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SCIENTIFIC OPINION

Guidance on the environmental risk assessment of genetically modified plants[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This document provides guidance for the environmental risk assessment (ERA) of genetically modified (GM) plants submitted within the framework of Regulation (EC) No. 1829/2003 on GM food and feed or under Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms (GMOs). This document provides guidance for assessing potential effects of GM plants on the environment and the rationales for the data requirements for a comprehensive ERA of GM plants. The ERA should be carried out on a case-by-case basis, following a step-by-step assessment approach. This document describes the six steps for the ERA of GM plants, as indicated in Directive 2001/18/EC, starting with (1) problem formulation including hazard identification; (2) hazard characterisation; (3) exposure characterisation; (4) risk characterisation; (5) risk management strategies; and (6) an overall risk evaluation. The scientific Panel on Genetically Modified Organisms (of the European Food Safety Authority (EFSA GMO Panel) considers seven specific areas of concern to be addressed by applicants and risk assessors during the ERA (1) persistence and invasiveness of the GM plant , or its compatible relatives, including plant-to-plant gene transfer ; (2) plant-to-micro-organism gene transfer; (3) interaction of the GM plant with target organisms and (4) interaction of the GM plant with non-target organisms, including criteria for selection of appropriate species and relevant functional groups for risk assessment; (5) impact of the specific cultivation, management and harvesting techniques; including consideration of the production systems and the receiving environment(s); (6) effects on biogeochemical processes; and (7) effects on human and animal health. Each specific area of concern is considered in a structured and systematic way following the above-mentioned steps (1 to 6). In addition, the guidance document is supplemented with several general cross-cutting considerations (e.g. choice of comparator, receiving environment(s), general statistical principles, long-term effects) that need to be considered in the ERA. © European Food Safety Authority, 2010

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KEY WORDS

GM plant, GMO, guidance document, environmental risk assessment, environmental safety, import, processing, cultivation, Regulation (EC) No. 1829/2003, Directive 2001/18/EC.

SUMMARY

This document provides guidance for the environmental risk assessment (ERA) of genetically modified (GM) plants submitted within the framework of Regulation (EC) No 1829/2003 on GM food and feed or under Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms (GMOs).

The ERA of GM plants involves generating, collecting and assessing information on a GM plant in order to determine its impact on human/animal health and the environment relative to non-GMOs, and thus assessing its relative safety.

The present document provides guidance to risk assessors for assessing potential effects of GM plants into the environment and the rationales for data requirements in order to complete a comprehensive ERA, and to draw conclusions for the post-market environmental monitoring (PMEM).

The ERA should be carried out in a scientifically sound manner based on available scientific and technical data and on common methodology for the identification, gathering and interpretation of the relevant data. Tests, and measurements, and data generated should be clearly described as well as the assumptions made during the ERA. In addition, the use of scientifically sound modelling approaches could provide further useful information for the ERA. Sufficient scientific data must be available in order to arrive at qualitative/quantitative risk estimates.

The ERA should follow a step-by-step assessment approach. The EFSA GMO Panel describes the six steps for the ERA of GM plants, as indicated in Directive 2001/18/EC, starting with: (1) problem formulation including hazard identification; (2) hazard characterisation; (3) exposure characterisation; (4) risk characterisation; (5) risk management strategies; and (6) an overall risk evaluation.

Each risk assessment begins with problem formulation in which the most important questions that merit detailed risk characterisation are identified. Problem formulation helps to make the risk assessment process transparent by explicitly stating the assumptions underlying the risk assessment. At the end, the overall risk evaluation should result in informed qualitative and, if possible, quantitative advice to risk managers, outlining the nature and magnitude of uncertainties associated with the identified risks. The implications of the risk assessment for risk management measures should also be assessed.

The EFSA GMO Panel considers that seven specific areas of concern should be addressed by applicants and risk assessors during the ERA (1) persistence and invasiveness of the GM plant , or its compatible relatives, including plant-to-plant gene transfer ; (2) plant-to-micro-organism gene transfer; (3) interaction of the GM plant with target organisms; (4) interaction of the GM plant with non-target organisms, including criteria for selection of appropriate species and relevant functional groups for risk assessment; (5) impact of the specific cultivation, management and harvesting techniques; including consideration of the production systems and the receiving environment(s); (6) effects on biogeochemical processes; and (7) effects on human and animal health. Each specific area of concern is considered in a structured and systematic way following the above-mentioned steps (1 to 6).

The ERA should follow a weight-of-evidence approach considering intended and unintended effects.

The ERA should be carried out on a case-by-case basis, meaning that the required information may vary depending on the type of the GM plants and trait(s) concerned, their intended use(s), and the potential receiving environment(s). Information for ERA can be collected via (1) field-generated data (from field trials, field surveys, semi-field trials, and/or agronomic field trials), (2) molecular characterisation data, (3) compositional data, (4) ecotoxicological testing, (5) modelling, and/or (6) desk and literature studies.

In addition, the Guidance Document is supplemented with several general cross-cutting considerations (e.g. choice of comparator, receiving environment(s), general statistical principles, long-term effects) that need to be considered in the ERA.

The scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) proposes a step-wise selection process of relevant receiving environments to be addressed for ERA of a GM plant in question. Applicants should follow general statistical principles as outlined in this document. If experimental studies are being used they should allow testing for difference and equivalence. The EFSA GMO Panel also provides statistical guidance for specification of effect size, limits of concern, power analysis, experimental design, analysis and reporting. Recommendations are given how to address uncertainty.

The assessment of long-term effects requires specific information sources and techniques, including experimental or theoretical methodologies, and recommendations for establishing relevant baseline information. Further, GM plants containing stacked events are considered with respect to specific areas of risk.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION AND EFSA

The scientific Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) regularly reviews its Guidance Document (GD) in the light of experience gained, technological progress and scientific developments.

The Guidance Document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed, adopted on 24 September 2004, was in 2005, with a chapter on general surveillance of unanticipated effects of genetically modified (GM) plants as part of the post-market environmental monitoring (published in May 2006) and further in 2008 (EFSA, 2009f). Moreover, in May 2008, for the food and feed safety assessment, the EFSA GMO Panel adopted a version of the Guidance Document for the Risk Assessment of GM Plants and Derived Food and Feed. This version, which did not include a revision of the ERA chapter, was submitted for public consultation (EFSA, 2009f).

The present GD on ERA of GM plants has been prepared by expanding and completing most sections of the previous GMO Panel GD (EFSA, 2006a,b) in accordance with current legislation, experience gained during the evaluation of the risk assessment of past applications, the outcome of a self-tasking activity on non-target organisms[4], the outcome of the sub-working group on statistics ERA guidance, additional guidance on stacked events[5] and in response to a mandate[6] from the European Commission (see Terms of Reference).

In parallel, the EFSA GMO Panel developed and adopted a scientific opinion on the assessment of potential impacts of GM plants on non-target organisms (NTOs) (EFSA, 2010c). The present GD on ERA of GM plants contains in a condensed format of the scientific opinion which provides guidance to applicants on the assessment of potential impacts of GM plants on NTOs. The scientific opinion on NTOs further describes the data requirements and gives the scientific rationale as well as examples of methodologies in order to complete a comprehensive ERA for NTOs.

A draft EFSA GMO Panel GD for the ERA of GM plants as well as the draft EFSA scientific opinion on assessment of potential impacts of GM plants on NTOs went for public consultation for a two-month period (from 5th March 2010 to 30th April 2010). The outcomes of both public consultations are published on the EFSA website (EFSA, 2010b,a).

The present ERA GD provides detailed update on the ERA of GM plants by the EFSA GMO Panel, consisting of the following members:

Hans Christer Andersson, Salvatore Arpaia, Detlef Bartsch, Josep Casacuberta, Howard Davies, Patrick du Jardin, Gerhard Flachowsky, Lieve Herman, Huw Jones, Sirpa Kärenlampi (vice-chair), Jozsef Kiss, Gijs Kleter, Harry Kuiper (chair), Antoine Messéan, Kaare Magne Nielsen, Joe Perry (vice-chair), Annette Pöting, Jeremy Sweet, Christoph Tebbe, Atte Johannes von Wright and Jean-Michel Wal.

EFSA established three specific Working Groups, which worked in parallel and close collaboration, to address the following mandates:

- The sub-Environmental Risk Assessment Working Group on the ERA GMO Panel Guidance Document (sub-ERA GD WG): was established in October 2008. The sub-ERA GD WG was responsible for the update of the chapters of the GD linked to the four issues mentioned in the mandate from the European Commission as well as the update of most other chapters of the ERA

[4] ESA-Q-2008-089

5 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902599859.htm

6 EFSA-Q-2008-262

GD. The WG was composed of: Detlef Bartsch (chair) (GMO Panel member), Cristina Chueca, Adinda De Schrijver, Yann Devos (EFSA GMO unit), Achim Gathmann, Rosie Hails, Antoine Messéan (GMO Panel member), Joe Perry (GMO Panel member), Lucia Roda, Angela Sessitsch, Geoff Squire and Jeremy Sweet (vice-chair) (GMO Panel member). The WG was supported by Karine Lheureux and Sylvie Mestdagh from the EFSA GMO Unit.

- The Self-Tasking Working Group on Environmental Impacts of GM plants on Non-Target Organisms (NTO WG): was established in March 2008 following the recommendations made on NTO testing during the EFSA scientific colloquium entitled "Environmental risk assessment of GM plants – challenges and approaches" held in June 2007 (EFSA, 2008). The NTO WG was responsible for harmonising different NTO testing approaches and for the development of more detailed guidance in this area. The NTO WG contributed to the development of selection of criteria for species and ecological functional group selection, experimental design of field studies for NTO tests, statistical analyses of NTO tests and considerations of the receiving environment(s). The WG was composed of: Salvatore Arpaia (chair) (GMO Panel member), Detlef Bartsch (GMO Panel member), Marc Delos, Achim Gathmann, Rosie Hails, Jozsef Kiss (vice-chair) (GMO Panel member), Paul Hening Krogh, Barbara Manachini, Joe Perry (GMO Panel member), Jeremy Sweet (GMO Panel member) and Claudia Zwahlen. The WG was supported by Sylvie Mestdagh from the EFSA GMO Unit.

- The Statistics ERA Guidance Document Working Group (Statistics ERA GD WG): A Statistics WG was established in November 2005 to provide support in the update of the EFSA GMO Panel GD (food-feed chapter). In March 2009, the Statistics ERA GD WG was established to provide support to the NTO WG and sub-ERA GD WG in particular to address the development of criteria for field trials and statistical analysis. The WG was composed of: Salvatore Arpaia (GMO Panel member), Marco Acutis, Detlef Bartsch (GMO Panel member), Rosie Hails, Antoine Messéan (GMO Panel member), Joe Perry (chair) (GMO Panel member), Jeremy Sweet (GMO Panel member) and Hilko Van Der Voet. The WG was supported by Claudia Paoletti from the EFSA GMO Unit.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION AND EFSA

On 17 March, 2008, the EFSA GMO Panel received a 24-month mandate from the European Commission to further develop and update its guidelines as regards the environmental risk assessment and to cover the following points:

1. Environmental risk assessment of potential effects of genetically modified plants on non-target organisms through
    - Development of criteria for the selection of non-target organisms and representative species thereof, focusing on arthropods and other invertebrates, and also considering other relevant non-target organisms in different trophic levels;
    - Selection and recommendation of appropriate method to study the potential effects on these non-target organisms;
2. Development of criteria for field trials to assess the potential ecological effects of the GM plants in receiving environments (including experimental design and analysis to ensure sufficient statistical power);

3. Identification of the EU geographical regions where the GM plants (combination crop + trait) may be released and the selection of representative receiving environment(s) which reflect the appropriate meteorological, ecological and agricultural conditions;

4. Selection of appropriate techniques to assess potential long-term effects of GM plants including experimental and theoretical methodologies, and recommendations for establishing baseline information.

In addition to the points raised by the mandate of the European Commission, EFSA requested the EFSA GMO Panel to

- revise/update most sections of the ERA GD in the light of the experience gained during the evaluation of ERA of applications that included cultivation in their scope, and of the latest scientific development;
- include the outcome of the self-tasking working group on NTOs;
- include a section related to GM herbicide tolerant crops;
- include the outcome of the self-tasking working group on stacked events.

The present ERAGD expands and completes all sections of the ERA part of the EFSA GD (EFSA, 2006a) in accordance with current legislation, experience gained during the evaluation of the ERA in applications, the outcome of a self-tasking activity on Non-Target Organisms[7],the outcome of the sub-working group on statistics ERA GD, additional guidance on stacked events[8] and in response to a mandate from the European Commission[9]. The chapter on post-market environmental monitoring has not been revised and is similar to the GD of the EFSA GMO Panel issued in 2006.

