenary meetings.

GMO market approval applications

Currently, approximately 50 GM plants and derived products are approved for commercial use in the EU (http://ec.europa.eu/food/dyna/gm_register/index_en.cfm). The approval decision has a validity of 10 years. Since 2002, the EFSA GMO Panel has issued 69 scientific opinions on GM plant applications, of which 62 for import and processing for food and feed uses, six for cultivation and one for the use of pollen (as or in food). Sixteen of these opinions concerned renewal applications for which the applicant wanted to continue to market an authorised GMO following the original ten year approval decision. At the moment 51 GM plant applications are under assessment by EFSA (http://www.efsa.europa.eu/en/request/requests.htm). GM plant applications are submitted under Regulation (EC) No 1829/2003 on GM food and feed, or previously under Directive 2001/18/EC on the deliberate release into the environment of GMOs and Regulation (EC) No 258/97 on novel foods and novel food ingredients. The scope of most GM plant applications is limited to import and processing for food and feed uses or for industrial uses and does not include cultivation in the EU.

GM plant applications cover seven crops; mostly maize, followed by cotton and soybean, and—to a lesser extent—oilseed rape, potato, sugar beet and rice (Table 1). The predominant traits are resistance to insect pests and tolerance against certain herbicidal active substances, but they also include modified composition (e.g., altered fatty acid profile), tolerance to drought or reduced amylose content. About half of the GM plant applications concern so called "stacked" events, in which two or more single transformation events have been combined by conventional crossing, in order to introduce several traits into one plant.

In the light of the complexity of evaluations of GM plant applications, the EFSA GMO Panel is supported by three standing working groups, each focusing on specific areas of the risk assessment. The standing working group on applications—*Molecular Characterisation* considers all relevant scientific data on the molecular characterisation of the GM plant. Detailed information is evaluated on the source and function of the donor DNA, the transformation method, the organisation of the inserted DNA at the insertion site(s), and the expression and stability of the insert. The standing working group on applications—*Food/Feed Risk Assessment* focuses its evaluation on the agronomic and phenotypic characteristics, composition, toxicity, allergenicity and nutritional value of the GM plant and/or derived food and feed. The standing working group on applications—*Environmental Risk Assessment* considers elements such as potential changes in interactions of the GM plant with the biotic and abiotic environment resulting from the genetic modification and whether these changes cause environmental harm. Changes in the persistence (weediness) and invasiveness ability of the GM plant, potential for gene transfer and its environmental consequences, interactions between the GM plant and target and non-target organisms, effects on biogeochemical processes and the abiotic environment, as well as impacts of specific cultivation, management and harvesting techniques associated with the cultivation of the GM plant are also evaluated. For the environmental risk assessment, potential direct and indirect, as well as immediate, delayed and cumulative long-term adverse effects are considered on a case-by-case basis taking into account the plant species, traits, receiving environments and intended uses, and the combination of these characteristics. In its evaluations, EFSA carefully considers the content of applications, scientific comments raised by EU Member States on the applications, the applicant's responses to requests for further information, and all relevant scientific publications.

Table 1 Number of GM plant applications submitted to EFSA under Regulation (EC) 1829/2003 for risk assessment purposes (withdrawn applications are not included; August 2013)

Plant	Traits				Intended uses		Total
	Herbicide tolerance + insect resistance	Herbicide tolerance	Insect resistance	Other traits	Import and processing for food and feed uses	Cultivation	
Cotton	9	7	4	0	19	1	20
Maize	33	11	15	2	48	13	61
Oilseed rape	0	9	0	0	9	0	9
Potato	0	0	0	1	1	0	1
Rice	0	1	0	0	1	0	1
Soybean	2	19	2	1	23	1	24
Sugar beet	0	2	0	0	1	1	2
Total	44	49	21	4	102	16	118

The number of applications is presented per crop, trait and intended uses

During the evaluation of GM plant applications for cultivation, EFSA works in close collaboration with specific EU Member States who volunteer to take responsibility for the initial evaluation of the environmental risk assessment provided by the applicant (Lheureux et al. 2008). Several EU Member States have performed this initial evaluation (Table 2). The environmental risk assessment reports produced by those EU Member States are considered throughout EFSA's scientific opinions. The EFSA GMO Panel issued scientific opinions on the cultivation of amylose reduced potato (event EH92-527-1), herbicide tolerant maize (events NK603, MON 88017 and GA21) and soybean (event 40-3-2), as well as insect resistant maize (events Bt11, 1507, MON 810, MON 88017 and 59122). In all cases, the implementation of specific risk mitigation measures to mitigate identified risks were recommended and several proposals to revise the post-market environmental monitoring plan proposed by applicants were made (EFSA 2005a, b, 2006d, 2008b, 2009b, c, 2011g, k, 2012g, EFSA 2013a).

In accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA endeavours to respect a time limit of six months from the acknowledgement of the valid GM plant application, in order to deliver its scientific advice. As additional information is usually requested from applicants, this time frame is frequently extended. On average, the time required for the risk assessment of GM plant applications, which includes the time taken by applicants to generate and/or collate the additional information, is 25 months. EFSA's scientific advice on GM plant applications is then passed to the European Commission and EU Member States, who decide whether the GM plant and/or derived food and feed products can be put on the EU market. Within three months after receiving EFSA's scientific advice, the European Commission submits a draft decision to the Standing Committee on the Food Chain and Animal Health (SCFCAH). If the national representatives in the SCFCAH give a favourable opinion by qualified majority, then the European Commission adopts the decision. However, if no qualified majority is reached, the draft decision is sent to an Appeal Committee where EU Member States can adopt or reject the proposal by qualified majority. If the Appeal Committee does not act within three months or does not obtain a qualified majority for adoption or rejection of the European Commission's draft decision, it is the European Commission itself that adopts the decision. Additional factors such as the scope of the application (i.e., whether it includes cultivation or not) may further delay the authorisation process.

Approximately 39 GM microorganism applications have been assessed, or are under assessment by EFSA. Further details about GM microorganism applications are given in the Electronic Supplementary Material. Currently, no GM animal applications have been submitted to EFSA.

Guidelines for the risk assessment and monitoring of GMOs

Several guidelines for the risk assessment and monitoring of GMOs, as well as on specific aspects of risk

Table 2 Status of GM plant applications for cultivation submitted to EFSA (August 2013)

Application reference	Plant	Transformation event	EU Member State(*); status	EFSA status
Submitted under Directive 2001/18/EC				
C/F/96/05.10	Maize	Bt11	–	✔
C/SE/96/3501	Potato	EH92-527-1	–	✔
C/ES/01/01	Maize	1507	–	✔
Submitted under Regulation (EC) No 1829/2003				
UK-2005-17	Maize	1507 × NK603	ES; ✔	∞
NL-2005-22 + RX-NK603	Maize	NK603	ES; ✔	✔; likely to be withdrawn
NL-2005-23	Maize	59122	NL; ✔	✔
NL-2005-24	Soybean	40-3-2	DE; ✔	✔; likely to be withdrawn
NL-2005-26	Maize	MON810 × NK603	ES; ✔	✘; withdrawn in 2013
NL-2005-28	Maize	1507 × 59122	NL; ✔	∞
UK-2006-30	Maize	59122 × 1507 × NK603	BE; ∞	∞
RX-MON810	Maize	MON810	ES; ✔	✔
NL-2007-46 + RX-T25	Maize	T25	UK; ✔	✘; scope revised in 2013 to exclude cultivation
CZ-2008-54	Maize	MON88017	BE; ✔	✔; likely to be withdrawn
UK-2008-60	Maize	GA21	CZ; ✔	✔
DE-2008-63	Sugar beet	H7-1	DE; ✔	∞
NL-2009-69	Potato	AV43-6-G7	SE; ∞	✘; withdrawn in 2013
BE-2009-71	Maize	MON89034 × MON88017	BE; ✔	∞; likely to be withdrawn
NL-2009-72	Maize	MON89034 × NK603	NL; ∞	∞; likely to be withdrawn
UK-2010-83	Maize	MIR604	DE; ∞	∞
UK-2010-84	Maize	Bt11 × MIR604 × GA21	DE; ∞	∞
SE-2010-88	Potato	AM04-1020	FI; ∞	✘; withdrawn in 2013
BE-2011-90	Maize	MON89034	BE; ✔	∞; likely to be withdrawn
UK-2011-102	Potato	PH05-026-0048	NL; ∞	✘; withdrawn in 2013
ES-2012-104	Cotton	GHB614	ES; ∞	∞

EU Member State abbreviations: *BE* Belgium, *CZ* Czech Republic, *DE* Germany, *ES* Spain, *FI* Finland, *NL* the Netherlands, *SE* Sweden, *UK* United Kingdom

Codes: (*) EU Member State performing initial evaluation of environmental risk assessment provided by applicant; ∞ risk assessment ongoing; ✔ risk assessment finalised; ✘ risk assessment (for cultivation) stopped; RX renewal applications for the continued marketing of the GM plant

assessment such as the selection of comparators have been developed by the EFSA GMO Panel with the support of its working groups and EFSA's GMO Unit (Table 3). These guidelines assist applicants in the preparation and presentation of their applications by describing elements and data requirements for the risk assessment and monitoring of GMOs. EFSA uses these guidelines in the evaluation of risk assessments and post-market environmental monitoring plans submitted by applicants as part of their GMO applications.

During the development of guidelines, EFSA consulted EU Member States and all relevant stakeholders via online public consultations. Scientific comments received during these consultations were considered when finalising the guidelines. In specific

Table 3 Overview of guidelines on the risk assessment and monitoring of GMOs issued by the EFSA GMO Panel (August 2013)

Years	Food and feed safety assessment (including molecular characterisation)	Environmental risk assessment	Post-market environmental monitoring	Other	References
2003					
2004	GMPs	GMPs	GMPs		–
2005					
2006	GMPs (revised)/ GMMs	GMPs (revised)/ GMMs	GMPs (revised)/ GMMs	Renewal of authorisation of existing GMOs	EFSA (2006a,c,f,h)
2007				GMPs containing stacked events	EFSA (2007a)
2008	GMPs (revised)				EFSA (2008a)
2009				GMPs used for non-food/ feed purposes	EFSA (2009f)
2010		GMP (revised)		Statistical considerations/ allergenicity of GMPs & GMMs/NTOs	EFSA (2010a,b,c,d)
2011	GMPs (revised)/ GMMs (revised)	GMMs (revised)	GMPs (revised)/ GMMs (revised)	Selection of comparators	EFSA (2011a,b,c,e)
2012	GMAs				EFSA (2012a)
2013		GMAs	GMAs		EFSA (2013b)

Abbreviations: GMA GM animal, *GMM* GM microorganism, *GMP* GM plant, *NTO* non-target organism

cases, workshops were also held to discuss and clarify received comments with involved EU Member States and relevant stakeholders.

Risk assessment of GM plants and derived food and feed

The first guidelines for the risk assessment of GM plants and derived food and feed developed by EFSA were adopted in 2004, revised in 2005, and published in 2006 (EFSA 2006a). Since 2006, initiatives were taken to further update the guidelines for the risk assessment of food and feed from GM plants (EFSA 2011b), and to incorporate the various scientific outputs delivering guidelines on specific aspects, such as: (1) the risk assessment of stacked events (EFSA 2007a); (2) the selection of comparators for the risk assessment of GM plants and derived food and feed (EFSA 2011a); (3) considerations for the generation, analysis and interpretation of compositional data to support the comparative analysis of GM plants (EFSA 2010a, see below); (4) the role of animal feeding trials in the safety and nutritional assessment of GM plants and derived food and feed (EFSA 2008b); and (5) the assessment of allergenicity of GM plants and derived food and feed (EFSA 2010b, see below) (Table 3).

The updated guidelines (EFSA 2011b) outline the principles of the risk assessment of food and feed containing, consisting or produced from GM plants, and provide definitions of the different steps and objectives of the risk assessment process.