Given the complexity of the topic and the large number of public comments expected, the duration of the mandate to deliver a scientific opinion on the GD for the ERA of GM plants was extended till November 10, 2010.

7 ESA-Q-2008-089
8 http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902599859.htm
9 EFSA-Q-2008-262

ASSESSMENT

1. Introduction

This document provides guidance for the ERA of GM plants submitted within the framework of Regulation (EC) No. 1829/2003 on GM food and feed (EC, 2003) or under Directive 2001/18/EC on the deliberate release into the environment of GMOs (EC, 2001). It covers the ERA in case of cultivation of GM plants and in case of import of food and feed containing or consisting of GM plants or produced from GM plants. The document provides in particular guidance on drawing up of information to supplement Annex III B of Directive 2001/18/EC, on the preparation of the conclusion of the ERA as described in Annex II of that Directive and on the set up of a post-market environmental monitoring plan according to Annex VII thereof.

These ERA GD of the EFSA GMO Panel on the Risk Assessment of GM plants and/or Derived Food and Feed replaces the ERA and supporting chapters in the 'Guidance Document for the Risk Assessment of GM Plants and Derived Food and Feed' of May 2006 (EFSA, 2006b).

This ERA GD provides detailed guidance for the applicant in the preparation and presentation of the ERA part of applications, according to Articles 5(8) and 17(8) of Regulation (EC) No. 1829/2003. This document addresses the requirements set out in Articles 5(5)(a) and (b) and 17(5)(a) and (b) of that Regulation, e.g. taking into account Annexes III B, II D2 and VII of Directive 2001/18/EC. The ERA GD is a 'stand-alone' document, meaning that all environmental issues are addressed in this document, while all molecular characterisation issues and food and feed safety issues (such as toxicology, allergenicity, nutritional aspects) are addressed in the EFSA Guidance Document for the Risk Assessment of GM Plants and Derived Food and Feed (see update EFSA, 2009f). Cross-references between these two GDs are made wherever necessary.

This ERA GD does not consider issues related to traceability, labelling, or co-existence.Socio-economic and ethical issues are also outside the scope of this guidance. The overall risk/benefit is out of the remit of the EFSA mandate. The ERA should primarily focus on potential environmental risks arising from the GM plants. The chapter on post-market environmental monitoring has not been revised and is similar to the GD of the EFSA GMO Panel issued in 2006.

This GD does not cover the deliberate release into the environment of GMOs for experimental purposes (Part B notifications under Directive 2001/18/EC). Nor does it cover the deliberate or non-deliberate environmental release or the contained use of genetically modified micro-organisms (GMMs), or the placing on the market of food and/or feed consisting of, containing, or produced from GMMs (Directive 2001/18/EC, Annex III A). For food and feed containing, consisting of or produced from GMMs, a GD is provided by the EFSA GMO Panel (EFSA, 2006a). For the RA of GM plants used for non-food or non-feed purposes, a parallel GD is provided by the EFSA GMO Panel (EFSA, 2009d).

The EFSA GMO Panel considered various sources of information and references from scientific reviews, conference reports, and expert consultation in preparing this GD.

An overview of the structure of the ERA GD is presented in Figure 1.

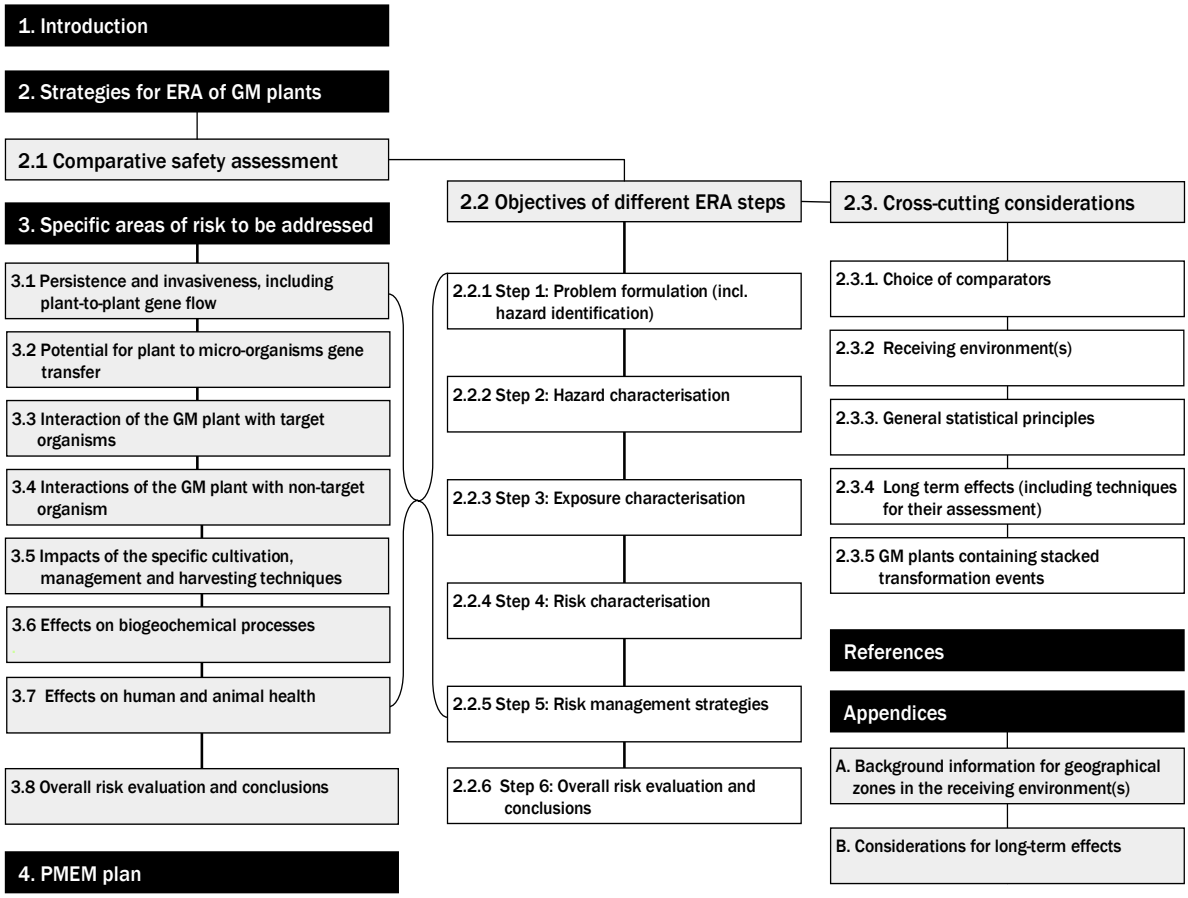

Figure 1: Structure of the ERA GD with chapter assignments and relationships between different steps in the ERA. Chapter 1 is the introduction. Chapter 2 describes the general strategies for the ERA. After the description of the comparative safety assessment in sub-chapter 2.1, the six steps of the ERA are described in general terms in sub-chapter 2.2. These steps are further elaborated in more detail in chapter 3. The cross-connectivity between chapters 2.2 and 3 is visualised by curly brackets. Chapter 2.3 introduces cross-cutting considerations for chapter 3. Chapter 4 describes the post-market environmental monitoring (PMEM) plan taking into account the results of the ERA. References and appendices complete the GD.

2. Strategies for ERA of GM plants

The purpose of the ERA is to assess if the introduction of the GM plant into the environment would have adverse effects on human and animal health and the environment. The ERA of GM plants involves generating, collecting and assessing information on a GM plant in order to determine its potential adverse impact relative to its non-GM plant comparator, and thus assessing its comparative safety. The underlying assumption of the comparative assessment for GM plants is that the biology of traditionally cultivated plants from which the GM plants have been derived, and the appropriate comparators is well known. To this end the concept of familiarity was developed by the OECD (OECD, 1993). In the ERA, it is appropriate to draw on previous knowledge and experience and to use the appropriate comparator in order to highlight differences associated with the GM plant in the receiving environment(s). The ERA for GM plants containing events combined by conventional breeding (stacked events) may also involve comparison with GM events as well as appropriate comparators (chapter 2.3.5).

The ERA should be carried out in a scientifically sound and transparent manner. The ERA should include any relevant data (e.g. research data, scientific publications, monitoring reports) obtained prior to and/or during the risk assessment process. The purpose of the performed studies, the data and their interpretation, as well as the assumptions made during the ERA, should be clearly described. In addition, the use of models could provide further information useful for ERA. The final risk evaluation should result in qualitative and if possible quantitative conclusions on risk that inform risk managers and allow decision-making. Any uncertainties associated with the identified risks should be outlined.

The ERA should be carried out on a case-by-case basis, meaning that the required information may vary depending on the species of GM plants concerned, the introduced genes, their intended use(s) and the potential receiving environment(s), taking into account specific cultivation requirements and the presence of other GM plants in the environment.

2.1. Comparative safety assessment as a general principle for the risk assessment of GM plants

The risk assessment strategy for GM plants seeks to use appropriate methods to compare the GM plant and derived products with their appropriate comparator (see chapter 2.3.1). Thus non-GM plants serve as comparators for the ERA of GM plants. The comparative safety assessment is being followed in order to identify differences caused by either intended or unintended effects.

Comparative safety assessment includes molecular characterisation, the agronomic and phenotypic characteristics of the GM plant in question, as well as its compositional analysis (OECD, 1993, FAO/WHO, 1996). In addition, the comparative safety assessment within ERA shall use information on the interactions of the GM plant with its receiving environment(s) (see chapter 2.3.2).

Any type of genetic modification of plants results in intended effects, but may also result in unintended effects. The ERA is focused on the identification and characterisation of both effects with respect to possible adverse impacts on human and animal health and the environment. Effects can be direct and indirect, immediate and delayed, including cumulative long-term effects.

Intended effects are those that are designed to occur and which fulfil the original objectives of the genetic modification. Alterations in the phenotype may be identified through a comparative analysis of growth performance, yield, pest and disease resistance, *etc*. Intended alterations in the composition of a GM plant compared to its appropriate comparator, may be identified by measurements of single compounds.

Unintended effects of the genetic modification are considered to be consistent (non-transient) differences between the GM plant and its appropriate comparator, which go beyond the primary intended effect(s) of introducing the transgene(s). Since these unintended effects are *event-specific*, applicants must supply data on the specific event. Sources of data[10] that may reveal such effects are:

1. Molecular characterisation: A starting point in the identification of potential unintended effects is analysis of the DNA construct and insertion site to establish whether the insertion is likely to have potential effects other than the intent of the original genetic modification (e.g. unintended effect(s) could be due to loss of function of an endogenous gene at the insertion site).

2. Compositional analysis: Unintended effects may be detected through the comparison of the compositional characteristics of the GM plant with its appropriate comparator (e.g. unintended effect(s) could potentially be linked to metabolic perturbations).

[10] Further guidance on the type and relevance of laboratory, semi-field, and field generated data is provided in chapter 2.3.3.4.

3. Agronomic and phenotypic characterisation: Unintended effects may also be detected through the comparison of the phenotypic and agronomic characteristics of the GM plant with its appropriate comparator (e.g. unintended effects could be linked to morphological alterations).

4. GM plant-environment interactions: Unintended effects may be detected through comparisons of biotic and abiotic interactions of the GM plant and its appropriate comparator with components of their receiving environment(s). *In planta* data are the fundamental source of information (e.g. unintended effects could be linked to changes in the interaction of the GM plant on functionality of NTO guilds).

Statistically significant differences between the GM plant and its appropriate comparators, which are not due to the intended modification, may indicate the occurrence of unintended effects, and should be assessed specifically with respect to their biological relevance and potentially hazardous environmental implications. The outcome of the comparative safety assessment allows the determination of those "identified" characteristics that need to be assessed for their potential adverse effects in the environment, regardless of whether they were intended or unintended, and will thus further structure the ERA.

The level and routes of environmental exposure to the GM plants shall be taken into account (e.g. in relation to the scope of the application: cultivation in the EU versus import and processing). Comparisons should be made between the GM plant and its appropriate comparators (see chapter 2.3.1), wherever applicable, grown in relevant production systems and similar environments (see chapters 2.3.3 and 3.5).

2.2. Objectives of the different steps of the environmental risk assessment

The objective of the ERA is on a case-by-case basis to identify and evaluate potential adverse effects of the GM plant, direct and indirect, immediate or delayed (including cumulative long-term effects) on the receiving environment(s) where the GM plant will be released. The ERA consists of the six steps described in Directive 2001/18/EC:

1. Problem formulation including hazard identification,
2. Hazard characterisation,
3. Exposure characterisation,
4. Risk characterisation,
5. Risk management strategies,
6. Overall risk evaluation and conclusions.

The ERA is conducted starting with step 1 and moving towards step 6; step 2 and 3 can, however, be carried out in parallel (see Figure 2).