Molecular characterisation Each GM plant application must contain an appropriate molecular characterisation of the GM plant intended to be placed on the market. Molecular characterisation data inform on the genetic aspects of the GM plant, from the origin of the sequences conferring the trait, the method used to introduce them into the plant's genome, the characterisation of the sequences inserted, to the expression and stability of the trait. The updated guidelines define the requirements for the initial molecular characterisation dataset (EFSA 2011b). As the risk assessment is carried out on a case-by-case basis, additional or specific information may be needed on certain aspects to allow EFSA to draw a conclusion. Further details about the data requirements for the molecular characterisation of

GM plants are given in the Electronic Supplementary Material.

The assessment of data in the molecular characterisation may flag aspects that require further evaluation. Potential safety issues identified with the molecular characterisation should be addressed in the relevant complementary part(s) of the risk assessment (e.g., toxicological, nutrition). Depending on the outcome of these studies, further toxicological and nutritional information may be requested.

Comparative analysis The risk assessment of GM plants includes a comparative analysis in which appropriate methods are used to compare the GM plant with its conventional counterpart (e.g., Paoletti et al. 2008). The underlying assumption of the comparative approach is that traditionally cultivated non-GM crops have gained a history of safe use for consumers and/or animals. This approach enables to place the importance of risks posed by a GM plant and derived food and feed products in the context of those posed by its comparator, by assessing whether intentionally and unintentionally modified properties of the GM plant alter the level of risk or give rise to additional risks. The relevance of the observed intended and unintended changes to human and animal health is further assessed by investigating the toxicological, allergenic and nutritional properties of the GM plant.

In the comparative analysis, a two-step approach is followed, which starts with the identification of possible differences between the GM plant and its appropriately selected comparator (=proof of difference); and which is then followed by an assessment of whether the characteristics of the GM plant fall within the range of natural variation estimated from a set of non-GM reference varieties with a history of safe use (=proof of equivalence) (van der Voet et al. 2011). These two steps are complementary, as statistically significant differences may point to biological changes caused by the genetic modification, but these may or may not be meaningful in terms of harm to humans, animals and the environment (EFSA 2011f). Should a statistically significant difference be within the range known for the plant, then it can be considered biologically insignificant; otherwise, it will constitute an unintended change that requires consideration in the risk assessment.

The updated guidelines give special attention to the design and statistical analysis of field trials that are performed to generate compositional and agronomic/phenotypic data to support the comparative analysis of GM plants (see Electronic Supplementary Material for more details). The given recommendations will ensure that future compositional and agronomic/phenotypic field trials have sufficient statistical power to detect intended and unintended changes in the GM plant, and enable a reliable estimation of natural variability.

To support the implementation of the statistical analysis of compositional and agronomic/phenotypic data in line with the requirements laid down in the updated guidelines, EFSA commissioned the development of user-friendly software that can be used to analyse the data derived from the compositional and agronomic/phenotypic field trials according to the EFSA requirements (EFSA 2010a). The software is expected to be released on EFSA's website during the course of 2014, and it will be freely available to all stakeholders that would need to analyse compositional and agronomic/phenotypic datasets provided as part of GM plant applications. To ease EFSA's appraisal of the compositional and agronomic/phenotypic analyses, applicants are required to provide raw data in a suitable electronic format.

Toxicology The approach followed by EFSA in the toxicological risk assessment of GM plants is consistent with that of chemicals: hazards associated with biologically relevant changes in the GMO are characterised and the exposure levels are determined, in order to make an estimation of the risk. In the updated guidelines, reference is made to the internationally agreed protocols for the toxicological assessment of chemicals that can be selectively used for the assessment of GMOs, focusing on the newly expressed proteins, other new constituents, and natural compounds the levels of which may have been altered due to the genetic modification (EFSA 2011b).

Depending on the evaluation of the novel proteins and the outcome of the comparative assessment, further toxicological testing of the whole GM food and feed may be required on a case-by-case basis (EFSA 2011b). EFSA published two separate guidelines that provide recommendations on the use of animal feeding trials in characterising hazards in the GMO risk assessment and detecting any possible toxicological effects of the test diet compared to the control diet (EFSA 2008b), as well as on the protocol to be followed when whole food and feed is to be

tested in a 90-day repeated dose toxicity study in rodents (EFSA 2011i). The guidelines recommend the use of a randomised block test design to maximise the power of the experiment, while avoiding unnecessary use of test animals. To reduce stress in the test animals, housing rodents as pairs was advised.

Allergenicity The proposed approach to assess allergenicity of GM plants and derived food and feed in the updated guidelines focuses on IgE-mediated food allergy (e.g., allergic reactions to peanuts or soy), as it provokes the most severe allergic reactions including anaphylaxis (EFSA 2010b, 2011b). Because there is not a single test that can predict the allergenicity properties of a protein, a weight-of-evidence approach is recommended to be followed, in order to assess the allergenicity of newly expressed proteins (Liu et al. 2013). The cumulative body of evidence necessary for this assessment is based on amino acid sequence similarity comparison (bioinformatics search), specific serum screening, pepsin resistance test, other in vitro digestibility tests and, possibly, other studies (e.g., in vitro assays and in vivo models). EFSA (2010b, 2011b) also underlines the importance of including an adjuvanticity assessment of the newly expressed proteins in the allergenicity assessment, as the combined exposure to an adjuvant and an antigen may boost the immune response of an allergic individual/animal to that particular antigen causing more severe adverse reactions than when exposed to the antigen only.

When the plant receiving the new gene(s) is known to be allergenic, its allergenicity is to be compared with that of the appropriate comparator(s), taking into account natural variation (Fernandez et al. 2013). Although the approach to be followed depends on the available information on the allergenicity of the recipient plant, the updated guidelines recommend: the use of analytical methodologies in conjunction with sera of allergenic humans; the inclusion of allergens among the endpoints tested in the compositional analysis; and the immunological testing with sera collected from animals experimentally sensitised (EFSA 2011b).

The updated guidelines also consider the assessment of non-IgE-mediated adverse reactions (e.g., allergic eosinophilic gastroenteropathies) on a case-by-case basis (EFSA 2011b). Because the guidelines do not provide detailed information on how and in which cases the latter assessment should be performed, EFSA recently commissioned a literature review of non-IgE-mediated adverse reactions as well as their relevance in the field of food/feed safety. In addition, a literature review was requested on protocols to be followed in the in vitro digestibility tests to reflect the physiological conditions of digestion and to assess the interaction between the newly expressed protein(s) and the food matrix. The outcomes of these reviews will be considered by the EFSA GMO Panel for further discussions.

Recently, EFSA jointly organised a workshop with the Competent Authorities of Austria and Norway to initiate an in-depth discussion on the possible routine inclusion of allergens in the compositional analysis for the allergenicity assessment of GM plants (EFSA 2012u).

Nutrition Food and feed derived from GM plants intended to be placed on the EU market should not be nutritionally disadvantageous to humans and animals. The nutritional assessment as described in the updated guidelines should address the nutritional relevance in the total diet for the consumers/animals of the newly expressed protein(s), as well as the nutritional relevance of other possible new constituents and of changes in the levels of endogenous constituents in the GM plant and derived food and feed (EFSA 2011b). If the comparative compositional assessment does not indicate any relevant difference between the GM plant and its appropriately selected comparator (usually its near-isogenic non-GM counterpart), except for the introduced trait(s), nutritional equivalence can be inferred and no further nutritional studies are needed. However, when this cannot be demonstrated, properly designed animal feeding studies are necessary. For GM plants with an altered content of nutrients, animal studies with model or target species (e.g., poultry, pigs, ruminants, fish) are to be performed, in order to determine the bioavailability of individual nutrients and their impact on animal performance and feed safety (EFSA 2011b; Liu et al. 2013).

Implementing regulation The updated guidelines for the risk assessment of GM plants and derived food and feed (EFSA 2011b) were used by the European Commission and EU Member States as a basis for the preparation of the Implementing Regulation on applications for authorisation of GM food and feed in

accordance with Regulation (EC) No 1829/2003 of the European Parliament and the Council. This Regulation has been adopted in February 2013 by EU Member States and will come into force six months after its publication (EC 2013). GM plant applications submitted to EFSA after the entry into force of the Regulation will need to comply with it. In this Regulation, there are some important differences in data requirements compared to EFSA's updated guidelines. These differences include the requirement: (1) to perform a 90-day feeding study in rodents with whole GM food/feed, irrespective of the quality of the available data provided for the risk assessment and case-by-case principle advocated by the EFSA GMO Panel (see also Kuiper et al. 2013; Herman and Ekmay 2013); (2) to reduce the amount of inserted DNA not necessary to achieve the desired trait(s); (3) to aim for the development of GMOs without antibiotic resistance marker genes (see below); (4) to assess potential effects on off-target genes, in the case of RNA interference (RNAi)-based traits; (5) to re-sequence DNA inserts and the regions flanking DNA inserts of GM plants containing stacked transformation events and compare them with the nucleotide sequence of the respective single events; and (6) to perform a systematic review of studies, which consider potential adverse effects on human and animal health of the GM food and feed, published in the scientific literature.

Environmental risk assessment of GM plants

Some details of approaches to be used to assess the potential risks that the cultivation of GM plants and their associated farm management and cropping practices may pose to the environment remain a point of debate in the EU (e.g., EFSA 2008a; Dolezel et al. 2011; Graef et al. 2012). In a continuous effort to improve the environmental risk assessment of GM plants and to account for the latest developments in the field, several working groups contributed to elaborate the updated guidelines for the environmental risk assessment of GM plants (EFSA 2010d). The focus was on four areas: potential effects on non-target organisms; new criteria for design and analysis of field trials; characterisation of different relevant receiving environments within the EU where GM plants may have environmental effects; and techniques to assess potential long-term effects.

The work on non-target organisms resulted in separate guidelines (EFSA 2010c) from which the essence was integrated into the guidelines on the environmental risk assessment of GM plants (EFSA 2010d). Specific requirements were given for data to evaluate possible adverse effects on non-target organisms. For the assessment of potential adverse effects on non-target organisms caused by the intended genetic modification (e.g., the expression of the *Bt*-protein) in the GM plant, a tiered testing approach needs to be followed that progresses from highly controlled worst-case exposure lower-tier studies in the laboratory over greenhouse studies to more realistic but less controlled higher-tier studies in the field (Romeis et al. 2008). The guidelines also require an assessment of potential adverse effects arising from unintended changes in the GM plant, which go beyond the primary objectives of the genetic modification. For the assessment of such effects, a weight-of-evidence approach is proposed that relies on *in planta* (event-specific) data; field or laboratory studies with *in planta* material are considered suitable means to retrieve data on interactions of the GM plant and its comparator with non-target organisms. In these studies, one focal species of each relevant functional group needs to be tested. The updated guidelines require 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 (Perry et al. 2009).

For the selection of focal species for risk assessment, and if required, for testing purposes, a four-step approach combining the strengths of two existing species selection approaches—the ecological and ecotoxicological approach—is proposed (Andow and Hilbeck 2004; Romeis et al. 2008): step 1: to identify functional groups being directly or indirectly exposed to the GM plant; step 2: to list non-target organisms associated to the functional groups identified in step 1; step 3: to identify and prioritise species of non-target organisms from the list built in step 2 based on ecological criteria (such as species exposure to the GM plant under field conditions, relevance to ecosystem functioning); and step 4: to select focal non-target organism species per functional group from the list built in step 3 based on practical considerations (such as testability, species abundance, conservation status).

To facilitate the identification of most abundant and widespread arthropod species, as well as the contribution of taxa to particular valued ecosystem services, EFSA commissioned the compilation of a database on arthropods found in different crops in the EU in 2010. The fauna database provides a detailed overview of the composition of the arthropod fauna and the abundance of species found in maize, oilseed rape, potato, sugar/fodder beet, soybean, cotton and rice, and in different geographic regions across Europe. Species attributes and abundance data, retrieved from over 1000 publications, give ecological information for 3030 arthropod species and 14762 abundance records from 31 European countries (Meissle et al. 2012).