Any uncertainty inherent to the different steps of the ERA should be highlighted and quantified as much as possible (for more background on uncertainties see chapter 2.3.3.7).

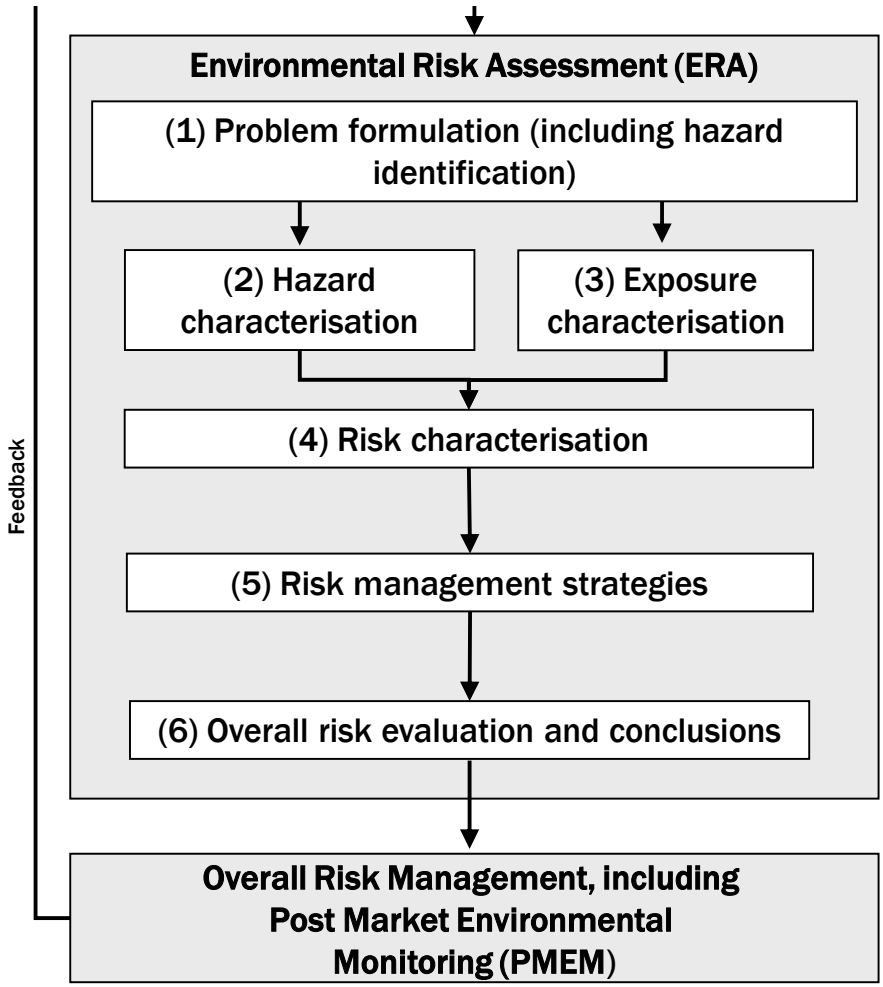

Figure 2: Six steps within the environmental risk assessment (ERA) and relationship to risk management, including monitoring, according to Directive 2001/18/EC and Regulation (EC) No. 1829/2003.

2.2.1. Step 1: Problem formulation: including hazard identification

The risk assessment begins with problem formulation in which all important questions for the risk characterisation are identified. Problem formulation helps to make the risk assessment process transparent by explicitly stating the assumptions underlying the risk assessment.

In this document, problem formulation includes the identification of characteristics of the GM plant capable of causing potential adverse effects to the environment (hazards), of the nature of these effects, and of pathways of exposure through which the GM plant may adversely affect the environment. It also includes defining assessment endpoints and setting of specific hypotheses to guide the generation and evaluation of data in the next risk assessment steps (hazard and exposure characterisation). In this process, both existing scientific knowledge and knowledge gaps (such as scientific uncertainties) are considered.

Problem formulation starts with the identification of hazards of the GM plant and its use. A comparison of the characteristics of the GM plant with those of its appropriate comparator (plant) enables the identification of differences in the GM plant that may lead to harm (see chapter 2.1). These

differences are identified in the problem formulation process in order to focus the ERA on the potential environmental consequences of these differences. While some differences may be deemed irrelevant to the assessment, others will need to be assessed for their potential to cause harm. More detailed guidance for applicants on how to apply problem formulation on specific areas of risk to be addressed in the ERA is provided in chapter 3 of this document.

After identifying the hazards and potential adverse effects that warrant further consideration, problem formulation then considers the available information on exposure through which the GM plant may interact with the environment. Depending upon the intended uses of a GM plant, such as import, processing, food, feed and/or cultivation, the pathways and levels of exposure of the GM plant to the environment will vary. In the case where the use of GM plant does not include cultivation in the EU, the problem formulation will consider exposure (1) via the accidental release into the environment of propagules, such as seeds, of the GM plant during transportation and processing potentially leading to sporadic feral GM plants and (2) indirect exposure, for example, through manure and faeces from the gastrointestinal tracts mainly of animals fed the GM plant, and/or (3) organic plant matter either imported as a fertiliser or soil amendment or derived from other bioproducts of industrial processes. In the case where the GM plant use includes cultivation in the EU, the problem formulation will consider exposure resulting from the expected cultivation of the GM plant in the receiving environment(s).

A crucial step in problem formulation is to identify the aspects of the environment that need to be protected from harm according to environmental protection goals set out by EU legislation (for background information, see table 1). Because protection goals are general concepts, they should be translated into measurable assessment endpoints (Suter, 2000, Romeis et al., 2008, Sanvido O, 2009, EFSA, 2010f, Wolt et al., 2010). Defining assessment endpoints is necessary to focus the risk assessment on assessable/measurable aspects of the environment – a natural resource (e.g. natural enemies) or natural resource service (e.g. biological control functions of pest populations performed by natural enemies) that could adversely be affected by the GM plant and that require protection from harm. Examples on how to consider protection goals for the ERA of GM plants are provided in the NTO Scientific Opinion in chapter 1.2 and appendix 1 (roadmap).

Subsequently, within the problem formulation, the identified potential adverse effects need to be linked to assessment endpoints in order to derive testable hypotheses that allow quantitative evaluation of the harm posed to those assessment endpoints. The hypotheses are of importance as they will further guide the setting up of a methodological approach[11] on how to evaluate the magnitude of harm. Through hypothesis, assessment endpoints are translated into quantitatively measurable endpoints, termed measurement endpoints (such as measurements of mortality, reproduction, abundance). A measurement endpoint can be regarded as an indicator of change in the assessment endpoint, and constitutes measures of hazard (chapter 2.2.2) and exposure (chapter 2.2.3).

Finally, for each measurement endpoint, the level of environmental protection to be preserved is expressed through the setting of 'limits of concern' which may take one of two forms. For studies in the environment(s) that are controlled (see chapter 3.4) the limits of concern will usually be trigger values which, if exceeded, will either lead to conclusions on risks or the need for further assessment in receiving environment(s). For field studies, the limits of concern will reflect more directly the minimum effect that is considered to potentially lead to harm (see also chapter 2.3.3). If these limits are exceeded, then detailed quantitative modelling of exposure may be required to scale up effects at the field level both temporally and spatially. Limits of concern can be defined by e.g. literature data, modelling, existing knowledge and policy goals.

The information considered in problem formulation can take many forms, including published scientific literature, scientific and expert opinions, and/or research data. Available data from analyses

[11] Problem formulation is generally performed on the basis of a conceptual model and an analysis plan (EPA, 1998, Hill and Sendashonga, 2003, Raybould and Cooper, 2005, Raybould, 2006, 2007, Romeis et al., 2008, Storkey et al., 2008, Raybould, 2009, Raybould et al., 2009, Wolt, 2009, Wolt et al., 2010).

performed to characterise the GM plant, including molecular, compositional, agronomic/phenotypic analysis and plant-environment interactions, shall also address the occurrence of unintended effects. Data generated outside Europe with the GM plant might be used by the applicant only if its relevance for the European environment(s) is justified.

Problem formulation should on a case-specific basis:

- Identify characteristics of the GM plant and, where appropriate, the associated production and management systems capable of causing potential adverse effects to the environment;
- Identify the potential adverse effects linked to those harmful characteristics;
- Identify exposure pathways through which the GM plant may adversely affect the environment;
- Define assessment endpoints being representative of the aspects of the environment that need to be protected from harm according to protection goals set out by EU legislation and their translation into national policies, and describe criteria used for the selection of assessment endpoints (e.g. relevance, practicality);
- Define measurement endpoints that can be used to assess the potential harm to assessment endpoints defined;
- Formulate testable hypotheses that are clearly phrased and easily transferable to data to be generated or evaluated;
- Set the limits of concern for each measurement endpoint;
- Consider knowledge gaps (such as scientific uncertainties)

Table 1: Examples of environmental protection goals and their legal bases in the European Union (EU). Directive 2001/18/EC specifically applies to GM plants. Other legislations as listed below should be considered by the applicant, even though GM plants may not be specifically mentioned.

Protection goals		**Legal basis**	
Areas of protection		**Background**	**Scope**
Biodiversity conservation	Species of conservation or cultural value; red list species // Protected habitats; landscapes	Directive 2004/35/EC[(b)]	Environmental liability
		Directive 92/43/EEC[(c)]	Conservation of natural habitats and of wild fauna and flora
		Directive 2009/147/EC[(d)]	Conservation of wild birds
		Regulation 338/97[(e)]	Protection of endangered wild fauna and flora
		Action plan for biodiversity[(f)]	Conservation of biodiversity
		Biodiversity strategy[(g)]	Conservation of biodiversity
		Biodiversity action plan for the conservation of natural resources[(h)]	Conservation of natural resources
		Biodiversity action plan for agriculture[(i)]	Conservation of biodiversity
		Bern convention[(j)]	Conservation of European wildlife and natural habitats
		Convention on biological diversity[(k)]	Conservation of biological diversity
Ecological functions	Soil	Directive 2004/35/EC	Environmental liability
		Thematic strategy for soil protection[(l)]	Preservation of soil functions
	Water	Directive 2000/60/EC[(m)]	Water protection
	Production systems; plant health	Regulation 1107/2009[(n)]	Marketing of plant protection products
		Directive 2009/128/EC[(o)]	Sustainable use of plant protection product
		Biodiversity strategy	Sustainable use of biodiversity
		Thematic strategy on the sustainable use of natural resources[(p)]	Sustainable use of natural resources

(a): Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC
(b): Directive 2004/35/CE of the European Parliament and of the Council of 21 April 2004 on environmental liability with regard to the prevention and remedying of environmental damage
(c): Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora
(d): Council Directive 2009/147/EC of 30 November 2009 on the conservation of wild birds
(e): Council Regulation (EC) No 338/97 of 9 December 1996 on the protection of species of wild fauna and flora by regulating trade therein
(f): Commission Communication of 22 May 2006 "Halting the loss of biodiversity by 2010 - and beyond - Sustaining ecosystem services for human well-being" COM(2006) 216
(g): Communication from the Commission to the Council and the European Parliament of 4 February 1998 on a European Community biodiversity strategy COM(1998) 42
(h): Commission Communication of 27 March 2001 to the Council and the European Parliament: Biodiversity Action Plan for the Conservation of Natural Resources (Volume II) COM(2001) 162
(i): Commission Communication of 27 March 2001 to the Council and the European Parliament: Biodiversity Action Plan for Agriculture (Volume III) COM(2001) 162
(j): Council Decision 82/72/EEC of 3 December 1981 concerning the conclusion of the Convention on the conservation of European wildlife and natural habitats (Bern Convention)
(k): Council Decision 93/626/EEC of 25 October 1993 concerning the conclusion of the Convention on Biological Diversity
(l): Commission Communication of 22 September 2006 entitled "Thematic strategy for soil protection" COM(2006) 231
(m): Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy
(n): Regulation (EC) No 1107/2009 of the European Parliament and of Council of 21 October 2009 concerning the placing of plant protection products and repealing Council Directives 79/117/EEC and 91/414/EEC
(o): Directive 2009/128/EC of the European Parliament and of the Council of 21 October 2009 establishing a framework for Community action to achieve the sustainable use of pesticides

(p): Communication from the Commission of 21 December 2005 - Thematic Strategy on the sustainable use of natural resources COM(2005) 670

2.2.2. Step 2: Hazard characterisation

Hazard characterisation in this GD is defined as the qualitative and/or quantitative evaluation of environmental harm associated with the hazard as set out in one or more hypotheses derived from problem formulation.