The structure of the environmental risk assessment 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 environmental risk assessment process starts with the crucial first step of problem formulation which facilitates a structured approach to identifying potential risks and scientific uncertainties (Raybould 2006; Wolt et al. 2010; Gray 2012). Problem formulation is then followed by five further steps in which all other relevant issues are addressed. The six-step procedure is applied to each of the eight areas of risk listed in the Directive. For each area of risk, applicants should specify which protection goals are applicable to their environmental risk assessment, and what assessment and measurement endpoints they use (see also Nienstedt et al. 2012; Sanvido et al. 2012). The guidelines for the environmental risk assessment of GM plants are currently used by the European Commission and EU Member States as a basis for the revision of the annexes of Directive 2001/18/EC (in progress). Further details about the environmental risk assessment of GM plants are given in the Electronic Supplementary Material.

Risk assessment of GM plants used for non-food or non-feed purposes

EFSA's remit includes products other than food and feed relating to GMOs as defined by Directive 2001/18/EC, which excludes medicinal products for human and veterinary use. Therefore, GM plant applications could include plants used for ornamental purposes, phytoremediation, the production of non-food enzymes, or as biofuels. To account for specific consideration for such applications, guidelines for the risk assessment of GM plants used for non-food or non-feed purposes were developed and issued in 2009 (EFSA 2009f). Until now, EFSA has not given scientific advice on GM 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.

Post-market environmental monitoring of GM plants

In 2006, EFSA published guidelines on post-market environmental monitoring (PMEM) (EFSA 2006c), which were updated in 2011 (EFSA 2011e). In the EU, the objectives of PMEM according to Annex VII of Directive 2001/18/EC and the Council Decision 2002/811/EC are: to confirm that any assumptions regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment are correct; to identify possible unanticipated adverse effects on human health or the environment which could arise directly or indirectly from GM plants and which were not anticipated in the environmental risk assessment; and to further inform the environmental risk assessment. The scientific knowledge obtained during monitoring of GMOs, along with the experience gained from their marketing/cultivation as well as any other new knowledge generated through research, can provide valuable information to risk assessors to update environmental risk assessments and to resolve any remaining scientific uncertainty. The updated guidelines stress how information from PMEM may feed back into and strengthen the original environmental risk assessment.

The updated guidelines make recommendations for both case-specific monitoring and general surveillance, and indicate how the conclusions of the environmental risk assessment (including implemented risk mitigation measures) determine the requirements for case-specific monitoring to resolve the remaining scientific uncertainty. Case-specific monitoring is not obligatory but may be required when the environmental risk assessment identifies a particular potential risk that needs mitigation during cultivation, or for which scientific uncertainty remains. General surveillance is mandatory and aims to detect unanticipated adverse effects. Due to different objectives of case-specific monitoring and general surveillance, their underlying concepts differ

(Sanvido et al. 2005). Case-specific monitoring is designed to determine whether, and to what extent, potential anticipated adverse effects occur. It is mainly triggered by scientific uncertainties that were identified in the environmental risk assessment. Therefore, a hypothesis is established that can be tested on the basis of newly collected monitoring data. New methodology proposed for case-specific monitoring in the updated guidelines includes requirements for experimental design and analysis. The objective of general surveillance is to detect any unanticipated adverse effects on the environment including biodiversity and ecosystem services that may be due to the cultivation of the GM plant. By nature, the prediction of unanticipated effects does not lend itself to the formulation of defined scientific hypotheses. Therefore, the general status of the environment that is associated with the release of the GM plant into the environment is monitored without any preconceived hypothesis. Should any such effects be observed, they are to be studied in more detail to determine whether the effect is adverse, and whether it is associated with the use of a GM plant or the result of other environmental and cultivation factors.

The updated guidelines describe various sources of information that can deliver general surveillance data and make recommendations on how their use, design and analysis can be best optimised. General surveillance data can originate from farmer questionnaires, existing monitoring/surveillance networks (e.g., plant health surveys, soil surveys, ecological and environmental observations), scientific literature, industry stewardship programs and alert issues (Sanvido et al. 2005, 2011). Further details about the type of sources that can deliver general surveillance data are given in the Electronic Supplementary Material.

The updated guidelines also make proposals for the establishment of harmonised reporting centres for collecting and analysing monitoring/surveillance data at the EU Member State level.

Submission guidelines for GM plant applications

To complement the guidelines for the risk assessment of food and feed from GM plants (EFSA 2011b) and for the environmental risk assessment of GM plants (EFSA 2010d), EFSA established guidelines on the submission of GM plant applications in 2011 that were updated in 2012 (EFSA 2011d). The guidelines describe the community procedures for processing GM plant applications in the EU, and provide instructions to applicants on how to prepare and present data in an application to be submitted to EFSA. It is supplemented with ten appendices that give examples of data presentation and a completeness checklist to be completed by applicants. This checklist clearly distinguishes minimum requirements from requirements for non-mandatory studies. It should ensure that applications fulfil the scientific requirements at their submission to EFSA, as this eases checking the completeness of GM plant applications. The other appendices of the submission guidelines aim to harmonise the structure of GM plant applications and to summarise the large amount of data contained in a GM plant application in a succinct manner. The recent publication of the Implementing Regulation (EC, 2013) necessitates the revision of EFSA's guidelines, in order to mirror the new data requirements outlined in the Implementing Regulation (see above).

Risk assessment of GM microorganisms and derived products intended for food and feed use

Microorganisms are not in the scope of the peer-review journal Transgenic Research. Further details on the guidelines for the risk assessment of GM microorganisms and their products intended for food and feed use (EFSA 2006f, 2011c) are therefore given in the Electronic Supplementary Material.

Risk assessment of GM animals

The EFSA GMO Panel, together with EFSA's AHAW (Animal Health and Welfare) Panel, developed guidelines on the food and feed risk assessment of GM animals, including health and welfare aspects (EFSA 2012a). The proposed strategy for the health and welfare assessment of GM animals and the risk assessment of GM animal-derived food and feed seeks to deploy appropriate approaches to compare GM animals and derived food and feed with their respective comparators. The underlying assumption of this comparative approach is that traditionally-bred animals have a history of consumption as food and feed for the average consumer or animal to which the animal-derived products are fed. Therefore, traditionally-bred animals can serve as a baseline for the food and feed safety assessment of GM animals or their products and the welfare assessment of GM animals. These guidelines also describe data

requirements pertaining to the molecular characterisation of GM animals, the comparative analysis of phenotypic characteristics, the toxicological and allergenicity assessment of newly expressed proteins as well as of the whole food derived from GM animals, and the nutritional assessment, in order to evaluate whether products derived from GM animals are as safe as those from traditionally-bred animals. Scientific requirements for the assessment of health and welfare of GM animals are provided too. The health status of a food and feed producing animal has traditionally been considered as an important indicator of the safety of derived food and feed and therefore an important component in the risk assessment. This assessment is made in terms of the effective functioning of their body systems in a given environment. More precise information may be gained by comparing the health and welfare of GM animals with those of their comparators. Where no comparator can be identified, an assessment of health and welfare of the GM animal itself is considered.

The guidelines for the environmental risk assessment of GM animals contain separate chapters addressing the principal aspects for fish, insects, terrestrial mammals and birds; elaborate case studies; and give generic considerations on the choice of comparators, the use of non-GM surrogates, the long-term effects, the uncertainty analysis, modelling requirements and statistical principles (EFSA 2013b). General guidelines for drawing conclusions on the post-market environmental monitoring of GM animals are also provided.

In order to gather the necessary background information in the area of the environmental risk assessment of GM animals, EFSA commissioned three external scientific reports about GM fish, GM insects, GM mammals and GM birds that may be the subject of EU applications for market release within the next decade. Moreover, relevant fields of expertise as well as the essential elements to be considered when performing an environmental risk assessment were identified. The considerations provided in these external scientific reports by Cowx et al. (2010) for GM fish, Benedict et al. (2010) for GM insects, and Henry et al. (2010) for GM mammals and GM birds served as a basis for the identification of scientists with relevant expertise and the development of the guidelines. EFSA also organised workshops, in order to discuss and review the draft external reports on GM fish, GM mammals and GM birds.

Specific issues

In addition to the evaluation of the scientific risk assessment of GMO applications and the development of risk assessment and monitoring guidelines, EFSA provides scientific advice on specific issues upon request of the European Commission; examples are national safeguard clause and emergency measures, annual PMEM reports, the consideration of potential risks associated with new biotechnology-based plant breeding techniques, evaluations of previously assessed GMOs in the light of new scientific publications, and the use of antibiotic resistance marker genes in GM plants. In accordance with Article 31 of Regulation (EC) No 178/2002, EFSA has also been active in providing assistance to urgent requests of the European Commission and EU Member States on public health risks or emergency situations (Deluyker and Silano 2012). A recent example in the area of GMO risk assessment is the assessment of the scientific publications by Séralini et al. (2012a,b).

National safeguard clause and emergency measures

Based on new scientific evidence related to the safety of a GM product, EU Member States can invoke safeguard clause measures under Article 23 of Directive 2001/18/EC or emergency measures under Article 34 of Regulation (EC) 1829/2003, in order to provisionally restrict or prohibit the commercial use of previously authorised GMOs on their territory. So far, safeguard clause and/or emergency measures have been invoked by Austria, France, Greece, Germany, Hungary, Italy and Luxemburg for several GM maize, oilseed rape and potato events (Table 4), with most of such measures invoked for maize MON 810 (Sabalza et al. 2011; Devos et al. 2012; Raybould 2012; Kuntz et al. 2013). For all cases where EFSA has been asked by the European Commission to evaluate whether the invocation was justifiable on the basis of the scientific information submitted in support of a safeguard clause or emergency measure, it concluded that there was no new specific scientific evidence in terms of risk to humans and animals and the environment that would support the invocation of such measures and that would invalidate its previous risk assessment conclusions (EFSA 2004a, b, 2005c, 2006e,g, EFSA 2008c, d, e, g, 2009d, e, 2012f, h, i, j, k, t, 2013c,d). It has been argued that the recurrent debate on safeguard clause

and emergency measures is driven by a dispute over values owing to an ambiguous interpretation of what constitutes harm. As long as there is dispute over values, natural sciences can offer little to resolve value differences, and instead of a scientific debate, a political one takes place (Raybould 2012; Sanvido et al. 2012).

Annual post-market environmental monitoring reports

Since 2007, EFSA has been asked to play an increasing role in recommendations for risk management for GM plants. Therefore, in 2010, EFSA established a working group to evaluate the yearly reports on the PMEM for all cultivated GM plants in the EU. These monitoring activities and their annual reporting are designed to detect and limit possible adverse environmental effects, including those that are long-term. In the EU, maize MON 810 has been cultivated since 1999, with approximately 129000 ha grown in 2012 [mainly in Spain (90 %), followed by Portugal (7 %), the Czech Republic (2 %), and Romania and Slovakia (both <1 %)]. Potato EH92-527-1 has been grown in 2010 and 2011 on a maximum acreage of 225 ha in Sweden, Czech Republic and Germany, but its cultivation was discontinued in 2012. This working group has assessed the appropriateness of the methodology used for the annual PMEM reports for the 2009 and 2010 cultivation seasons of maize MON 810 and for the 2010 and 2011 cultivation seasons of potato EH92-527-1 (EFSA 2011g, 2012b, d, p), with the technical support from EFSA's Scientific Assessment Support (SAS) Unit. The annual PMEM reports are available on the European Commission's website (http://ec.europa.eu/food/food/biotechnology/index_en.htm).

EFSA gave recommendations on how case-specific monitoring, the use of farmer questionnaires and surveillance/monitoring networks and the reviewing of scientific literature could be optimised further. With regard to the use of farmer questionnaires, the procedure for the identification and sampling of farmers to be surveyed should ensure that the monitored area is proportional to and representative of the total regional area under GMO cultivation. Therefore, the largest proportion of farmers should be those that had previously cultivated the GM plant and come from regions with a high uptake of the GM plant. Overall, the analysis of the results of farmer questionnaires, the observations by national surveillance/monitoring networks and the review of the scientific literature did not indicate any adverse environmental impacts associated with the cultivation of maize MON 810 and potato EH92-527-1 (EFSA 2011g, 2012b, d, p).

New biotechnology-based plant breeding techniques

Technological advances in genetics and genomics led to the development of a cohort of new biotechnology-based plant breeding techniques that differ in part substantially from those used in the last two decades to develop GM plants. These new techniques enable the

Table 4 Safeguard clause and emergency measures invoked by EU Member States and for which the EFSA GMO Panel was asked to provide a scientific advice (August 2013)

Transformation event	Plant	EU Member States							Total per event
		AT	DE	EL	FR	HU	IT	LU	
MON810	Maize	2×		3×	2×	2×	1×	1×	11×
MON863	Maize	2×							2×
Bt176	Maize	1×	1×					1×	3×
T25	Maize	2×							2×
GT73	Oilseed rape	3×							3×
Ms8, Rf3 + Ms8xRf3	Oilseed rape	3×							3×
Topas 19/2	Oilseed rape			1×	1×				2×
Ms1, Rf1 + Ms1xRf1	Oilseed rape				1×				1×
EH92-527-1	Potato	1×				1×		1×	3×
Total per EU Member State		14×	1×	4×	4×	3×	1×	3×	30×

EU Member State abbreviations: *AT* Austria, *DE* Germany, *EL* Greece, *FR* France, *HU* Hungary, *IT* Italy, *LU* Luxembourg

transfer of limited amounts of DNA between related genotypes from the breeders' gene pool, as well as the introduction of specific modifications to plant genomes through targeted mutagenesis by using tailor-made molecular tools such as zinc-finger nucleases or oligonucleotides (Gaj et al. 2013; Podevin et al. 2013). In some cases (for example, zinc finger nuclease type 3), the insert is highly targeted within the plant genome, unlike in transgenesis. They also allow breeders to modify traits without making changes to genome sequences—for instance, through epigenetic changes by inducing DNA methylation or by reconstituting a desired plant variety through reverse breeding. In addition, these new techniques enable breeders to create so-called 'cisgenic' or 'intragenic' plants by inserting a sequence from a sexually compatible species (Podevin et al. 2012).

Because some of the new plant products obtained by using these new biotechnology-based breeding techniques have been subject to field trials in the EU and a number of them are now approaching commercialisation, the European Commission established a working group to explore the potential regulatory status of eight new biotechnology-based plant breeding techniques. The techniques under assessment were: (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; and (8) synthetic biology. Appointed scientific experts from EU Member States considered the full spectrum of new techniques, and assessed whether the use of these techniques would lead to a GMO as defined under Directive 2001/18/EC. In 2012, the working group finished the evaluation and circulated its opinion for comments. In the meantime, a working group led by the Joint Research Centre of the European Commission reported on the state of the art and prospects from commercial development of new biotechnology-based plant breeding techniques (Lusser et al. 2012).

In 2011, EFSA was requested by the European Commission to address two questions with regard to the 8 new biotechnology-based plant breeding techniques. The first question was to determine whether there is a need for new guidelines or whether the existing guidelines on risk assessment should be updated or further elaborated in advance of such products entering the marketplace. The second one was to assess the risks in terms of impact on humans and animals and the environment that the use of the techniques could pose, irrespective of whether or not they fall under the GMO legislation. The techniques will be addressed in a phased approach, and techniques considered so far are cisgenesis, intragenesis and zinc finger nuclease technology. In its scientific opinion, the EFSA GMO Panel was of the opinion that cisgenic and conventionally-bred plants represent similar hazards, whereas intragenic and transgenic plants could raise new hazards. Whether or not identified hazards translate into risks to human and animal health and the environment depends on exposure; for instance, the extent to which the plant is cultivated or its derived products are consumed. However, if cisgenic plants were to be considered GM plants in the EU, the EFSA GMO Panel considered that its existing risk assessment guidelines for plants and products developed through transgenesis would generally apply to cisgenic and intragenic plants, but that they would require less event-specific data, depending on the specific case (EFSA 2012c). Similar conclusions were drawn for plants developed through zinc finger nuclease type 3 and other Site-Directed Nucleases with similar function (EFSA 2012n).

Evaluation of previously assessed GMOs in the light of new scientific publications

Scientific publications are monitored continually by EFSA and relevant new work is examined to determine whether it raises any new safety concern. At the request of the European Commission, EFSA recently reconsidered the validity of its previous risk assessment conclusions and risk management recommendations on maize MON 810, Bt11 and 1507 in the light of new scientific publications (EFSA 2006b, 2008f, 2011h, 2012m, 2012r, s). Overall, no new peer-reviewed scientific publications were identified reporting information that would invalidate EFSA's previous conclusions on the safety of maize MON 810, Bt11 and 1507. Furthermore, using a mathematical model of exposure to better quantify the potential risk to the larvae of non-target Lepidoptera due to the ingestion of *Bt*-maize pollen deposited on their host plants, under representative EU cultivation conditions (EFSA 2009c; Perry et al. 2010, 2011, 2012, 2013), the risk for species with a range of susceptibilities to

Bt-proteins and the efficacy of simulated mitigation measures consisting of non-*Bt*-maize strips of different width placed around the *Bt*-maize field edge were estimated (EFSA 2011h, j, 2012q). The model accounts for three types of parameters: (1) parameters concerned with mortality (considering five assumed levels of susceptibility ranging from below-average to extremely high levels of susceptibility to the *Bt*-protein); (2) small-scale parameters (considering two assumed within-crop host-plant densities and a range of nine levels of mitigation in the form of sown strips of non-*Bt*-maize); and (3) five large-scale parameters. Mortality is estimated in two phases; firstly locally, using the 'small-scale' parameters, and then globally, using the 'large-scale' parameters. The model enables one to identify under which situations non-target lepidopteran species with different levels of susceptibility to a *Bt*-protein might be at risk and for which situations risk mitigation and monitoring may be required or not. No model is ever complete, and in this case the availability of data for EU Lepidoptera is limited. However, steps to collect appropriate data are currently being taken in the context of the EU-funded AMIGA project (www.amigaproject.eu).

At the urgent request of the European Commission, EFSA reviewed the recent publication by Séralini et al. (2012a) taking into consideration assessments conducted by EU Member States (Annex 1 of EFSA 2012o) and any clarification given by the authors (Séralini et al. 2012b). Séralini et al. (2012a) reported on a two-year feeding study in rats investigating the health effects of maize NK603 with and without Roundup WeatherMAX® and Roundup® GT Plus alone (both contain the herbicidal active substance glyphosate). The assessments of EU Member States and EFSA revealed an overall agreement, namely that the study as reported by Séralini et al. (2012a) was found to be inadequately designed, analysed and reported, in spite of the additional clarifications provided by the authors (Séralini et al. 2012b). Therefore, no conclusions can be drawn on the difference in tumour incidence between treatment groups on the basis of the design, the analysis and the results as reported (see also the review by Arjó et al. 2013). EFSA found that the study as reported by Séralini et al. (2012a) is of insufficient scientific quality for safety assessments. EFSA therefore concluded that the currently available evidence does not impact on the ongoing re-evaluation of glyphosate and does not call for the reopening of the safety evaluations of maize NK603 and its related stacks (EFSA 2012l, o). Previously, the Séralini et al. (2007) publication on the statistical evaluation of a 90-day animal feeding study with maize MON 863 was also reviewed by EFSA at the request of the European Commission, in order to identify any consequences for its risk assessment of maize MON 863. The publication presented an alternative statistical analysis of the 90-day rat study that was considered in the original risk assessment. Following a detailed statistical review and analysis, it was concluded that the reanalysis of the data did not raise any new safety concerns (EFSA 2007b).

Antibiotic resistance marker genes in GM plants

Marker genes encoding resistance to specific antibiotics may be used in genetic modification to help identify GM cells among the untransformed cells. A possible hazard associated with the presence of antibiotic resistance marker (ARM) genes in GM plants is the acquisition and dissemination of those genes by pathogenic bacteria, as this could increase the environmental pool of antibiotic resistance and ultimately compromise clinical therapy of infections (Nielsen et al. 1998). The potential of horizontal gene transfer of ARM genes in GM plants to microorganisms is to be considered in the risk assessment. Such risk assessments have generally concluded that: (1) the frequency of horizontal gene transfer from GM plants towards microbial populations, if occurring, is considered to be very low compared with gene transfer between bacteria; (2) the risk arising from any such gene transfer is, at worst, slight; but that (3) their use should be strongly discouraged when they could compromise the use of clinically relevant antibiotics (Ramessar et al. 2007; Keese 2008).

In June 2009, EFSA published a consolidated overview on the use of ARM genes in GM plants, including a joint scientific opinion by EFSA's GMO and BIOHAZ Panels. The Panels concluded that, according to information currently available, adverse effects on human health and the environment resulting from the transfer of the two ARM genes, *nptII* (kanamycin/neomycin resistance) and *aadA* (streptomycin/spectinomycin resistance), from GM plants to bacteria, associated with use of GM plants, are unlikely (EFSA 2009a). The rationale leading to this

conclusion built on the fact that the transfer of ARM genes from GM plants to bacteria has not been shown to occur either in natural conditions or in the laboratory in the absence of sequence identity in the recipient bacterial cell. Sequence identity is necessary to allow homologous recombination between the transformed DNA in the plant and bacterial DNA. Moreover, DNA transfer from GM plants to bacteria, if occurring, would be at a frequency several orders of magnitude lower compared with gene transfer between bacteria; metagenomic analyses of total bacterial populations (including non-cultivable bacteria) have demonstrated that resistance determinants of kanamycin, neomycin and streptomycin are present in all environments investigated. Such resistance genes may be selected from this environmental reservoir and disseminated among bacteria. The Panels also pointed out that the presence of antibiotics and antibiotic usage in different environments are key factors in driving the selection and dissemination of antibiotic resistance genes among bacteria.

It was acknowledged by the GMO and BIOHAZ Panels that the increasing occurrence worldwide of "extensively drug-resistant" (XDR) isolates of tuberculosis (TB) with resistance to second-line antibiotics such as kanamycin is a cause for global concern. However, the *npt*II gene has not been implicated in such resistance. Resistance to kanamycin in XDR-TB strains is chromosomally encoded and has not developed as a result of the transfer of aminoglycoside resistance genes, such as *npt*II (Johnson et al. 2006; Zaunbrecher et al. 2009; Georghiou et al. 2012).

The remaining scientific uncertainties regarding the risk associated with the presence of ARM genes in GM plants are due to limitations related, among others, to sampling and detection, as well as challenges in estimating exposure levels and the inability to assign transferable resistance genes to a defined source.

Other activities

EFSA is involved in risk communication tasks pertaining to GMOs, contributes to expand scientific cooperation with EU Member States and national risk assessment bodies, and responds to external questions related to the risk assessment of GMOs. Such questions come from the European Commission, members of the European Parliament, EU Member States, the general public, as well as from various stakeholders, such as applicants and environmental non-governmental organisations.

On a yearly basis, EFSA engages in meetings with stakeholders active in the GMO field. The purpose of these meetings is to update participants on the continuing work carried out by EFSA in this area, and to ensure that interested groups have a dedicated forum for discussion with EFSA. On the one hand, EFSA invites representatives from the industry for a discussion on technical aspects of GMO applications in order to streamline the workflow of validation and assessment of such applications. On the other hand, EFSA meets with non-governmental organisations, including consumers' groups, which have an interest on the safety of GMOs, to promote an interactive dialogue on different aspects of EFSA's work.

EFSA also proactively engages with EU Member States. In 2010, EFSA launched the *Scientific Network for Risk Assessment of GMOs*, a forum in which appointed experts representing national agencies, authorities and ministries of all EU Member States can exchange information and expertise in GMO risk assessment. The aim of the Network is to enhance cooperation between scientists involved in GMO risk assessment at EFSA and in the EU Member States in order to harmonise risk assessment practices within the EU. The Network meets on a yearly basis, and delegates of the European Commission and of EU candidate and pre-candidate countries are invited as observers. GMO Panel members and other experts may be invited to deliver presentations and speeches on the issues discussed.

EFSA also participates in conferences, meetings and workshops organised by EU and non-EU safety assessment bodies and scientific organisations. Scientific officers explain the principles underlying the GMO risk assessment made by EFSA and inform and update on the status of the work.