The magnitude of each potential adverse environmental effect should, if possible, be expressed in quantitative rather than qualitative terms. Ordered categorical descriptions such as "*high*", "*moderate*", "*low*" or "*negligible*", where the ordering is from *'high'* at one end to *'negligible'* at the other (Liu and Agresti, 2005), may be used to place identified hazard on a scale of severity. If at all possible, these terms should themselves be defined in quantitative terms, as precisely as possible[12]. If the expression of magnitude is not made in quantitative term, but solely using the "ordered categorical description", a justification for this categorisation is necessary and should be provided.

2.2.3. Step 3: Exposure characterisation

This step is to evaluate the exposure, *i.e.* likelihood of adverse effects occurring, and to estimate the exposure quantitatively.

For each hazard identified and characterised, it may not be possible to estimate the exposure (likelihood) precisely. Likelihood of exposure can be expressed either qualitatively using an ordered categorical description (such as "*high*", "*moderate*", "*low*" or "*negligible*") or quantitatively as a relative measure of probability (from zero to one, where zero represents impossibility and one certainty). However, if qualitative terms are used to express such likelihoods, then the link between likelihood and probability should be accounted for. Thus, whatever term is chosen, an indication should be given of the range, within a numeric scale of 0 to 1, to which the term is intended to refer. For example, "the likelihood of exposure of a non-target lepidopteran species to Bt toxin (Cry1Ab protein) in field margins was estimated to be moderate, where 'moderate' in this context means within the range 0.1 to 0.4".

[12] The following classifications are extracted from the Commission Decision 2002/623/EC (EC, 2002) and are suggested as illustrative and qualitative examples in a very broad sense. They are not intended to be definitive or exclusive, but to give an indication of the considerations that might be taken into account when weighing up the consequences:

"*high level consequences*" might be significant changes in the numbers of one or more species of other organisms, including endangered and beneficial species in the short or long-term. Such changes might include a reduction in or complete eradication of a species leading to a negative effect on the functioning of the ecosystem and/or other connected ecosystems. Such changes would probably not be readily reversible and any recovery of the ecosystem that did take place would probably be slow;

"*moderate consequences*" might be significant changes in population densities of other organisms, but not a change which could result in the total eradication of a species or any significant effect on endangered or beneficial species. Transient and substantial changes in populations might be included if likely to be reversible. There could be long-term effects, provided there are no serious negative effects on the functioning of the ecosystem;

"*low level consequences*" might be non-significant changes in population densities of other organisms, which do not result in the total eradication of any population or species of other organisms and have no negative effects on functioning of the ecosystem. The only organisms that might be affected would be non-endangered, non-beneficial species in the short or long-term;

"*negligible consequences*" would mean that no significant changes had been caused in any of the populations in the environment or in any ecosystems.

2.2.4. Step 4: Risk characterisation

Risk is characterised by combining the magnitude of the consequences of a hazard and the likelihood that the consequences occur (EC, 2002). It is described in this GD as the quantitative or semi-quantitative estimate, of the probability of occurrence and severity of harmful effect(s) based on problem formulation, hazard and exposure characterisation. It is important that the values obtained for each measurement endpoint are related to the limits of concern to test whether the observed effect falls within those limit and, thereby, to aid in the assessment of the biological relevance of the observed effect (see 2.3.3).

On the basis of the conclusions reached in steps 2 and 3, an estimate of the risk of adverse effects should be made for each hazard identified in step 1. If a hazard has more than one adverse effect, the magnitude and likelihood of each individual adverse effect should be assessed. Where precise quantitative evaluation of risk is not possible, terms should be defined where possible. The evaluation for each risk should consider:

- The magnitude of the consequences of the hazard ("*high*", "*moderate*", "*low*" or "*negligible*", with an explanation of what is meant by these terms);
- The likelihood of the consequences related to hazard occurring ("*high*", "*moderate*", "*low*" or "*negligible*", with quantified definitions of terms, using ranges of probability) in the receiving environment(s);
- The risk characterised by combining the magnitude of the consequence of the hazard and its likelihood.

The uncertainty for each identified risk should be described where relevant, possibly including documentation relating to:

- Assumptions and extrapolations made at various levels in the ERA;
- Different scientific assessments;
- Specified uncertainties (see also chapter 2.3.3.7);
- Conclusions that can be derived from the data.

The risk characterisation should indicate whether the problem formulation (including hazard identification), hazard characterisation and exposure characterisation are complete.

2.2.5. Step 5: Risk management strategies

When the risk characterisation (step 4) identifies risks, then applicants should propose measures to manage them. These risk management strategies should aim to reduce the identified risks associated with the GM plant to a level of no concern and should consider defined areas of uncertainty. Applicants should describe the risk management in terms of reducing hazard and/or exposure, and the consequent reduction in risk should be quantified (when possible). Where applicants have identified risk management characteristics (e.g. reduced fertility) in the GM plant which can reduce these risks, then the reliability and efficacy of these characteristics should be assessed. In addition, if applicants place restrictions or conditions on the release of a GM plant in order to reduce risks, then the efficacy and reliability of these measures should be assessed.

Applicants should also state the measures they will put in place post-commercialisation in order to monitor and verify the efficacy of the risk management measures and to allow changes in risk

management strategies in case circumstances change, or new data become available which require changes to the risk management (see also PMEM plan in chapter 4).

2.2.6. Step 6: Overall risk evaluation and conclusions

An evaluation of the overall risk of the GM plant(s) should be made taking into account the results of the ERA and associated levels of uncertainty, the weight of evidence and the risk management strategies proposed (step 5) in the receiving environment(s).

The overall risk evaluation should result in informed qualitative and, if possible, quantitative guidance to risk managers. The applicants should explain clearly what assumptions have been made during the ERA and what is the nature and magnitude of uncertainties associated with the risk(s). When risks are identified in the overall risk evaluation, applicants should indicate why certain levels of risk might be acceptable.

The overall risk evaluation, including risk management strategies, may give indications for the requirement of specific activities within PMEM of GM plants. ERA and environmental monitoring are closely linked. The ERA provides the basis for the monitoring plans, which focus on detecting any adverse effects on human health and the environment in the receiving environment(s). PMEM may provide data on long-term, potentially adverse effects of GM plants. Monitoring results may confirm the assumptions of the ERA or may lead to its re-evaluation (see chapter 4).

ERA is an iterative process. If new information on the GM plant and its effects on human health or the environment becomes available, the ERA may need to be re-addressed in order to (1) determine whether the risk characterisation has changed; and (2) determine whether it is necessary to amend the risk management.

2.3. Cross-cutting considerations

2.3.1. Choice of comparators[13]

Single events

Where feasible and appropriate, similarities and differences in the interactions between the GM plant and the environment due to genetic modification and induced changes in management should be estimated in relation to a conventional counterpart (see also chapter 2.3.3).

In the case of vegetatively propagated crops, the conventional counterpart shall, in principle, be the near-isogenic variety used to generate the transgenic lines (EFSA, 2009e).

In the case of crops that reproduce sexually, the conventional counterpart shall have a genetic background comparable to the GM plant (EFSA, 2009e). Since many crops used to produce food and feed are developed using back-crossing, a conventional counterpart with a genetic background that is as close as possible to the GM plant shall be selected. On a case-by-case basis, and if there is explicit justification, applicants may instead consider the use of a non-GM variety with as similar agronomic properties to the GM plant as possible, as the appropriate comparator for ERA. In all cases, information on the breeding scheme (pedigree) in relation to both the GM plant and all chosen comparator(s) and justification for the use of the selected use of all chosen comparator(s) shall be provided.

[13] More specific guidance on the selection of appropriate comparators is currently under consideration by the EFSA GMO Panel Working Group on comparators (http://www.efsa.europa.eu/en/gmo/gmowgs.htm).

For certain assessment issues, such as the effects of management, cultivation and harvesting, applicants should consider the inclusion of an additional comparator(s). It is imperative to consider the use of different current management techniques that help to place any effects of the genetic modification into context, particularly concerning the agronomic management of both the GM plant and the chosen comparator(s). For example, for insect-resistant GM plants, equivalence with a conventional counterpart is highly unlikely, if the latter is managed without the pest control that would be typically applied to conventional non-GM plants. Hence, for such crops a conventional counterpart managed without pest control, and the same conventional counterpart managed with pest control that would be typically applied in the area are recommended. The management techniques applied shall be compatible with the principles of good agricultural practice and Integrated Pest Management that are being introduced by Member States under the Directive on the sustainable use of pesticides in the EU (see http://ec.europa.eu/environment/ppps/home.htm). When more than one management technique is employed, the principal comparison for inferences regarding environmental harm should be representative management techniques, rather than 'untreated' regimes which may be agronomically less realistic. In some circumstances, it may be advantageous for the ERA to include an *additional* comparator with a closer genetic background to the GM plant than the conventional counterpart (such as a negative segregant). In all cases where an additional comparator is used, the motivation and choice shall be justified explicitly.

It is recognised that appropriate management is site- and year-specific; management should therefore follow standard farming practices and clearly document deviations. Applicants must provide detailed management records and carry out independent agronomic audits by trained personnel to give sufficient confidence that management practices are appropriate (Champion et al., 2003). Any additional treatments and/or comparators should be fully integrated within the experimental design, randomised and replicated in the same way as the GM plant and its conventional counterpart.

Furthermore, whereas in general parlance the term 'comparator' applies to the plant, ERA must account for the production system as a whole. The production system includes the following scales: spatially - the landscape and region as well as the field; agronomically - the rotation and crop as well as the plant; temporally - the long-term, rotational and yearly effects as well as the seasonal. So for ERA, the impacts of management, cultivation and harvesting must be considered at larger temporal and spatial scales than apply to relatively small-scale experiments. For ERA, upscaling, modelling, simulation and analysis of production systems will typically be required, in addition to analysis of the smaller-scale experiments referred to in this document, that provide parameter values for such modelling (EFSA, 2008). Allowance shall be made that a range of management options are possible in production systems using GM or conventional plants, and a range of comparisons might therefore be necessary.

The ERA of effects of persistence and invasiveness requires a wide variety of information from specific experiments which tend to be of a case-specific, research-driven nature rather than of a routine nature (see chapter 3.1). The effects studied include: reproduction, germination, seed persistence, invasiveness, hybridisation. Selection of the comparator should therefore be done on a case-by-case basis.

In the case of herbicide-tolerant GM plants incorporating a single event, at least three test materials (EFSA, 2009e) are recommended: the GM plant exposed to the intended herbicide and associated weed management, the conventional counterpart treated with current weed management regimes and the GM plant treated with the same weed management. Such comparison allows the assessment of whether the expected agricultural practices influence the expression of the studied assessment endpoints.

If no extra comparator is employed, it may still be necessary to consider the use of some form of positive control (Perry et al., 2009) in order to demonstrate *post-hoc* that the study was capable of detecting the desired effects (for example that there was a sufficient population density of organisms available in the experimental area to be sampled). If the positive control is external to the experiment,

for example on a single unrandomised plot, then data from the control may not enter the statistical analysis in any form.

In this ERA GD, the term 'GM plant' refers to the specific GM event for which approval is requested. However, in practice, commercially available GM varieties are often produced from crosses of this event with other varieties. The applicants should discuss potential risks arising from the genetic background of varieties which might subsequently include the GM event and how these might alter the conclusions of the risk assessment. On a case-by-case basis, depending on the nature of the event and according to the scope of the application, data may be required on the safety of the event when present in different genetic backgrounds.

Stacked events

Stacked events combined by conventional crossing pose particular challenges for ERA; comparators should be selected to establish whether the combination of events raises safety concerns with regard to stability and/or interactions. In addition, the ERA should consider to what extent the combination of events results in changes in management systems which could lead to additional environmental impacts compared to the single events. For stacked events, the conventional counterpart, if available, should be used as the comparator.

Note that in an *n*-event stack, there are, in addition to the stacked events itself, the negative segregant and the *n* single events, a further $2^n - n - 2$ different possible sub-combinations of events. For example, for a 5-stacked events, there are a further 25 possible sub-combinations, and therefore a multiplicity of interactions that might give rise to potential risks. For ERA, field trials for comparative analysis will normally comprise the stacked event under assessment and its conventional counterpart. Selection of comparators for ERA must take account of the need for relatively large plots, consequent relatively restricted ability to increase replication, and, crucially, the consequent need to restrict the number of treatments compared to a minimum (often of two). It is recognised that if concerns over stability and/or interactions are indicated by such initial experiments then further more detailed experimentation encompassing a greater number of treatments may be required. Whilst the most relevant study for ERA is the observed potential adverse effect itself, rather than the potential interaction that is the cause, it may well be useful to identify the source of the interaction.

It is very unlikely that any scientific rationale could justify the absence of experimental data for ERA, because there would need to be considerable evidence from previous risk assessments to rule out *ab initio* interactions between the events on biota, even if the proteins themselves could be shown not to interact. Furthermore, for cultivation, it should be stressed that consideration of management is essential.