Future challenges

In the next decade, EFSA will be faced with a number of challenges that may require the adjustment and revision of its current risk assessment guidelines or the development of new risk assessment strategies. The most important challenge relates to the increasing complexity and diversity of the next generation of GMOs. The landscape of GMOs is changing quickly with several new products being at various stages of

development or close to commercialisation. The development of GM plants targeted towards major compositional changes is progressing rapidly (Zhu et al. 2013). This includes, for example, the development of crops with modified metabolism and physiology to provide improved quality and enhanced nutritional profiles. The composition of these plants may be modified substantially, which raises challenges for the comparative analysis, as no appropriate comparator may be identified. Although a comprehensive safety assessment of the GM plant per se is advocated in cases where appropriate comparators are not available (e.g., where significant compositional changes have been targeted), little information is available on how such an assessment may work in practice (ADAS 2013). GM plants with stress tolerant phenotypes are now being widely tested in field trials around the world, with the first and most advanced drought tolerant maize being launched commercially in the United States in 2013. These plants may raise specific issues for the environmental risk assessment that will be more challenging to resolve compared with the first generation of GM plants (Wilkinson and Tepfer 2009; Rüdelsheim and Smets 2010). Another example, holding great promise for novel opportunities to manage target insect pests, is the use of RNAi (Burand and Hunter 2012). The ingestion of double stranded RNA (dsRNA) has been demonstrated to be effective in triggering RNAi in western corn rootworm (*Diabrotica virgifera virgifera* LeConte) (Baum et al. 2007; Rangasamy and Siegfried 2011; Bolognesi et al. 2012), a major coleopteran maize pest and a serious threat to agriculture in North America and the EU. RNA-based mechanisms have also been exploited in the production of several GM plants such as a virus resistant squash, papaya, plum, bean and potato, a delayed ripening tomato, and a soybean with altered oil composition. These mechanisms have also been used to improve crop nutritional values, reduce allergen levels, and improve agronomic characteristics (Parrott et al. 2010; Heinemann et al. 2013; Petrick et al. 2013).

Besides new traits, the next generation of GMOs will consist of new types of organisms that have not been previously assessed by EFSA, such as GM trees, GM animals and GM algae.

- Although GM tree applications in the EU are not expected in the near future, they might be in the next decade. A few GM trees are already commercially available outside the EU and the application of genetic modification techniques to trees is currently at an advanced stage, with experimental field trials being conducted in various countries (Walter et al. 2010; Häggman et al. 2013). Trees differ from crop plants in several important characteristics, and these differences will have to be accounted for during their risk assessment (Aguilera et al. 2013; Häggman et al. 2013). Trees are generally perennial, woody, long lived species with long life cycles taking several years to reach sexual maturity and commence reproduction. When mature, some can produce large amounts of seed and pollen that can disperse over long distances. The purpose of the GM tree will also determine relevant aspects to consider in the assessment. For example, cultivated forest and plantation trees are normally very similar to wild types and exchange genes with them (Fladung et al. 2012). In addition, they have complex interactions with a range of biota often supporting large diverse communities and food webs. They also have important roles in geographical and physical features of landscapes e.g. in stabilising hillsides, preventing erosion or influencing microclimates (Aguilera et al. 2013). With respect to GM orchard fruit trees, their cultivation techniques and management systems may differ from the conventional ones. Initiatives in the EU have already been taken to address the biosafety of GM trees (Fladung et al. 2012) and some risk assessment guidelines are already available from international discussions (OECD). Nevertheless, more detailed guidelines on challenging issues like stability, number and design of field trials, and choice of comparators to estimate current variation in commercial systems might be needed.
- No GM animals or derived products from GM animals are legally on the EU market, nor have GM animal applications been submitted to EFSA. Yet, future GMO applications may include the marketing of food and feed products derived from GM animals, and the release of GM animals, including companion animals, into the environment. The traits involved may be related to disease resistance, growth enhancement, sterility,

population suppression, cold tolerance, dietary performance and ornamental uses (Benedict et al. 2010; Cowx et al. 2010; Henry et al. 2010; Beech et al. 2012). The major environmental challenges centre on the peculiar characteristics of animals in comparison with plants, such as superior mobility and social behaviour (EFSA 2013b), and the fact that GM animals can have complex and context-specific interactions with wild populations (Van Eenennaam and Muir 2011; Oke et al. 2013). 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 animal with which it may be compared.

- The use of algae in biotechnological research and industry is increasing due to their potential to fix atmospheric carbon dioxide during photosynthesis, their growth potential on non-arable land and the short harvesting times. Algal products are used as food additives, animal feed (including aquaculture), vitamins, pigments, pharmaceutical compounds, cosmetics and potentially as a biofuel source (Adarme-Vega et al. 2012). Metabolic engineering is currently under intensive investigation to optimise and increase the production efficiency (Qin et al. 2012). Examples are the metabolic engineering of algae to increase the production of omega-3 fatty acids used for addition in infant milk formula or other food (Adarme-Vega et al. 2012); the pathway engineering for optimised production of beneficial carotenoids as lycopene, β-carotene, zeaxanthin, canthaxanthin and astaxanthin (Ye and Bhatia 2012); and the improved photosynthesis for algal biofuels (Stephenson et al. 2011) for which the possibilities of synthetic biology are exploited (Georgianna and Mayfield 2012).

As these new GMOs approach commercialisation, their safety will have to be investigated fully, urging the need to proactively evaluate whether currently applied risk assessment strategies remain appropriate, or whether new or complementary risk assessment strategies should be developed (e.g., CERA 2011; COGEM 2012; Fladung et al. 2012; Snow and Smith 2012; Bachman et al. 2013; FSANZ 2013; Häggman et al. 2013; Henley et al. 2013; Lundgren and Duan 2013; Petrick et al. 2013).

Conclusions

The creation of the European Food Safety Authority (EFSA) in 2002 in response to multiple food crises and the establishment of its GMO Panel in 2003 have been an important landmark for the food and feed safety in the EU. Over the years EFSA and its GMO Panel have taken a prominent role in the scientific evaluation of GMO applications and in the development of risk assessment and monitoring guidelines, contributing to improvements in the risk assessment process in the EU. EFSA's work builds on and complements that of (inter)national authorities responsible for the risk assessment of GMOs, and informs risk managers in the European Commission, the European Parliament and EU Member States on any possible risks that the use of GMOs may pose to human and animal health and the environment. However, risk assessment strategies developed by EFSA and followed in its scientific advice on GMO applications are not unanimously accepted by all EU Member States and stakeholders (e.g., Meyer 2011; Séralini et al. 2011; Ward et al. 2012; de Jong and Rong 2013; Goodman et al. 2013; Herman and Price 2013; Panda et al. 2013). During the development of guidelines some consulted EU Member States and stakeholders considered the new elements proposed useful, but in some cases insufficient. Others found the guidelines excessive or too precautionary. A recurrent comment is that the updated guidelines are more specific than previous ones, but still not sufficiently prescriptive in some cases, leaving room for interpretation. With the growing experience gained with the risk assessment of GMOs and with the scientific developments made in the field of GMO risk assessment, EFSA will take initiatives to further update the contents of its guidelines in the future. It is therefore important that the dialogue with interested parties on principles of risk assessment of GMOs continues, and that EFSA's presence at and contribution to relevant (inter)national fora should be further expanded.

EFSA is committed to ensuring that the European food policy is underpinned by a robust evidence base and that European consumers are fully protected and informed. The provision of independent scientific evidence to Europe's risk managers will continue to be a crucial element of policy in food and feed. EFSA's Science Strategy for 2012-2016 will guide EFSA in

the coming years laying out the vision for its scientific development, focussing on four key strategic objectives: (1) further develop the excellence of EFSA's scientific advice; (2) optimise the use of risk assessment capacity in the EU; (3) develop and harmonise methodologies and approaches to assess risks associated with the food/feed chain; and (4) strengthen the scientific evidence for risk assessment and risk monitoring. Taking stock of what has been achieved in its first 10 years of existence and exploring the drivers for future progress and change, the Science Strategy will ensure that EFSA continues to support the European food safety system through up-to-date, science-based risk assessments and to contribute to improving human health, animal health and welfare as well as plant health (Deluyker and Silano 2012).

Acknowledgments We thank current and previous members of the EFSA GMO Panel (http://www.efsa.europa.eu/en/gmomembers/gmopreviousmembers.htm), the experts of the various Working Groups of the EFSA GMO Panel (http://www.efsa.europa.eu/en/gmo/gmowgs.htm), as well as EFSA GMO Unit colleagues (http://www.efsa.europa.eu/en/staffdirectory/docs/staffdirectory.pdf) for inspiring discussions that helped to develop this publication. We thank two anonymous reviewers for insightful comments that helped to improve this publication.

References

Adarme-Vega TC, Lim DKY, Timmins M, Vernen F, Li Y, Schenk PM (2012) Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production. Microb Cell Fact 11:96

ADAS (2013) Review of the strategies for the comprehensive food and feed safety and nutritional assessment of GM plants per se (in press)

Aguilera J, Nielsen KM, Sweet J (2013) Risk assessment of GM trees in the EU: current regulatory framework and guidance. iForest 6:127–131

Andow DA, Hilbeck A (2004) Science-based risk assessment for non target effects of transgenic crops. Bioscience 54:637–649

Arjó G, Portero M, Piñol C, Viñas J, Matias-Guiu X, Capell T, Bartholomaeus A, Parrott W, Christou P (2013) Plurality of opinion, scientific discourse and pseudoscience: an in depth analysis of the Séralini et al. study claiming that Roundup™ Ready corn or the herbicide Roundup™ cause cancer in rats. Transgenic Res 22:255–267

Bachman PM, Bolognesi R, Moar WJ, Mueller GM, Paradise MS, Ramaseshadri P, Tan J, Uffman JP, Warren JA, Wiggins BE, Levine SL (2013) Characterization of the spectrum of insecticidal activity of a double-stranded RNA with targeted activity against western corn rootworm (*Diabrotica virgifera virgifera* LeConte). Transgenic Res. doi:10.1007/s11248-013-9716-5

Baum JA, Bogaert T, Clinton W, Heck GR, Feldmann P, Ilagan O, Johnson S, Plaetinck G, Munyikwa T, Pleau M, Vaughn T, Roberts J (2007) Control of coleopteran insect pests through RNA interference. Nat Biotechnol 25:1322–1326

Beech CJ, Koukidou M, Morrison NI, Alphey L (2012) Genetically modified insects: science, use, status and regulation. Collect Biosaf Rev 6:66–124

Benedict M, Eckerstorfer M, Franz G, Gaugitsch H, Greiter A, Heissenberger A, Knols B, Kumschick S, Nentwig W, Rabitsch W (2010) Defining environmental risk assessment criteria for genetically modified insects to be placed on the EU market. Supporting Publications 2010:EN-71 [200 pp], EFSA. http://www.efsa.europa.eu/en/supporting/doc/71e.pdf

Bolognesi R, Ramaseshadri P, Anderson J, Bachman P, Clinton W, Flannagan R, Ilagan O, Lawrence C, Levine S, Moar W, Mueller G, Tan J, Uffman J, Wiggins E, Heck G, Segers G (2012) Characterizing the mechanism of action of double-stranded RNA activity against western corn rootworm (*Diabrotica virgifera virgifera* LeConte). PLoS ONE 7(10):e47534

Burand JP, Hunter WB (2012) RNAi: future in insect management. J Invertebrat Pathol 112:S68–S74

CERA (2011) Problem formulation for the environmental risk assessment of RNAi plants: conference proceedings. http://cera-gmc.org/docs/cera_publications/pub_08_2011.pdf

COGEM (2012) Algae and genetic modification. Research, production and risks. Research report commissioned by COGEM (CGM 2012-05). http://www.cogem.net/index.cfm/en/publications/publicatie/research-report-algae-and-genetic-modification-research-production-and-risks

Cowx IG, Bolland JD, Nunn AD, Kerins G, Stein J, Blackburn J, Hart A, Henry C, Britton JR, Copp G, Peeler E (2010) Defining environmental risk assessment criteria for genetically modified fishes to be placed on the EU market. Supporting Publications 2010:EN-69 [187 pp], EFSA. http://www.efsa.europa.eu/en/supporting/doc/69e.pdf

de Jong TJ, Rong J (2013) Crop to wild gene flow: does more sophisticated research provide better risk assessment? Environ Sci Pol 27:135–140