As comparators should be selected to establish whether the combination of events raises safety concerns with regards to stability and/or interactions, protein expression levels relating to single events from only historic data, i.e. not obtained from concurrent data in trials of the higher stacked events, may not be acceptable for ERA of that higher stacked events and/or its sub-combinations. To assess interactions between events that could impact on protein expression levels, any set of events which have all been risk assessed, and which contain between them all the events present in the stacked events, should be included as comparators.

In case a conventional counterpart is not available, different comparator(s) may be appropriate depending upon the issue(s) under consideration. In particular, where studies on target organisms or other issues utilise data arising from field trials for food and feed risk assessment (often used to assess agronomic and phenotypic characteristics) , the comparators will be identical to those referred to in the EC Regulation on implementing rules for GM food and feed.

To evaluate the impact on non-target organisms, effects of management, cultivation and harvest, and biogeochemical processes the conventional counterpart can be substituted, on a case by case basis, by either a non-GM line derived from the breeding scheme used to develop the GM plant, , or by a non-GM line with agronomic properties as similar as possible to the stacked events. Applicants must justify the choice explicitly in such cases.

To evaluate the effect of persistence and invasiveness the conventional counterpart can be substituted, on a case by case basis, by either a non GM line derived from the breeding scheme used to develop the GM plant, or by a non-GM line with agronomic properties as similar as possible to the stacked events. Since the assessment of the effects of persistence and invasiveness requires information from specific experiments which tend to be of a case-specific, research-driven nature, the selection of the appropriate comparator should be done on a case-by-case basis according to the effect studied. Applicants must justify the choice explicitly in such cases.

Applicants should consider whether the use of extra comparators, such as negative segregants or the parental lines, may be appropriate.

For herbicide-tolerant GM plants that are stacked events, GM plants treated with conventional herbicides are not required for field trials for ERA, because the primary concern of these trials is to provide data to establish that the combination of events does not raise any additional safety concerns over protein and trait expression compared with the single events. However, if these initial trials identify unintended effects that raise safety concerns then further, more detailed experimentation is required which includes additional comparator(s). However, on a case-by-case basis and, particularly, when assessing the effects of changes in management, it may be necessary to include GM plants treated with conventional herbicides as an additional comparator.

2.3.2. Receiving environment(s)

The receiving environment(s) is the environment into which the GM plant(s) will be released and into which the transgene(s) may spread. The receiving environment(s) is characterised by three components (see Figure 3):

- The GM plant (e.g. plant species, genetic modification(s) and intended uses(s));
- The Geographical Zones (e.g. the climate, altitude, soil, water, flora, fauna, habitats….);
- The Management Systems (e.g. land use and production systems, other cultivated GM plants, cultivation practices, integrated and other pest management, non-production activities and nature conservation activities).

In the component "Management Systems", land use, and production systems shall be considered as these systems can differ significantly between geographical regions (e.g. irrigated maize versus non-irrigated cultivation). Moreover, in a specific region, cultivation of GM plants for different purposes may have specific risk assessment implications (e.g. green maize for biogas or silage with early harvest compared to grain maize).

The three components listed above result in biotic and abiotic interactions that shall be considered by applicants when establishing representative scenarios considering receiving environment(s) for carrying out the ERA of a GM plant (Figure 3 and Table 2). A broad range of environments in terms of fauna and flora, climatic conditions, habitat composition and ecosystem functions and human interventions occurs in the EU. Accordingly, GM plants will potentially interact with those differing environments.

The ERA shall be carried out on a case-by-case basis, meaning that the required information varies depending on the types of the GM plants and trait(s) concerned, their intended use(s), and the potential receiving environment(s). There may be a broad range of environmental characteristics (regional-specific) to be taken into account. To support a case-by-case assessment, it may be useful to classify regional data, reflecting aspects of the receiving environment(s) relevant to the GM plant (e.g. botanical data on the occurrence of compatible relatives of GM plants in different agricultural or (semi) natural habitats of Europe, or effects of production systems on the interactions between the GM plant and the environment).

Relevant baseline(s) of the receiving environment(s), including production systems, indigenous biota and their interactions, should be established to identify any potentially (harmful) characteristics of the GM plant (see chapter 2, and specific areas of risk to be addressed in sub-chapters of chapter 3). Relevant baselines refer to current production systems for which generally published literature is available. These baseline(s) serve as a point of reference against which future changes can be compared. The baseline(s) will depend to a considerable extent on the receiving environment(s), including biotic and abiotic factors (for example, natural preserved habitats, agricultural farmland or contaminated land).

Both the plant and the transgenic trait(s) determine where the GM plant will most likely be grown (see Table 2). Some GM plants (e.g. cotton, rice) can realistically be cultivated in some geographical zones only, while others, like maize, may be cultivated more widely in Europe. Transgenic traits such as biotic (e.g. pest resistance) and abiotic (e.g. drought and salt) stress tolerance will also determine where GM plants are likely to be grown. Therefore, all these elements should be taken into account when defining the receiving environment(s) (e.g. considering geographical zones) for the ERA of each GM plant.

Applicants shall take into account the potential risk implications of the presence of any other GM plants that have been placed on the market in the same receiving environments, including interactions between the specific cultivation characteristics (e.g. use of plant protection products) associated with the different GM plants. In addition, applicants shall consider likely and/or predicted trends and changes to receiving environments, and how these might interact with the GM plants.

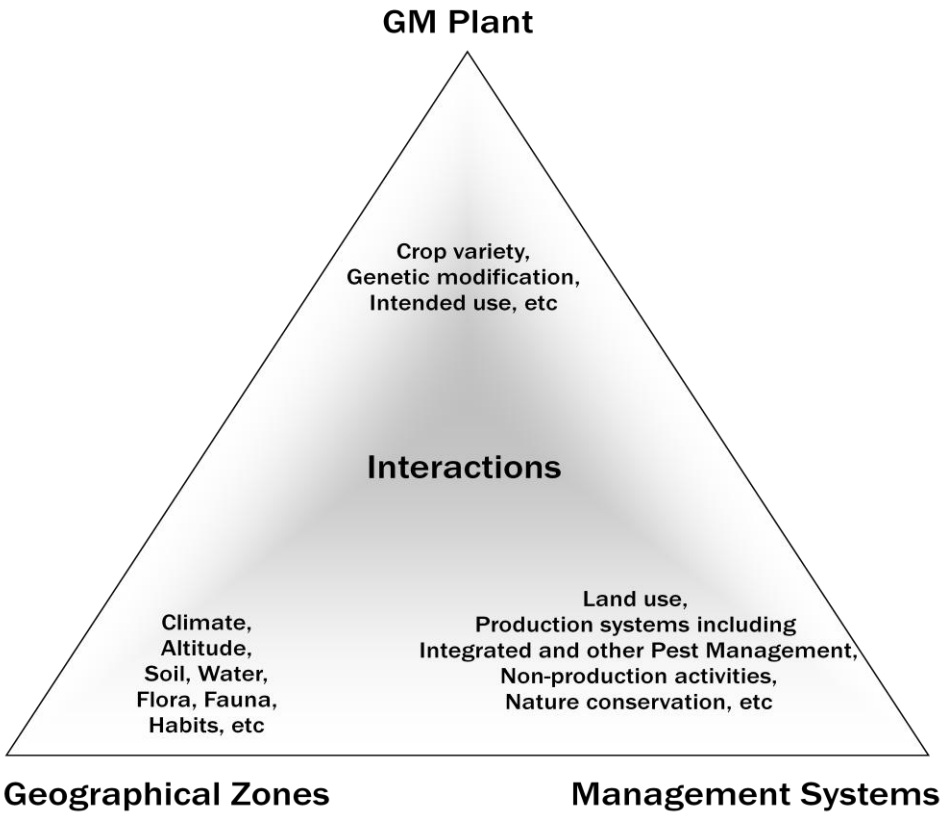

Figure 3: The receiving environment(s) is characterised by: (A) the GM plant including its intended uses, (B) the Geographical Zones, and (C) the Management Systems. Examples of attributes of (A), (B), and (C) that could interact are provided in the triangle.

There are many climatic, ecological, agricultural and political ways of defining geographical regions or zones in Europe and examples of existing definitions are provided in Appendix A. The variety of the methods and criteria used to define these zones reflects the diversity and multivariate nature of the characteristics of potential receiving environments of a GM plant. In some cases, such methods may assist applicants to select study sites. However, applicants should also consider selecting sites where the exposure and impacts are expected to be highest and where it is anticipated that if effects exist they will be detected. Applicants shall explain why the results of their studies in certain receiving environment(s) are considered representative for other receiving environment(s).

Applicants should initially consider representative scenarios for the GM plants, including a worst-case scenario where the exposure and impact are expected to be the highest. The receiving environment(s) is characterised by the GM plant, the geographical zones and the management systems (including production systems) (Figure 3). Cultivation areas may cover one or more geographical regions or zones[14] in Europe. Further background information on geographical zones is provided in Appendix A of this GD. Applicants may use the following step-wise approach (Table 2) to select appropriate receiving environment(s) for ERA.

[14] Region or zone is used here as spatial scale without defining its size characteristics.

Table 2: Selection process of relevant receiving environment(s) for ERA

Step 1 Plant	Consider the present distribution range of the plant species.
Step 2 Plant x trait	Revise present cultivation areas and their production systems according to the nature of the trait: - add potential future cultivation areas, - where relevant, consider changes in production systems, - according to the nature of the trait, concentrate on those areas and production systems in which the GM plant is most likely to be grown.
Step 3 Plant x trait x environmental issue of concern	Select appropriate receiving environment(s) for each environmental issue of concern identified in the problem formulation, taking into consideration assessment endpoints (see chapters 3.1 to 3.7).

For the set of selected receiving environment(s) identified in step 3 of Table 2, applicants shall describe:

- The characteristics of these receiving environments where the plant is likely to be distributed, specifically considering the transgenic trait(s) (e.g. that might induce farmers to adopt it);
- The representative management systems (e.g. use of the plant, crop rotation, other GM plants, production systems, cultivation techniques);
- The range of relevant biotic and abiotic interactions (e.g. the interactions between plants and target organisms (TO) and/or NTO) likely to occur in the receiving environment(s) taking into consideration the range of natural environmental conditions, protection goals (including those related to species differences across Europe) and production systems. Where appropriate, the presence of cross-compatible wild/weedy relatives nearby, the ability of the GM plant to form feral populations and hence the potential impacts on the receiving environment should be considered.

Based on the criteria listed above, applicants shall provide evidence that data generated are representative of the range of receiving environment(s) where the crop will be grown in the EU, e.g. for the selection of field trial sites according to chapter 2.3.3.5.

2.3.3. General statistical principles

This chapter applies to data collected from experiments in which specific hypotheses are tested. When such experiments are conducted in the field they are termed 'trials' throughout this chapter. This chapter does not apply to data obtained from surveys or observational data.

For ERA, applicants shall list explicitly *in words* all the questions that each study, be it a field trial, a trial in semi-field conditions or a laboratory study, was designed to address. In addition, each of these questions shall be re-stated *in formal terms*, in the form of the precise null hypothesis that was tested to answer the question. This shall apply equally to those studies that seek confirmatory data on unintended effects when some evidence already exists, as to those that take an ecotoxicological approach with a specific null hypothesis (see chapter 3.4). For field trials, applicants shall provide a clear and explicit statement concerning the minimum levels of abundance acceptable for each taxa sampled, below which results would lack credibility (for an example, see Heard et al., 2003 section 2F). Applicants shall supply justification for the values chosen. In mathematical modelling for the

assessment of long-term or large-scale effects, applicants shall state explicitly all assumptions made and provide justifications for each. The principles underlying the statistical tests of difference and equivalence (EFSA, 2009e) described below are to provide information with quantified uncertainty that may be used by biologists in risk characterisation of those endpoints for which differences or lack of equivalence are found. In order to place differences or lack of equivalence into context, allowance must be made for the distinction between statistical and biological significance. The two approaches are complementary: statistically significant differences may point to biological changes caused by the genetic modification, but these may or may not be relevant on safety grounds (see limits of concern, below). For risk assessment it is not the function of statistical analysis to provide results that lead automatically to a particular decision; instead, the case-by-case approach shall remain paramount.

The ERA is often hampered by the difficulty of conducting experiments with sufficient statistical power (see below). The use of meta-analysis (Marvier et al., 2007) is an option for applicants to consider, but is not mandatory. It may be useful to quantify studies that may not all have the power to be individually significant, in the statistical sense, and also to provide an overview of broad patterns when individual studies appear to contradict each other.

The comparative analysis referred to above shall involve two approaches: (1) a proof of difference, to verify whether the GM plant is different from its conventional counterpart(s) and might therefore be considered a potential risk depending on the type of the identified difference, extent and pattern of exposure; and (2) a proof of equivalence to verify whether the GM plant is equivalent or not to its conventional counterpart(s) (Perry et al., 2009) within bounds defined by so-called *'limits of concern'* (see below). For each measurement endpoint, the level of environmental protection to be preserved is expressed, directly or indirectly, through the setting of 'limits of concern' which may take one of two forms. For lower-tier studies (see chapter 3.4) the limits of concern will usually be trigger values which, if exceeded, will usually lead to further studies at higher tiers. Then the relationship of the limits of concern to environmental protection goals is indirect. For higher tier studies, especially field studies, the limits of concern shall reflect more directly the minimum ecological effects (in positive and negative directions) that are deemed biologically relevant. For field studies, at least one of the limits of concern shall represent the minimum effect that is considered by applicants potentially to lead to environmental harm (see also chapter 2.3.3.2). If this limit is exceeded then detailed quantitative modelling of exposure may be required to scale up adverse effects at the field level both temporally (to seasons, generations, rotations) and spatially (to farms, landscapes, regions and ecosystems) (EFSA, 2008). Baseline data can be used to define the limits of concern. Purely as a guide, for laboratory studies, a multiplicative effect size of 20% is often taken as the trigger value for further, higher-tier studies. Similarly, for semi-field testing, a trigger value of 30% has been used previously. For field studies, several studies, both in the USA and in the EU (Heard et al., 2003), have adopted 50% as a limit of concern, which is a reasonable level. By contrast, the effect size threshold for classification set by IUCN for butterflies is a reduction in population size of at least 30% over three generations (but here 'population' is defined at a larger than field scale). Note that, unless there is explicit justification, limits of concern for lower-tier studies shall usually be less than those for higher-tier studies, since it makes no sense for the results from laboratory studies to exclude from further study effects that might be manifest in the field. Whatever are the limits of concern adopted, applicants shall state their value and justify the choice explicitly, for each measurement endpoint. For field studies, it will usually be the lower limit, which might correspond for example to a decrease in the abundance of a particular species in the presence of the GM plant relative to that for the conventional counterpart, that will be defined as the threshold effect deemed to be of just sufficient magnitude to cause environmental harm. Notwithstanding this general approach, it is acknowledged that the multiplicity and diversity of questions that might be posed in an ERA may demand alternative statistical approaches, on a case-by-case basis.

All test materials, the GM plant and conventional counterpart(s), whether in the field, in semi-field conditions or in the laboratory, shall be fully randomised to the experimental units. Other aspects of experimental design are addressed below.

Whether analysis is of field, semi-field or laboratory data, results shall be presented in a clear format, using standardised scientific units. Applicants shall provide the raw data and the programming code used for the statistical analysis in an editable form. Other aspects of reporting and analysis are addressed below.

2.3.3.1. Testing for difference and equivalence

In testing for a difference the null hypothesis is that there is no difference between the GM plant and its conventional counterpart, against the alternative hypothesis that a difference exists. In testing for equivalence the null hypothesis is that there is lack of equivalence, in the sense that the difference between the GM plant and its conventional counterpart is at least as great as a specified minimum size, against the alternative hypothesis that there is no difference or a smaller difference than the specified minimum between the GM plant and its conventional counterpart. Rejection of the null hypothesis (*i.e.* a finding that the difference is no greater than this minimum size) is required in order to conclude that the GM plant and the conventional counterpart are unambiguously equivalent for the measurement endpoint considered. The two approaches are complementary: statistically significant differences may point to biological changes caused by the genetic modification, but these may or may not be relevant from the viewpoint of environmental harm. For studies that use extra comparators, the analysis shall encompass separate difference tests (between the GM plant and each of its different comparators) and separate equivalence tests (between the GM plant and each of its different comparators), and these shall be reported similarly. Further discussion of the principles of equivalence testing, with practical examples, is given in EFSA (EFSA, 2009e).

2.3.3.2. Specification of the effect size and the limits of concern

Major parts of the risk assessment dossier are problem formulation and risk characterisation. Notwithstanding the well-known distinction between biological relevance and statistical significance (Perry, 1986) risk characterisation cannot be done without relating effects to potential harm. Therefore, it is essential to specify for each variable studied a minimum effect size which is considered to potentially have a relevant impact on the receiving environment(s). Based on such effect sizes, power analyses aid transparency and may engender public confidence that risk to the consumer is well-defined and low (Marvier, 2002); these require specification of the magnitude of the effect size that the study is designed to detect. Good scientific studies are planned carefully enough for the experimenters to have a reasonable idea of the size of effect that the study is capable of detecting. For all these reasons, for each study, whether in the field, in semi-field conditions or in the laboratory, applicants shall state explicitly the size of the effect that it is desired to detect in the study, for each measured endpoint. The effect size may be asymmetric, and in particular may be set as zero in one direction to yield a non-inferiority form of the equivalence test (Laster and Johnson, 2003). The magnitude of the effect size that the study is designed to detect will generally be greater for trials designed to provide confirmatory field data for the assessment of unintended effects on non-target organisms than for specific hypotheses (see chapter 3.4). The effect size will often be placed on the multiplicative scale; however, the natural scale or some other scales are admissible alternatives, on a case-by-case basis. In principle, where more than one comparator is used different effect sizes may be specified for the different comparators; however, this is unlikely to be necessary in practice. Applicants shall provide a full justification for all effect sizes chosen.

The applicant shall state explicitly how the chosen effect size(s) relates to the limits of concern through the minimum relevant ecological effect that is deemed biologically relevant. Usually, these quantities will be identical; applicants shall justify cases where this is not so. Applicants shall state explicitly the limits of concern that were used for each equivalence test. If justified appropriately, more than one pair of limits of concern may be set for each measurement endpoint; an equivalence test shall then be performed for each pair of limits.

2.3.3.3. Power analysis

For each study, be it a field trial, a trial in semi-field conditions or a laboratory study, applicants shall ensure that the design is such that the difference test has sufficient statistical power to provide reasonable evidence (Perry et al., 2009). Statistical power is the probability of detecting an effect of a given size, when such a real effect exists. In medical science, a level of 80% is usually considered to be an acceptable level for statistical power, but it is recognised that for ecological GM field trials the restriction on the land available for experimentation combined with unavoidable environmental heterogeneity usually necessitates some compromise between the replication required for high power and the experimental resources available (Perry et al., 2003). Notwithstanding, optimal experimental design shall be directed to attain power as high as possible.

For each study, applicants shall provide an analysis that estimates the power for each difference test on each measurement endpoint, based on the stated effect size and assuming a 5% type I error rate. The analysis shall be done at the planning stage of the study. The power analysis shall use only information verifiable as available prior to the study; under no circumstances shall data from the study itself be used. For field trials, since each field trial at a site on a particular occasion shall have sufficient replication to be able to yield a stand-alone analysis if required (see below), this power analysis shall relate to a single site, not to the entire set of trials. For situations where many species are sampled such as in field trials, the power analysis is required only for those species of prime importance and those expected to be the most abundant.

2.3.3.4. Experimental environment

The first decision in conducting a study is whether the questions asked are best answered by data produced in the laboratory, mesocosm, semi-field, field or region.

As is clear from chapters 2.2.1 and 3.4.1.4, the effect of plant-environment interactions **can** be studied starting from studies that encompass a range of environmental scales. For this, hazards are evaluated within environments that progress from worst-case scenario conditions with laboratory experiments, up to ecological field trials with relatively large plots.

The laboratory environment is favoured for studies where it is important to control and define closely the conditions for tested organisms. Since environmental variability and interfering factors which can mask potential effects are minimised, laboratory studies yield results of relatively high precision. The laboratory environment is used particularly for the identification of acute and direct impacts of GM products and metabolites on individuals. In particular dose-response relationships may be well described. It also provides the possibility to study indirect and multi-trophic effects at small scales. Trait-environment interactions may be studied in the laboratory, but only to a limited extent. The laboratory is often used as an initial environment in the tiered approach, particularly for tier 1 studies (see chapter 3.4.1.4). In a laboratory study, decisions must be made whether test materials should be of synthetic or *in-planta* form (see chapter 3.4.1.4).

Semi-field trials are manipulative test systems that are designed to control the inherent variability of the environment. They usually incorporate some form of protected environment or containment, such as field cages or screen houses, designed both to isolate the organisms under test and exclude unwanted biotic (e.g. predators) or non-biotic (e.g. rainfall) factors. Semi-field trials allow exposure to ambient weather and light conditions. The larger cages may result in more natural behavioural interactions between the organisms and plants tested. The semi-field environment is not subject to large variations in the ecology of habitats, and any variability due to different receiving environments is suppressed. Semi-field trials may have greater sensitivity than less-controlled open field trials and it may be that lower levels of statistically significant differences may therefore be detected. Examples

include studies on possible indirect effects on non-target pollinators using bees in screen house trials (see chapter 3.4.). Mesocosms are experimental ecosystems that can be used to perform tests under realistic semi-field conditions. Examples include studies of biogeochemical cycles using residue decomposition (see chapter 3.6), although litterbag experiments within field trials provide a more realistic alternative.

Field trials allow the study of indirect and multi-trophic effects at larger scales, including at some cases the population level. Trait-environment interactions may be tested validly. Although they must, by definition, suffer from less ability to control environmental conditions and therefore produce results subject to greater environmental variability, they provide the only way in which relevant lower-tiered results may be validated under natural conditions. They allow experimental tests of parameters of importance in ecosystem functioning (such as the predation and/or parasitism rate of a species, the decomposition rate of plant residues, etc.) and the estimation of overall ecosystem functions (such as pollination, natural pest control, etc.). Another advantage of field trials is that genotype x environment interactions may be studied in the receiving environment(s).

Field surveys are scientifically designed studies without a hypothesis and where there is no experimental imposition of treatments. However, data are collected in the receiving environment(s). For example, these may provide appropriate data relevant to the identification of unintended effects on non-target organisms (see chapter 3.4.1) and to changes in plant fitness (see chapter 3.1.1).

The importance of field trials in the ERA of GM plants is widely accepted (EFSA, 2008). One crucial aspect is the increase in ecological realism that can be achieved as the scale of tests move up from laboratory through mesocosm to semi-field, field and region. For example, when any organism is in contact with a GM plant within a multi-trophic context, identification of the impacts on ecological functioning is facilitated by an increase of scale of the experimental arena.

Field studies (semi-field, field trials and field surveys) for environmental effects of GM plants is of special importance because there are organisms for which particular ecological or behavioural tests in the laboratory fail to encompass realistic conditions (for example in some studies of species that are highly mobile, such as adult butterflies or bees; or species for which rearing methods are inadequate; see chapter 3.4). Field testing allows a wide range of arthropod characteristics to be assessed (such as species number, life stages, exposure to abiotic and biotic stress, complexity of trophic interactions) that cannot easily be reproduced in laboratory settings. Conversely, laboratory studies may incorporate controlled conditions that are impossible to reproduce in the field, which may prevent the identification of causal relationships. Attention shall therefore be paid to the differences in inferences that may be drawn between standardised tests and field testing. For example, due to the lack of well-defined standards, the number of standard laboratory tests on necrotrophic decomposers is very limited and, in particular, some biogeochemical processes cannot be investigated in artificial environments, such as pot experiments. Therefore, field trials may be essential to produce results in such cases.

2.3.3.5. Experimental design

Experimental designs for laboratory experiments shall conform to accepted international standards and protocols such as those published, for example, by OECD or similar organisations specialising in ecotoxicology.

For field trials, the principle shall be followed that each field trial at a site on a particular occasion shall have sufficient replication to be able to yield a stand-alone analysis if required, although the main analysis shall derive inferences from averages over the complete set of field trials at all sites and years. The level of within-site replication shall be informed by the power analysis referred to above. Notwithstanding this, it is most unlikely that less than three replicates per site would provide an adequate design. A completely randomized or randomized block experimental design is usually appropriate; appropriate extensions to these designs are discussed by (Perry et al., 2009). Applicants

shall justify explicitly why the different sites selected for the trials are considered to be representative of the range of receiving environments where the crop will be grown, reflecting relevant meteorological, ecological, soil and agronomic conditions. The choice of plant varieties shall be appropriate for the chosen sites and shall also be justified explicitly (see chapter 2.3.2). Within each site the GM plant and its conventional counterpart(s) and any additional test material, where appropriate, shall be identical for all replicates. Environmental variation is manifest at two scales: site-to-site and year-to-year. The primary concern is not environmental variation *per* e.g., but whether potential differences between the test materials vary across environmental conditions (*i.e.* statistical interactions between test material and environmental factors, often referred to as genotype by environment (GxE) interactions). Hence, in addition to within-field replication there is a need to replicate over sites and years to achieve representativeness across geography and climate. Unless explicit appropriate justification is given by applicants, each field trial shall be replicated over at least two years, within each of which there shall be replication over at least three sites. In the case that sites cover a very restricted geographic range, further replication of trials, over more than two years, may be required. The use of data from different continents may be informative, but applicants must justify explicitly why the sites within these continents are representative of the range of receiving environments where the GM plant will be grown, reflecting relevant meteorological, ecological, soil and agronomic conditions. In particular, applicants must provide explicit reasons when data from field trials in EU Member States are not available.