Deluyker H, Silano V (2012) The first ten years of activity of EFSA: A success story. EFSA J 10(10):1–6 [se101]. http://www.efsa.europa.eu/en/efsajournal/doc/es101.pdf

Devos Y, Hails RS, Messéan A, Perry JN, Squire GR (2012) Feral genetically modified herbicide tolerant oilseed rape from seed import spills: are concerns scientifically justified? Transgenic Res 21:1–21

Dolezel M, Miklau M, Hilbeck A, Otto M, Eckerstorfer M, Heissenberger A, Tappeser B, Gaugitsch H (2011) Scrutinizing the current practice of the environmental risk assessment of GM maize applications for cultivation in the EU. Environ Sci Eur 23:33

EC (2013) Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. Off J EU L157:1–47. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:157:FULL:EN:PDF

EFSA (2004a) Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Austrian invoke of Article 23 of Directive 2001/18/EC. EFSA J 78:1–13. http://www.efsa.europa.eu/en/efsajournal/doc/78.pdf

EFSA (2004b) Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the Greek invoke of Article 23 of Directive 2001/18/EC. EFSA J 79:1–8. http://www.efsa.europa.eu/en/efsajournal/doc/78a.pdf

EFSA (2005a) Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/ES/01/01) for the placing on the market of insect-tolerant genetically modified maize 1507 for import, feed and industrial processing and cultivation. EFSA J 181:1–33. http://www.efsa.europa.eu/en/efsajournal/doc/181.pdf

EFSA (2005b) Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/F/96/05.10) for the placing on the market of insect-tolerant genetically modified maize Bt11, for cultivation, feed and industrial processing, under Part C of Directive 2001/18/EC from Syngenta Seeds. EFSA J 213:1–33. http://www.efsa.europa.eu/en/efsajournal/doc/213.pdf

EFSA (2005c) Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safeguard clause invoked by Hungary according to Article 23 of Directive 2001/18/EC. EFSA J 228:1–14. http://www.efsa.europa.eu/en/efsajournal/doc/228.pdf

EFSA (2006a) Guidance document for the risk assessment of genetically modified plants and derived food and feed by the Scientific Panel on Genetically Modified Organisms (GMO). EFSA J 99:1–100. http://www.efsa.europa.eu/en/efsajournal/doc/99.pdf

EFSA (2006b) Clarifications of the Scientific Panel on Genetically Modified Organisms following a request from the Commission related to the opinions on insect resistant genetically modified Bt11 (Reference C/F/96/05.10) and 1507 (Reference C/ES/01/01) maize. EFSA J 181:1–33. http://www.efsa.europa.eu/en/efsajournal/doc/1561.pdf

EFSA (2006c) Opinion of the Scientific Panel on Genetically Modified Organisms on the Post Market Environmental Monitoring (PMEM) of genetically modified plants. EFSA J 319:1–27. http://www.efsa.europa.eu/en/efsajournal/doc/319.pdf

EFSA (2006d) Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the notification (Reference C/SE/96/3501) for the placing on the market of genetically modified potato EH92-527-1 with altered starch composition, for cultivation and production of starch, under Part C of Directive 2001/18/EC from BASF Plant Science. EFSA J 323:1–20. http://www.efsa.europa.eu/en/efsajournal/doc/323.pdf

EFSA (2006e) Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to genetically modified crops (Bt176 maize, MON 810 maize, T25 maize, Topas 19/2 oilseed rape and Ms1xRf1 oilseed rape) subject to safeguard clauses invoked according to Article 16 of Directive 90/220/EEC. EFSA J 338:1–15. http://www.efsa.europa.eu/en/efsajournal/doc/338.pdf

EFSA (2006f) Guidance document for the risk assessment of genetically modified microorganisms and their derived products intended for food and feed use by the Scientific Panel on Genetically Modified Organisms (GMO). EFSA J 374:1–115. http://www.efsa.europa.eu/en/efsajournal/doc/374.pdf

EFSA (2006g) Opinion of the Scientific Panel on Genetically Modified Organisms on a request from the Commission related to the safeguard clause invoked by Greece according to Article 23 of Directive 2001/18/EC and to Article 18 of Directive 2002/53/EC. EFSA J 411:1–26. http://www.efsa.europa.eu/en/efsajournal/doc/411.pdf

EFSA (2006h) Guidance document of the Scientific Panel on Genetically Modified Organisms for renewal of authorisations of existing GMO products lawfully placed on the market, notified according to Articles 8 and 20 of Regulation (EC) No 1829/2003. EFSA J 435:1–4. http://www.efsa.europa.eu/en/efsajournal/doc/435.pdf

EFSA (2007a) Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events. EFSA J 512:1–5. http://www.efsa.europa.eu/en/efsajournal/doc/512.pdf

EFSA (2007b) EFSA review of statistical analyses conducted for the assessment of the MON 863 90-day rat feeding study. Statistical report. http://www.efsa.europa.eu/en/efsajournal/doc/19r.pdf

EFSA (2008a) Environmental risk assessment of genetically modified plants—challenges and approaches. EFSA Scientific Colloquium Series 8 (June 2007). http://www.efsa.europa.eu/en/supporting/doc/colloquiagmoera.pdf

EFSA (2008b) Safety and nutritional assessment of GM plants and derived food and feed: the role of animal feeding trials. Food Chem Toxicol 46:S2–S70

EFSA (2008c) Request from the European Commission related to the safeguard clause invoked by Hungary on maize MON 810 according to Article 23 of Directive 2001/18/EC. EFSA J 756:1–18. http://www.efsa.europa.eu/en/efsajournal/doc/756.pdf

EFSA (2008d) Request from the European Commission related to the safeguard clause invoked by Greece on maize MON 810 according to Article 23 of Directive 2001/18/EC. EFSA J 757:1–12. http://www.efsa.europa.eu/en/efsajournal/doc/757.pdf

EFSA (2008e) Scientific Opinion of the Panel on Genetically Modified Organisms related to a request from the European Commission related to the safeguard clause invoked by France on maize MON 810 according to Article 23 of Directive 2001/18/EC and the emergency measure according to Article 34 of Regulation (EC) No 1829/2003. EFSA J 850:1–46. http://www.efsa.europa.eu/en/efsajournal/doc/850.pdf

EFSA (2008f) Scientific Opinion of the Panel on Genetically Modified Organisms on a request from the European Commission to review scientific studies related to the impact on the environment of the cultivation of maize Bt11 and 1507. EFSA J 851:1–27. http://www.efsa.europa.eu/en/efsajournal/doc/851.pdf

EFSA (2008g) Scientific Opinion of the Panel on Genetically Modified Organisms on a request from the European Commission related to the safeguard clause invoked by Austria on maize MON 810 and T25 according to Article 23 of Directive 2001/18/EC. EFSA J 891:1–64. http://www.efsa.europa.eu/en/efsajournal/doc/891.pdf

EFSA (2009a) Statement of EFSA on the consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "use of antibiotic resistance genes as marker genes in genetically modified plants" and the Scientific Opinion of the GMO Panel on "consequences of the opinion on the use of antibiotic resistance genes as marker genes in genetically modified plants on previous EFSA assessments of individual GM plants". EFSA J 1108:1–8. http://www.efsa.europa.eu/cs/BlobServer/Statm_of_Efsa/gmo_biohaz_st_ej1108_ConsolidatedARG_en,0.pdf

EFSA (2009b) Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMO-NL-2005-22 and EFSA-GMO-RX-NK603) for the placing on the market of the genetically modified glyphosate tolerant maize NK603 for cultivation, food and feed uses and import and processing, and for renewal of the authorisation of maize NK603 as existing product. EFSA J 1137:1–50. http://www.efsa.europa.eu/en/efsajournal/doc/1137.pdf

EFSA (2009c) Scientific Opinion of the Panel on Genetically Modified Organisms on applications (EFSA-GMO-RX-MON810) for the renewal of authorisation for the continued marketing of (1) existing food and food ingredients produced from genetically modified insect resistant maize MON 810; (2) feed consisting of and/or containing maize MON 810, and maize MON 810 for feed use (including cultivation); and of (3) food additives and feed materials produced from maize MON 810, all under Regulation (EC) No 1829/2003 from Monsanto. EFSA J 1149:1–84. http://www.efsa.europa.eu/en/efsajournal/doc/1149.pdf

EFSA (2009d) Scientific Opinion of the Panel on Genetically Modified Organisms on a request from the European Commission related to the safeguard clause invoked by Austria on oilseed rape GT73 according to Article 23 of Directive 2001/18/EC. EFSA J 1151:1–16. http://www.efsa.europa.eu/en/efsajournal/doc/1151.pdf

EFSA (2009e) Scientific Opinion of the Panel on Genetically Modified Organisms on a request from the European Commission related to the safeguard clause invoked by Austria on oilseed rape MS8, RF3 and MS8xRF3 according to Article 23 of Directive 2001/18/EC. EFSA J 1153:1–16. http://www.efsa.europa.eu/en/efsajournal/doc/1153.pdf

EFSA (2009f) Scientific Opinion on Guidance for the risk assessment of genetically modified plants used for non-food or non-feed purposes, on request of EFSA. EFSA J 1164:1–42. http://www.efsa.europa.eu/en/efsajournal/doc/1164.pdf

EFSA (2010a) Statistical considerations for the safety evaluation of GMOs. EFSA J 8(1):1–59 [1250]. http://www.efsa.europa.eu/en/efsajournal/doc/1250.pdf

EFSA (2010b) Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA J 8(7):1–168 [1700]. http://www.efsa.europa.eu/en/efsajournal/doc/1700.pdf

EFSA (2010c) Scientific Opinion on the assessment of potential impacts of GM plants on non-target organisms. EFSA J 8(11):1–72 [1877]. http://www.efsa.europa.eu/en/efsajournal/doc/1877.pdf

EFSA (2010d) Guidance on the environmental risk assessment of GM plants. EFSA J 8(11):1–111 [1879]. http://www.efsa.europa.eu/en/efsajournal/doc/1879.pdf

EFSA (2011a) Guidance document on selection of comparators for the risk assessment of GM plants. EFSA J 9(5):1–20 [2149]. http://www.efsa.europa.eu/en/efsajournal/doc/2149.pdf

EFSA (2011b) Guidance for risk assessment of food and feed from GM plants. EFSA J 9(5):1–37 [2150]. http://www.efsa.europa.eu/en/efsajournal/doc/2150.pdf

EFSA (2011c) Scientific Opinion on guidance on the risk assessment of genetically modified microorganisms and their products intended for food and feed use. EFSA J 9(6):1–54 [2193]. http://www.efsa.europa.eu/en/efsajournal/doc/2193.pdf

EFSA (2011d) EFSA guidance on the submission of applications for authorisation of genetically modified food and feed and genetically modified plants for food or feed uses under Regulation (EC) No 1829/2003. EFSA J 9(7):1–27 [2311]. http://www.efsa.europa.eu/en/efsajournal/doc/2311.pdf

EFSA (2011e) Guidance on the post-market environmental monitoring (PMEM) of genetically modified plants. EFSA J 9(8):1–40 [2316]. http://www.efsa.europa.eu/en/efsajournal/doc/2316.pdf

EFSA (2011f) Statistical significance and biological relevance. EFSA J 9(9):1–17 [2372]. http://www.efsa.europa.eu/en/efsajournal/doc/2372.pdf

EFSA (2011g) Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2009. EFSA J 9(10):1–66 [2376]. http://www.efsa.europa.eu/en/efsajournal/doc/2376.pdf

EFSA (2011h) Scientific Opinion updating the evaluation of the environmental risk assessment and risk management recommendations on insect resistant genetically modified maize 1507 for cultivation. EFSA J 9(12):1–73 [2429]. http://www.efsa.europa.eu/en/efsajournal/doc/2429.pdf

EFSA (2011i) EFSA guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. EFSA J 9(12):1–21 [2438]. http://www.efsa.europa.eu/en/efsajournal/doc/2438.pdf

EFSA (2011j) Statement supplementing the evaluation of the environmental risk assessment and risk management recommendations on insect resistant genetically modified maize Bt11 for cultivation. EFSA J 9(11):1–44 [2478]. http://www.efsa.europa.eu/en/efsajournal/doc/2478.pdf

EFSA (2011k) Scientific Opinion on application (EFSAGMO-UK-2008-60) for placing on the market of genetically modified herbicide tolerant maize GA21 for food and feed uses, import, processing and cultivation under Regulation (EC) No 1829/2003 from Syngenta Seeds. EFSA J 9(12):1–94 [2480]. http://www.efsa.europa.eu/en/efsajournal/doc/2480.pdf

EFSA (2011l) Scientific Opinion on application (EFSA-GMO-CZ-2008-54) for placing on the market of genetically modified insect resistant and herbicide tolerant maize MON 88017 for cultivation under Regulation (EC) No 1829/2003 from Monsanto. EFSA J 9(11):1–152 [2428]. http://www.efsa.europa.eu/en/efsajournal/doc/2428.pdf

EFSA (2012a) Guidance on the risk assessment of food and feed from genetically modified animals and animal health and welfare aspects. EFSA J 10(1):1–43 [2501]. http://www.efsa.europa.eu/en/efsajournal/doc/2501.pdf

EFSA (2012b) Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from BASF Plant Science Company GmbH on the cultivation of genetically modified potato EH92-527-1 in 2010. EFSA J 10(2):1–38 [2558]. http://www.efsa.europa.eu/en/efsajournal/doc/2558.pdf

EFSA (2012c) Scientific Opinion addressing the safety assessment of plants developed through cisgenesis and intragenesis. EFSA J 10(2):1–33 [2561]. http://www.efsa.europa.eu/en/efsajournal/doc/2561.pdf

EFSA (2012d) Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from Monsanto Europe S.A. on the cultivation of genetically modified maize MON 810 in 2010. EFSA J 10(4):1–35 [2610]. http://www.efsa.europa.eu/en/efsajournal/doc/2610.pdf

EFSA (2012e) Scientific Opinion on risk assessment terminology. EFSA J 10(5):1–43 [2664]. http://www.efsa.europa.eu/en/efsajournal/doc/2664.pdf

EFSA (2012f) Scientific Opinion on a request from the European Commission related to the emergency measure notified by France on genetically modified maize MON 810 according to Article 34 of Regulation (EC) No 1829/2003. EFSA J 10(5):1–21 [2705]. http://www.efsa.europa.eu/en/efsajournal/doc/2705.pdf

EFSA (2012g) Scientific Opinion on an application (EFSA-GMO-NL-2005-24) for the placing on the market of the herbicide tolerant genetically modified soybean 40-3-2 for cultivation under Regulation (EC) No 1829/2003 from Monsanto. EFSA J 10(6):1–110 [2753]. http://www.efsa.europa.eu/en/efsajournal/doc/2753.pdf

EFSA (2012h) Scientific Opinion on a request from the European Commission for the assessment of the scientific elements put forward by Luxembourg to support the prohibition for the placing on the market of GM potato EH92-527-1 for cultivation purposes in Luxembourg. EFSA J 10(9):1–9 [2874]. http://www.efsa.europa.eu/en/efsajournal/doc/2874.pdf

EFSA (2012i) Scientific Opinion on a request from the European Commission related to the prolongation of prohibition of the placing on the market of genetically modified oilseed rape event GT73 for import, processing and feed uses in Austria. EFSA J 10(9):1–33 [2876]. http://www.efsa.europa.eu/en/efsajournal/doc/2876.pdf

EFSA (2012j) Scientific Opinion on a request from the European Commission related to the safeguard clause notified by Greece on genetically modified maize MON 810 according to Article 23 of Directive 2001/18/EC. EFSA J 10(9):1–20 [2877]. http://www.efsa.europa.eu/en/efsajournal/doc/2877.pdf

EFSA (2012k) Scientific Opinion on a request from the European Commission related to the prolongation of prohibition of the placing on the market of genetically modified oilseed rape events Ms8, Rf3 and Ms8 x Rf3 for import, processing and feed uses in Austria. EFSA J 10(9):1–33 [2878]. http://www.efsa.europa.eu/en/efsajournal/doc/2878.pdf

EFSA (2012l) Review of the Séralini et al. (2012) publication on a 2-year rodent feeding study with glyphosate formulations and GM maize NK603 as published online on 19 September 2012 in Food and Chemical Toxicology. EFSA J 10(10):1–9 [2910]. http://www.efsa.europa.eu/en/efsajournal/doc/2910.pdf

EFSA (2012m) Scientific Opinion updating the risk assessment conclusions and risk management recommendations on the genetically modified insect resistant maize 1507. EFSA J 10(10):1–46 [2933]. http://www.efsa.europa.eu/en/efsajournal/doc/2933.pdf

EFSA (2012n) Scientific opinion addressing the safety assessment of plants developed using Zinc Finger Nuclease 3 and other Site-Directed Nucleases with similar function. EFSA J 10(10):1–31 [2943]. http://www.efsa.europa.eu/en/efsajournal/doc/2943.pdf

EFSA (2012o) Final review of the Séralini et al. (2012a) publication on a 2-year rodent feeding study with glyphosate formulations and GM maize NK603 as published online on 19 September 2012 in Food and Chemical Toxicology. EFSA J 10(11):1–10 [2986]. http://www.efsa.europa.eu/en/efsajournal/doc/2986.pdf

EFSA (2012p) Scientific Opinion on the annual Post-Market Environmental Monitoring (PMEM) report from BASF Plant Science Company GmbH on the cultivation of genetically modified potato EH92-527-1 in 2011. EFSA J 10(12):1–35 [3015]. http://www.efsa.europa.eu/en/efsajournal/pub/3015.pdf

EFSA (2012q) Scientific Opinion supplementing the conclusions of the environmental risk assessment and risk management recommendations for the cultivation of the genetically modified insect resistant maize Bt11 and MON 810. EFSA J 10(12):1–32 [3016]. http://www.efsa.europa.eu/en/efsajournal/pub/3016.pdf

EFSA (2012r) Scientific Opinion updating the risk assessment conclusions and risk management recommendations on the genetically modified insect resistant maize MON 810. EFSA J 10(12):1–98 [3017]. http://www.efsa.europa.eu/en/efsajournal/pub/3017.pdf

EFSA (2012s) Scientific Opinion updating the risk assessment conclusions and risk management recommendations on the genetically modified insect resistant maize Bt11. EFSA J 10(12):1–104 [3018]. http://www.efsa.europa.eu/en/efsajournal/pub/3018.pdf

EFSA (2012t) Scientific Opinion on a request from the European Commission for the assessment of the scientific elements put forward by Hungary to support the prohibition for the placing on the market of GM potato EH92-527-1 for cultivation purposes in Hungary. EFSA J 10(12):1–10 [3021]. http://www.efsa.europa.eu/en/efsajournal/pub/3021.pdf

EFSA (2012u) Workshop on key allergens and compositional analysis in the allergenicity assessment of genetically modified plants. Supporting Publications 2012:289 [11 pp], EFSA. http://www.efsa.europa.eu/en/supporting/doc/289e.pdf

EFSA (2013a) Scientific Opinion on application (EFSA-GMO-NL-2005-23) for placing on the market of genetically modified maize 59122 for food and feed uses, import, processing and cultivation under Regulation (EC) No 1829/2003 from Pioneer. EFSA J 11(3):1–103 [3135]. http://www.efsa.europa.eu/en/efsajournal/doc/3135.pdf

EFSA (2013b) Guidance on the environmental risk assessment of genetically modified animals. EFSA J 11(5):1–190 [3200]. http://www.efsa.europa.eu/en/efsajournal/doc/3200.pdf

EFSA (2013c) Scientific Opinion on a request from the European Commission related to the prolongation of prohibition of the placing on the market of genetically modified oilseed

rape event GT73 for import, processing and feed uses in Austria. EFSA J 11(4):1–38 [3201]. http://www.efsa.europa.eu/en/efsajournal/doc/3201.pdf

EFSA (2013d) Scientific Opinion on a request from the European Commission related to the prolongation of prohibition of the placing on the market of genetically modified oilseed rape events Ms8, Rf3 and Ms8 x Rf3 for import, processing and feed uses in Austria. EFSA J 11(4):1–38 [3202]. http://www.efsa.europa.eu/en/efsajournal/doc/3202.pdf

Fernandez A, Mills ENC, Lovik M, Spök A, Germini A, Mikalsen A, Wal JM (2013) Endogenous allergens and compositional analysis in the allergenicity assessment of genetically modified plants. Food Chem Toxicol (in press)

Fladung M, Altosaar I, Bartsch D, Baucher M, Boscaleri F, Gallardo F, Häggman H, Hoenicka H, Nielsen KM, Paffetti D, Séguin A, Stotzky G, Vettori C (2012) European discussion forum on transgenic tree biosafety. Nat Biotechnol 30:37–38

FSANZ (2013) Response to Heinemann et al on the regulation of GM crops and foods developed using gene silencing. http://www.foodstandards.govt.nz/consumer/gmfood/Documents/Heinemann%20Response%20210513.pdf

Gaj T, Gersbach CA, Barbas CF III (2013) ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol

Georghiou SB, Magana M, Garfein RS, Catanzaro DG, Catanzaro A, Rodwell TC (2012) Evaluation of genetic mutations associated with *Mycobacterium tuberculosis* resistance to amikacin, kanamycin and capreomycin: a systematic review. PLoS ONE 7:e33275

Georgianna DR, Mayfield SP (2012) Exploiting diversity and synthetic biology for the production of algal biofuels. Nature 488:329–335

Goodman RE, Panda R, Ariyarathna H (2013) Evaluation of endogenous allergens for the safety evaluation of genetically engineered food crops: review of potential risks, test methods, examples and relevance. J Agric Food Chem. doi:10.1021/jf400952y

Graef F, Römbke J, Binimelis R, Myhr AI, Hilbeck A, Breckling B, Dalgaard T, Stachow U, Catacora-Vargas G, Bøhn T, Quist D, Darvas B, Dudel G, Oehen B, Meyer H, Henle K, Wynne B, Metzger MJ, Knäbe S, Settele J, Székács A, Wurbs A, Bernard J, Murphy-Bokern D, Buiatti M, Giovannetti M, Debeljak M, Andersen E, Paetz A, Dzeroski S, Tappeser B, van Gestel CAM, Wosniok W, Séralini GE, Aslaksen I, Pesch R, Maly S, Werner A (2012) A framework for a European network for a systematic environmental impact assessment of genetically modified organisms (GMO). BioRisk 7:73–97

Gray A (2012) Problem formulation in environmental risk assessment for genetically modified crops: a practitioner's approach. Collect Biosaf Rev 6:10–65

Häggman H, Raybould A, Borem A, Fox T, Handley L, Hertzberg M, Lu M-Z, Macdonald P, Oguchi T, Pasquali G, Pearson L, Peter G, Quemada H, Séguin A, Tattersall K, Ulian E, Walter C, McLean M (2013) Genetically engineered trees for plantation forests: key considerations for environmental risk assessment. Plant Biotechnol J. doi:10.1111/pbi.12100

Heinemann JA, Agapito-Tengfen SZ, Carman J (2013) A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessments. Environ Intl 55:43–55

Henley WJ, Litaker RW, Novoveská L, Duke CS, Quemada HD, Sayre RT (2013) Initial risk assessment of genetically modified (GM) microalgae for commodity-scale biofuel cultivation. Algal Res 2:66–77

Henry H, Kerins G, Blackburn J, Stein J, Smith GC, Eyre D, Roy S, Parrott D, Hart A (2010) Defining environmental risk assessment criteria for genetically modified (GM) mammals and birds to be placed on the EU market. Supporting Publications 2012:EN-107 [187 pp], EFSA. http://www.efsa.europa.eu/en/supporting/doc/107e.pdf

Herman RA, Ekmay R (2013) Do whole-food animal feeding studies have any value in the safety assessment of GM crops? Regul Toxicol Pharmacol. doi:10.1016/j.yrtph.2013.007.003