However, these explicit requirements above for replication to achieve representativeness do not apply to confirmatory field data for the assessment of unintended effects, for example, on non-target organisms when some evidence already exists (see below and chapter 3.4), or to the great variety of field trials designed to provide data for a wide range of purposes, to assess aspects of potential persistence and invasiveness (see chapter 3.1). Many experimental designs used for research purposes are available in the literature as a guide for the very specific requirements for such trials. Data concerning phenotypic and agronomic characteristics of plants is often derived from the same trials designed to supply data for compositional analysis; statistical guidance (EFSA, 2009e,a) has already been prepared for compositional trials and the requirements above do not apply to them. However, for some non-food, non-feed applications for cultivation, such as potatoes modified to enhance the content of the amylopectin component of starch, compositional trials may not be conducted. Then, the experimental design of phenotypic and agronomic trials shall follow the guidance in this chapter.

For non-target organisms, plant performance and data on environmental measurement endpoints (e.g. agronomic characteristics, including herbivore interactions with the plant, responses to specific environmental exposure) may provide indications concerning the likelihood or otherwise of unintended effects (see chapter 3.4). This may, for example, include evidence for unchanged ecosystem functions. Under the weight of evidence approach (see chapter 2.2), data from field trials may be used to provide such confirmatory data to underpin conclusions that unintended effects are unlikely. While the requirement for statistical power for these field trials shall be carried out as outlined in chapter 2.3.3.3, the requirements for representativeness may be relaxed. Hence, as long as there is explicit justification, under these circumstances, there is no requirement for a minimum number of sites and/or years.

Experimental units (field plots) that are of the spatial scale of a whole or half-field are probably of most use for post-commercialisation studies, for monitoring or mitigation. For pre-commercialisation experimentation, smaller plots, where variation may be controlled and defined treatments imposed more easily, are more appropriate for experimental units (Perry et al., 2009). It is recommended to separate plots within sites, often by strips of bare soil of specified width, and to sample towards the centre of plots to avoid edge-effects. Unless the experiment is set up specifically to study residual effects from one season to the next or to study long-term effects, it is recommended not to utilise exactly the same plots over more than one year at a particular site (Perry et al., 2009).

When it is desirable to assess several different GM plants for one crop species (e.g. *Zea. mays*) the generation of data for the comparative assessment of these different GM varieties may be produced

simultaneously, at the same site and within the same field trial, by the placing of the different GM plants and their appropriate conventional counterparts in the same randomized block. This is subject to two conditions which shall be strictly met: (1) each of the appropriate counterpart(s) shall always occur together with its particular GM plant in the same block; (2) all the different GM plants and their counterpart(s) shall be fully randomized within each block. For further details, and for the use of partially balanced incomplete block designs see (EFSA, 2009e).

In general, it is easier to impose controlled conditions in semi-field trials, and these are not subject to environmental variability to the same extent as are field trials. However, if semi-field trials do not control conditions then the need to test in different environments (at different sites and/or in different years) shall be considered.

For some GM perennial plants (e.g. trees), the plants themselves may be more appropriate experimental units than are field plots (Petersen, 1994). Care should be taken to choose an experimental design that does not suffer unduly from loss of plants during the trial. Whilst it is largely unnecessary to control for positional variation, plant-to-plant variability should be minimised when selecting experimental material.

2.3.3.6. Analysis and reporting

It is recommended that applicants prepares an experimental design protocol and a statistical analysis protocol for each study (Perry et al., 2009 for a suggested checklist). It is recommended that the experimental design protocol comprises full information on the study, and includes but is not restricted to: (1) a list of the measurement endpoints, and why they were included; (2) a description of and justification for of the experimental design; (3) a description of the experimental units including dimensions; (4) the blocking structure of the experimental units, in terms of the factors that represent it, their levels and whether the factors are nested or crossed; (5) the sampling regime, within and between experimental units, and through time; (6) any repeated measurements made in the study; (7) the test materials and the justification for their inclusion; (8) the treatment structure of the study, in terms of the factors that represent it and their levels; (9) a list of the interactions, if any, that are of interest, and why they are; and (10) a description of how the treatment factors listed will be randomized to the experimental units specified in the blocking structure above.

It is recommended that the statistical analysis protocol comprises full information on the analysis, and includes but is not restricted to: (1) a description of the generic form of the analysis and why it was chosen; (2) the criteria for identifying outliers; (3) a description of the likely transformations planned, with reasons; (4) justification for any distributional assumptions; (5) the scale on which the effects in the experiment are assumed to be additive; and (6) justification for any other assumptions made in the analysis.

For field trials, the protocols shall also include: (1) details of the management of the fields before sowing including the cropping system and rotation; (2) the dates of sowing; (3) the soil types; (4) insecticide and herbicide use and use of any other plant protection products or techniques; (5) climatic and other cultivation/environmental conditions during growth, and where appropriate during harvest; (6) relevant details of the field margins and neighbouring fields; (7) brief descriptions of pest and disease infestations.

When many measurement endpoints have been included in a study (e.g. where the endpoints represent several NTO species), the results of all endpoints for which sufficient records have been obtained shall be reported, not just those deemed to be of particular biological or statistical interest. Data transformation may be necessary to ensure normality and to provide an appropriate scale on which statistical effects are additive. As is routine in ecological applications, for many measurement endpoint response variables, a logarithmic transformation (or a generalized linear model with a logarithmic link function) may be appropriate. In such cases, any difference between the GM plant and any other test

material is interpreted as a ratio on the natural scale. However, for other measurement endpoints the logarithmic transformation may not be optimal and the natural scale or another scale may be more suitable.

Allowance must be made for possible correlations between repeated measurements from the same experimental units. This is especially important (1) where sampling is repeated over several occasions during a season; and (2) where the GM plant is a perennial.

Analyses will involve a test for difference and a test for equivalence. Specifically, for a particular measurement endpoint, the mean difference(s) between the GM plant and its conventional counterpart(s) is computed and a 90% confidence interval constructed around it, as in (Perry et al., 2009). This mean(s), these confidence limits and all equivalence limits shall be displayed on a graph(s) similar to Figure 1 of (EFSA, 2009e), but where values are plotted relative to a zero baseline defined by the mean of the GM plant test materials (see Figure 3 of Perry et al., 2009) and example therein). The line of zero difference on the logarithmic scale corresponds to a multiplicative factor of unity on the natural scale. The horizontal axis shall be labelled with values that specify the change on the natural scale. In the case of logarithmic transformation, changes of 2x and ½x will appear equally spaced on either side of the line of zero difference.

Both the difference test and the equivalence test may be implemented using the well-known correspondence between hypothesis testing and the construction of confidence intervals. In the case of equivalence testing the approach used shall follow the two one-sided tests (TOST) methodology (*e.g.* Schuirmann, 1987) by rejecting the null hypothesis when the entire confidence interval falls between the equivalence limits. The choice of the 90% confidence interval corresponds to the customary 95% level for statistical testing of equivalence. Since the confidence interval graph is used also for the test of difference, each difference test will have a 90% confidence level. Although 1 in 10 of these tests is expected to yield a significant result by chance alone, applicants shall report and discuss all significant differences observed between the GM plant, its conventional counterpart and, where applicable, any other test material, focussing on their biological relevance within the context of risk characterisation. Regarding the simultaneous tests of difference and equivalence, each outcome from the graph shall be categorised and the respective appropriate conclusion shall be drawn, exactly as described in EFSA (EFSA, 2009e).

2.3.3.7. Statistical analysis of field trials

The main analysis shall address all field trials simultaneously and shall be based on the full dataset from all sites. Accordingly, the form of the equivalence test shall be that termed ‘average equivalence’ in the drug testing literature (Wellek, 2002). The use of a statistical mixed model is an important feature of analysis for food-feed assessments because of the need to estimate the natural variation of the commercial varieties. However, as stated in chapter 2.3.3.2 above, for ERA it is recommended that equivalence limits are set explicitly. Therefore, the use of commercial varieties for this purpose is not necessary, although it might be appropriate for other biological reasons. Hence it is not recommended that statistical mixed models be required forms of analysis, as they are for food-feed assessments (Perry et al., 2009). Indeed, it is recommended to use simple statistical models; effects due to environmental factors such as seasons and sites may be represented by fixed factors if desired. Applicants shall ensure that each analysis has the potential to identify any interactions between sites and years and the test materials. For each measurement endpoint studied, applicants shall make an explicit statement concerning the presence or absence of any such interactions. If interactions are found, the possible reasons for their existence and the implications for the inferences drawn from the trials shall be discussed. Applicants shall also provide a table or graph giving, for each site and year and for each (transformed) measurement endpoint, the means and standard errors of means of the GM plant and its conventional counterpart(s), and any other test material, where applicable.

Diversity indices are not recommended for general risk assessment in pre-commercialisation studies, because it is most unlikely that studies will yield sufficient samples of individuals to characterise indices adequately or that a sufficient degree of ecological background information will exist to give confidence that biodiversity can be represented adequately as a single number. By contrast, multivariate approaches may be useful, especially for summarising data and for analysing principal response curves (Perry et al., 2009).

Particular recommendations apply for the very wide range of possible studies of persistence and invasiveness, and the related estimation of selective advantage and disadvantage (see chapter 3.1).

Further discussions and motivations underpinning the above statistical guidance (chapters 2.3.3) may be found in Perry et al. (2009).

2.3.3.8. Uncertainties

Directive 2001/18/EC and the Guidance Notes supplementing Annex II to Directive 2001/18/EC define risk as the product of the magnitude of the consequences of the hazard and the likelihood of the adverse effect. Both the effect and the likelihood are measured with uncertainty.

ERA has to take into account uncertainty at various levels. Uncertainties may arise from problem formulation, limitations in the data (e.g. limited exposure data), gaps in the effect database, model choice, the limitation of the test systems and measurement endpoints selected, inadequacy of study designs and the uncertainties in extrapolating between species (EFSA, 2009b). Scientific uncertainty may also arise from differing interpretations of existing data, publication bias or lack of some relevant data. Uncertainty may relate to qualitative or quantitative elements of the analysis. The level of knowledge or data for a baseline is reflected by the level of uncertainty, which shall be discussed by applicants. Applicants shall in addition assess the degree of uncertainty within the ERA in comparison with the current uncertainties displayed in the scientific literature.

Although it may be impossible to identify all the uncertainties, the assessment shall include a description of the types of uncertainties encountered and considered during the different risk assessment steps. Their relative importance and their influence on the assessment outcome shall be described (EFSA, 2009b). Any uncertainties inherent in the different steps of the ERA (steps 1 to 5) shall be highlighted and quantified as far as possible; this might be done by adapting the methodology outlined by (Risbey and Kandlikar, 2007). Distinction shall be made between uncertainties that reflect natural variations in ecological and biological parameters (including variations in susceptibility in populations or varieties), and possible differences in responses between species. Estimation of uncertainties in experimental data shall be handled by proper statistical analysis, while quantification of uncertainties in assumptions (e.g. extrapolation from environmental laboratory studies to complex ecosystems) may be more difficult, but shall be discussed fully[15i] . The absence of data essential for the environmental risk assessment shall be indicated and the quality of existing data shall be discussed. It should be clear from the discussion how this body of information has been taken into account when the final risk characterisation is determined. Risk characterisation may be qualitative and, if possible, quantitative depending on the issue to be addressed and the available data. The terms for the expression of risks and associated uncertainties shall be as precise as possible. For instance, expressions like '*no/negligible/acceptable/significant risk*' need, where possible, further numerical quantification in terms of probability of exposure and/or occurrence of adverse effects (see also chapter 2.2.1).

It is recognised that an ERA is only as good as our state of scientific knowledge at the time it was conducted. Thus, under current EU legislation, ERAs are required to identify areas of uncertainty or

[15] (Morgan M. G., 1990, Finley, 1994)

risk which relate to areas outside current knowledge and the limited scope of the ERA. These include such factors as the impact of the large-scale exposure of different environments when GM plants are commercialised, the impact of exposure over long periods of time and cumulative long-term effects. When uncertainty factors (EFSA, 2009b) are used, an explanation of their basis and a justification of their appropriateness need to be provided, or a reference to documents where that information may be found shall be included. When point estimates are used for uncertain quantities, justification for the values chosen and assessment of their influence on the assessment shall be included (EFSA, 2009b).