Herman RA, Price WD (2013) Unintended compositional changes in genetically modified (GM) crops: 20 years of research. J Agric Food Chem. doi:10.1021/jf400135r

Johnson R, Streicher EM, Louw GE, Warren RM, van Helden PD, Victor TC (2006) Drug resistance in *Mycobacterium tuberculosis*. Curr Iss Mol Biol 8:97–111

Johnson KL, Raybould A, Hudoson MD, Poppy GM (2007) How does scientific risk assessment of GM crops fit within the wider risk analysis? Trends Plant Sci 12:1–5

Keese P (2008) Risks from GMOs due to horizontal gene transfer. Environ Biosaf Res 7:123–149

Kuiper H, Kok EJ, Davies HV (2013) New EU legislation for risk assessment of GM food: no scientific justification for mandatory animal feeding trials. Plant Biotechnol J. doi:10.1111/pbi.12091

Kuntz M, Davison J, Ricroch AE (2013) What the French ban of *Bt* MON810 maize means for science-based risk assessment. Nat Biotechnol 33:498–500

Lheureux K, Mestdagh S, Devos Y (2008) Collaboration between the EFSA GMO Panel and national EU competent authorities for the environmental risk assessment of GM crop cultivation applications. J Consum Prot Food Saf 3(2):51

Liu Y, Christodoulidou A, Fernandes Dumont A, Germini A, Paoletti C (2013) Guidance Documents for nutritional and safety assessment of feeds from GM plants. In: Flachowsky G (ed) Animal nutrition with transgenic plants, CABI Biotechnology Series (in press)

Lundgren JG, Duan JJ (2013) RNAi-based insecticidal crops: potential effects on nontarget species. Bioscience. doi:10.1525/bio.2013.63.8.8

Lusser M, Parisi C, Plan D, Rodriguez-Cerezo E (2012) Deployment of new biotechnologies in plant breeding. Nat Biotechnol 30:231–239

Meissle M, Álvarez-Alfageme F, Malone LA, Romeis J (2012) Establishing a database of bio-ecological information on non-target arthropod species to support the environmental risk assessment of genetically modified crops in the EU. Supporting Publications 2012:EN-334 [170 pp], EFSA. http://www.efsa.europa.eu/en/supporting/doc/334e.pdf

Meyer H (2011) Systemic risks of genetically modified crops: the need for new approaches to risk assessment. Environ Sci Eur 23:7

Nielsen KM, Bones AM, Smalla K, van Elsas JD (1998) Horizontal gene transfer from plants to terrestrial bacteria—a rare event? FEMS Microbiol Rev 22:79–103

Nienstedt KM, Brock TCM, van Wensum J, Montforts M, Hart A, Aagaard A, Alix A, Boesten J, Bopp SK, Brown C,

Capri E, Forbes V, Köpp H, Liess M, Luttik R, Maltby L, Sousa JP, Streissl F, Hardy AR (2012) Development of a framework based on an ecosystem services approach for deriving specific protection goals for environmental risk assessment of pesticides. Sci Tot Environ 415:31–38

Oke KB, Westley PAH, Moreau DTR, Fleming IA (2013) Hybridization between genetically modified Atlantic salmon and wild brown trout reveals novel ecological interactions. Proc R Soc B. doi:10.1098/rspb.2013.1047

Panda R, Ariyarathna H, Amnuaycheewa P, Tetteh A, Pramod SN, Taylor SL, Ballmer-Weber BK, Goodman RE (2013) Challenges in testing genetically modified crops for potential increases in endogenous allergen expression for safety. Allergy 68:142–151

Paoletti C, Flamm E, Yan W, Meek S, Renckens S, Fellous M, Kuiper H (2008) GMO risk assessment around the world: some examples. Trends Food Sci Technol 19:S66–S74

Parrott W, Chassy B, Ligon J, Meyer L, Petrick J, Zhou J, Herman R, Delaney B, Levine M (2010) Application of food and feed safety assessment principles to evaluate transgenic approaches to gene modulation in crops. Food Chem Toxicol 48:1773–1790

Perry JN, ter Braak CJF, Dixon PM, Duan JJ, Hails RS, Huesken A, Lavielle A, Marvier M, Scardi M, Schmidt K, Tothmeresz B, Schaarschmidt F, van der Voet H (2009) Statistical aspects of environmental risk assessment of GM plants for effects on non-target organisms. Environ Biosaf Res 8:65–78

Perry JN, Devos Y, Arpaia S, Bartsch D, Gathmann A, Hails RS, Kiss J, Lheureux K, Manachini B, Mestdagh S, Neemann G, Ortego F, Schiemann J, Sweet JB (2010) A mathematical model of exposure of non-target Lepidoptera to Bt-maize pollen expressing Cry1Ab within Europe. P Roy Soc B 277:1417–1425

Perry JN, Devos Y, Arpaia S, Bartsch D, Gathmann A, Hails RS, Kiss J, Lheureux K, Manachini B, Mestdagh S, Neemann G, Ortego F, Schiemann J, Sweet JB (2011) The usefulness of a mathematical model of exposure for environmental risk assessment. P Roy Soc B 278:982–984

Perry JN, Devos Y, Arpaia S, Bartsch B, Ehlert C, Gathmann A, Hails RS, Hendriksen NB, Kiss J, Messéan A, Mestdagh S, Neemann G, Nuti M, Sweet JB, Tebbe CC (2012) Estimating the effects of Cry1F *Bt*-maize pollen on non-target Lepidoptera using a mathematical model of exposure. J Appl Ecol 49:29–37

Perry JN, Arpaia S, Bartsch D, Birch ANE, Devos Y, Gathmann A, Gennaro A, Kiss J, Messéan A, Mestdagh S, Nuti M, Sweet JB, Tebbe CC (2013) No evidence requiring change in the risk assessment of *Inachis io* larvae. Ecol Model (in press)

Petrick JS, Brower-Toland B, Jackson AL, Kier LD (2013) Safety assessment of food and feed from biotechnology-derived crops employing RNA-mediated gene regulation to achieve desired traits: a scientific review. Regul Toxicol Pharmacol 66:167–176

Podevin N, Devos Y, Davies HV, Nielsen KM (2012) Transgenic or not? No simple answer!—new biotechnology-based plant breeding techniques and the regulatory landscape. EMBO Rep 13:1057–1061

Podevin N, Davies HV, Hartung F, Nogué F, Casacuberta JM (2013) Site-directed nucleases: a paradigm shift in predictable, knowledge-based plant breeding. Trends Biotechnol 31:375–383

Qin S, Lin H, Jiang P (2012) Advances in genetic engineering of marine algae. Biotechnol Adv 30:1602–1613

Ramessar K, Peremarti A, Gómez-Galera S, Naqvi S, Moralejo M, Muñoz M, Capell T, Christou P (2007) Biosafety and risk assessment framework for selectable marker genes in transgenic crop plants. A case of the science not supporting the politics. Transgenic Res 16:261–280

Rangasamy M, Siegfried BD (2011) Validation of RNA interference in western corn rootworm *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) adults. Pest Manag Sci 68:587–591

Raybould A (2006) Problem formulation and hypothesis testing for environmental risk assessments of genetically modified crops. Environ Biosaf Res 5:119–125

Raybould A (2012) Can science justify regulatory decisions about the cultivation of transgenic crops? Transgenic Res 21:691–698

Romeis J, Bartsch D, Bigler F, Candolfi MP, Gielkens M, Hartley SE, Hellmich RL, Huesing JE, Jepson PC, Layton R, Quemada H, Raybould A, Rose RI, Schiemann J, Sears MK, Shelton AM, Sweet J, Vaituzis Z, Wolt JD (2008) Nontarget arthropod risk assessment of insect-resistant GM crops. Nat Biotechnol 26:203–208

Rüdelsheim PLJ, Smets G (2010) Anticipating the environmental risk assessment of crops modified to enhance or preserve yield. COGEM report CGM 2010-05. http://www.cogem.net/index.cfm/nl/publicaties/publicatie/anticipating-the-environmental-risk-assessment-of-crops-modified-to-enhance-or-preserve-yield

Sabalza M, Miralpeix B, Twyman RM, Capell T, Christou P (2011) EU legitimizes GM crop exclusion zones. Nat Biotechnol 29:315–317

Sanvido O, Widmer F, Winzeler M, Bigler F (2005) A conceptual framework for the design of environmental post-market monitoring of genetically modified plants. Environ Biosaf Res 4:13–27

Sanvido O, Romeis J, Bigler F (2011) Environmental change challenges decision-making during post-market environmental monitoring of transgenic crops. Transgenic Res 20:1191–2101

Sanvido O, Romeis J, Gathmann A, Gielkens M, Raybould A, Bigler F (2012) Evaluating environmental risks of genetically modified crops: ecological harm criteria for regulatory decision-making. Environ Sci Pol 15:82–91

Séralini GE, Cellier D, de Vendomois JS (2007) New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity. Arch Environ Contam Toxicol 52:596–602

Séralini GE, Mesnage R, Clair E, Gress S, de Vendômois J, Cellier D (2011) Genetically modified crops safety assessments: present limits and possible improvements. Environ Sci Eur 23:10

Séralini GE, Clair E, Mesnage R, Gress S, Defarge N, Malatesta M, Hennequin D, de Vendômois JS (2012a) Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Food Chem Toxicol 50:4221–4231

Séralini GE, Mesnage R, Defarge N, Gress S, Hennequin D, Clair E, Malatesta M, de Vendômois JS (2012b) Answers

to critics: why there is a long term toxicity due to NK603 Roundup-tolerant genetically modified maize and to a Roundup herbicide. Food Chem Toxicol 53:476–483

Snow AA, Smith VH (2012) Genetically engineered algae for biofuels: a key role for Ecologists. Bioscience 62:765–768

Stephenson PG, Moore CM, Terry MJ, Zubkov MV, Bibby TS (2011) Improving photosynthesis for algal biofuels: toward a green revolution. Trends Biotechnol 29:615–623

van der Voet H, Perry JN, Amzal B, Paoletti C (2011) A statistical assessment of differences and equivalences between genetically modified and reference plant varieties. BMC Biotechnol 11:15

Van Eenennaam AL, Muir WM (2011) Transgenic salmon: a final leap to the grocery shelf? Nat Biotechnol 29:706–710

Waigmann E, Paoletti C, Davies H, Perry J, Kärenlampi S, Kuiper H (2012) Risk assessment of Genetically Modified Organisms (GMOs). EFSA J 10(10):1–7 [s1008]. http://www.efsa.europa.eu/en/efsajournal/doc/as1008.pdf

Walter C, Fladung M, Boerjan W (2010) The 20-year environmental safety record of GM trees. Nat Biotechnol 28:656–658

Ward KJ, Nemeth MA, Brownie C, Hong B, Herman RA, Oberdoerfer R (2012) Comments on the paper "A statistical assessment of differences and equivalences between genetically modified and reference plant varieties" by van der Voet et al. 2011. BMC Biotechnol 12:13

Wilkinson M, Tepfer M (2009) Fitness and beyond: preparing for the arrival of GM crops with ecologically important novel characters. Environ Biosaf Res 8:1–14

Wolt JD, Keese P, Raybould A, Fitzpatrick JW, Burachik M, Gray A, Olin SS, Schiemann J, Sears M, Wu F (2010) Problem formulation in the environmental risk assessment for genetically modified plants. Transgenic Res 19:425–436

Ye VM, Bhatia SK (2012) Pathway engineering strategies for production of beneficial carotenoids in microbial hosts. Biotechnol Lett 34:1405–1414

Zaunbrecher MA, Sikes RD Jr, Metchock B, Shinnick TM, Posey JE (2009) Overexpression of the chromosomally encoded aminoglycoside acetyltransferase *eis* confers kanamycin resistance in *Mycobacterium tuberculosis*. P Natl Acad Sci USA 106:20004–20009

Zhu C, Sanahuja G, Yuan D, Farré G, Arjó G, Berman J, Zorrilla-López U, Banakar R, Bai C, Pérez-Massot E, Bassie L, Capell T, Christou P (2013) Biofortification of plants with altered antioxidant content and composition: genetic engineering strategies. Plant Biotechnol J 11:129–141