Predicting impacts of GM plants on complex ecosystems which are continually in flux is difficult and largely based on experiences with other introductions and an understanding of the robustness of ecosystems. It is recognised that an environmental risk assessment is limited by the nature, scale and location of experimental releases, which biospheres have been studied and the length of time the studies were conducted. Probabilistic methods could be used to determine ranges of plausible values rather than single values or point estimates, which are subsequently combined in order to quantify the uncertainty in the end result. These methods could provide a powerful tool to quantify uncertainties associated with any steps in the environmental risk assessment. When such probabilistic approaches are used, the outcome of the environmental risk assessment should be characterised by reporting a distribution of the risk estimates[16]. However, the use of quantitative methods does not remove the need for a qualitative evaluation of the remaining uncertainties (EFSA, 2009b).

Scientific knowledge from the literature and experience gained from growing GM plants encompassed in PMEM following past applications and approvals may also inform the risk assessment process. Notwithstanding the requirement to fully assess all possible risks based on reliable data, this is but one example of the responsibility on applicants continually to update ERA in the light of new knowledge.

2.3.4. Long-term effects (including techniques for their assessment)

A general requirement of an ERA is that an analysis of the "*cumulative long-term effects relevant to the release and the placing on the market is to be carried out*" (EC, 2001). Predicting and assessing (adverse) long-term effects requires information about the GM plant and the receiving environment(s) (see also chapter 2.3.2), both in terms of the baseline conditions in the receiving environment(s) and temporal changes in these conditions independently of the GM plant and following GM plant introduction. The rate and degree to which the baseline is likely to change independently of the GM plant (e.g. as a result of new crops and agronomy) will vary among production systems. The consideration of long-term effects in the ERA should address effects that might arise up to a minimum of 10 years after the start of cultivation for annual plants, *i.e.* corresponding to the time frame of the consent authorisation (EC, 2001, EFSA, 2008), but possibly longer for perennial species, and should in all cases cover the time period over which progeny of the GM plant might persist and appear as volunteers or ferals. Thus, the analysis should be conducted case-by-case and applicants should fully justify their approach. Further background information and indicative examples are provided in Appendix B of this GD.

2.3.4.1. Categories of long-term effects

Long-term effects might result from a diversity of primary causes and secondary interactions, which make it difficult to generalise on methods of investigation. Such effects can be considered in two broad categories, however.

In the first category, long-term or chronic exposure to a particular GM plant or practice results in a delayed response by organisms or their progeny (Category I). It may be in some instances that a

[16] Examples of probabilistic approaches applied for ERA of pesticides may be found at http://www.eufram.com.

response occurs immediately, but is not detected by the measuring tools or the particular indicators employed. For example, exposure over time may affect a specie or community by suppressing certain functional forms in relation to others, or acting on natural mutations that exist at very low frequency such as occurs when pests develop resistance to a pesticide.

In the second category, effects occur as the result of an inevitable increase in spatial and temporal complexity, determined by the number of possible interactions that a GM plant would have with the biota and the physical and chemical environment as it is grown more widely throughout the landscape and in more extended sequences of cropping (Category II). There may not necessarily be a chronic or delayed effect as in the first category; rather, the effect occurs in certain contexts that are outside those experienced in the initial testing, or that have arisen as entirely new contexts due to global environmental change or the adoption of new forms of management. The latter may indeed arise as a downstream effect of the introduction of the GM plant cultivation itself, if this causes a change in the sequence or range of plants grown in the production system.

An estimate of whether long-term effects of both Categories are expected to occur and how PMEM should be followed after commercialisation should be given in any application. Based on the characteristics of the GM plant, the ERA should consider these long-term effects by reference to existing examples, long-term datasets, and in some instances modelling, as indicated below. The analysis and conclusions should be presented in the form of a desk study based on the interpretation of existing information.

2.3.4.2. Techniques and information required to assess long-term effects

Some effects of Category I might already have been investigated within constrained experimental systems maintained over several generations of the GM plant/trait combination under study. While some potential long-terms effects might be revealed by such studies, questions will still remain, as to how much the constrained system restricts the range of possible reactions or encourages untypical reactions, such as caused by a reduced choice in the foraging range and food available to invertebrates that are kept for months or years in controlled environment chambers or restricted to intensely managed field plots. Information from such studies might be useful for defining the primary mechanisms by which the GM plant might interact with other organisms and their abiotic environment, but would not be sufficient alone as a basis for assessment of long term effects in an agricultural or ecological context.

Category II, by definition, cannot be investigated through an initial experimental phase of testing, even at the scale of the field plot, half-field or paired field, none of which can provide the range of complexity experienced after full commercial release. For example, the unpredicted increase of grass weeds compared to broadleaf weeds in GM herbicide tolerant crop trials on winter oilseed rape, and the consequent reduction of the arable food web, were probably a combination of the timing of herbicide application, the local climate and the local weed profile - a context that had not been, and could not be, examined before large scale, multi-site trialling (see Appendix B). Category II effects can only be investigated by reference to existing examples and case histories that provide evidence of rates and magnitudes of environmental impact due to change in agricultural (*e.g.* pesticides, crop type) or external (e.g. extreme weather) factors, including GM cultivation where data are available.

Despite these uncertainties, there is now a great deal of information in the published literature, and in accessible reports and databases, on long term ecological and environmental effects due to agricultural change. Applicants should conduct appropriate desk-based studies to assess long-term environmental effects of the GM plant in relation to both categories of long-term effects. It is not the intention here to give precise instruction to applicants on which data, processes and indicators should be considered, since they will vary case-by-case. However, examples of the type of information that could be used in assessment are:

- Experience of cultivating the GM plant or long-term environmental exposure to GM cultivation in other regions;
- Experience from cultivation of similar plants (GM and non-GM);
- Long-term ecological or environmental datasets applicable to the receiving environment(s); e.g. government statistics on cropped areas, pesticide usage, nutrient inputs, agrochemicals in water; ecological surveys showing change in organisms range or abundance;
- The results of major field experiments on GM plants that have examined effects or GM events similar to those of the GM plant under assessment; e.g. the UK's field trials of GM herbicide tolerant crops (Firbank et al., 2003, Perry et al., 2003, Squire et al., 2003); and long-term field exposure studies to Bt maize in Spain (Farinos et al., 2004, de la Poza et al., 2005, de la Poza et al., 2008, Farinos et al., 2008);
- Quantitative examples of the degree to which previous agricultural change, even if not involving GM plants, has affected ecological and environmental indicators; e.g. response of plants and animals to change in pesticide usage, to change in season of cropping (winter replacing spring crops since the 1970s in many parts of Europe), and to expansion or contraction of cropped area (the rise of food-quality oilseed rape as a break crop; the rise and fall of set-aside);
- The results of meta-analyses drawing together data from different sources (e.g. Marvier et al., 2007, Duan et al., 2009, EC, 2009b);
- The use of models of ecological processes to explore or test scenarios: mathematical models of ecological processes are unlikely to be considered justification on their own, but may be used to argument or interpret data or to demonstrate that possibilities have been explored; descriptions would be necessary of the model, its verification using existing data, the input variables, etc;
- Foreknowledge of relevant change in the production system and wider environment that can be expected in the years following release; an example is the withdrawal of pesticides from commercial usage.

2.3.4.3. Other guidance for applicants

Applicants should conclude for each of the chapters 3.1 to 3.7 – where appropriate - on the outcomes of the risk assessment for long-term effects by summarising:

- The methods and approaches used to reach the conclusions, including the published long-term or large-scale experiments, reference datasets, analysis and models used directly in the assessment;
- The basis of and justification for a conclusion specific to the GM plant or its management (whether a conclusion is for or against the likelihood of a long-term effect);
- Identification of parts of the monitoring plan that are designed to detect possible long-term effects identified in the desk study.

Applicants should propose indicators and reference databases for appropriate EU-wide PMEM of long-term effects resulting from GM plant cultivation. Potential indicators should be further developed over time by applicants in cooperation with risk assessors and risk managers. The indicators for PMEM should be selected in accordance with the GM plant and its intended uses in the receiving environment(s) (see chapter 4).

2.3.5. Risk assessment of GM plants containing stacked transformation events

In the context of this GD, the term "*stacked transformation event" or "stacked events"* will refer to a GM plant derived from conventional crossing of GM plants consisting of one or more events[17]. The ERA of stacked events should follow the general strategies described in chapter 2.1 and 2.2 of this GD, *i.e.* it should include a comparative safety assessment and follow the 6 steps of the ERA (Figure 2).

In line with the EU implementing rules concerning applications for authorisation of GM food and feed in accordance with Regulation (EC) No 1829/2003 and the EFSA GD for the risk assessment of GM plants containing stacked events, the ERA of the single events is a pre-requisite for the risk assessment of stacked events. The ERA of stacked events shall start when the risk assessment of each single event is finalised. In case single events cannot exist separately, an alternative rationale for the risk assessment approach should be provided by applicants.

For GM plants containing stacked events, the primary concern for RA is to establish if the combination of events might result in interaction that would raise safety concerns compared with the single events or in case of stacked events containing three or more events combined by conventional crossing (defined as higher staked events), compared to already assessed sub-combinations (defined as lower stacked events). The ERA of a higher stacked events shall cover all sub-combinations of these events.

For application for import and processing, the ERA of higher stacked events shall cover all sub-combinations of these events as independent stacked events.

For application for cultivation of the higher stacked events only , applicants should consider the full range of environmental issues of concern (chapter 3.1 to 3.7) including change in management of the higher stacked events compared to lower stacked events or single events already risk assessed . In addition, the ERA of a higher stacked events shall consider all other sub-combinations of these events that may occur by natural segregation (e.g. volunteers).

For application for cultivation of the higher stacked events and specified sub-combinations (cultivation stack-n, stack-n-1,stack-n-2, etc.), applicants shall consider the full range of environmental issues of concern (chapter 3.1 to 3.7). In particular, applicants shall describe fully the management of each of the cultivated sub-combinations individually and assess their environmental impacts. In addition, the ERA of higher stacked events shall consider all other sub-combinations of these events that may occur by natural segregation (e.g. volunteers).

Applicants shall provide a scientific rationale justifying the range and extent of information used to support the risk assessment of sub-combinations.

The ERA of stacked events shall mainly focus on the characterisation and potential consequences of issues related to

- stability of the inserts;
- expression of the events;
- potential synergistic, additive or antagonistic effects resulting from the combination of the events;
- changes in management (if applicable).

[17] This excludes re-transformation and co-transformation (EFSA GMO Panel Working Group on comparators (http://www.efsa.europa.eu/en/gmo/gmowgs.htm).

The appropriate comparator for stacked events should be selected in accordance with the requirements defined in chapter 2.3.1. Applicants should justify the choice of all comparators.

Specific considerations for stacked events:

Areas of risk described in chapter 3 should be considered on a case-by-case basis. Some specific considerations for stacked events are mentioned below.

Persistence and invasiveness including plant-to-plant gene flow (see chapter 3.1)

For stacked events, applicants should consider, during the problem formulation phase, whether the combination of events may lead to enhanced persistence or invasiveness that is more than the expected from the simple product of the single traits. Additional field data may be required if changes are observed in phenotype or growth characteristics (e.g. such as behaviour, fitness, reproduction, survivability or dissemination).

Interactions of stacked events with target organisms (see chapter 3.3)

For stacked events, potential synergistic, additive or antagonistic effects of different biocidal substances should be taken into consideration. In order to confirm the absence of these potential effects, the potential impact on target organisms should be assessed. In addition, consequences of any interaction on the development of resistance in target organisms should also be assessed and considered when developing risk management strategies.

Interactions of stacked events with non-target organisms (see chapter 3.4)

For stacked events not expressing biocidal compounds, if scientific knowledge does not indicate possibility of synergistic, additive or antagonistic interactions between these compounds that may affect NTOs, then no specific testing is necessary.

Stacked events expressing more biocidial compounds that the single events, may have different adverse effects on NTOs than the single events due to synergistic, additive or antagonistic effects. Applicants shall perform studies (or provide existing data) with combined administration of proteins when the genetic modification results in the expression of two or more proteins in the GM plant. *In planta* tests with the stacked event shall be included in tier 1 studies. Testing should follow the same approach as described in 3.4.

Impacts of the specific cultivation, management and harvesting techniques (see chapter 3.5)

Applicants are requested to describe the specific cultivation, management and harvesting techniques of the GM plant containing stacked events and of each of the cultivated sub-combinations covered by the application and assess their potential environmental impacts with respect to the appropriate comparator. In this evaluation, any differences in the specific cultivation, management and harvesting techniques between: (1) the stacked events; (2) the single events contained in the stacked events; (3) the conventional counterparts if available; and (4) each of the cultivated sub-combination of stacked events shall be explicitly stated and assessed with regard to their environmental impacts.

Post-market environmental monitoring plan (see chapter 4)

The general principles of the PMEM as described in this GD are appropriate for applications concerning stacked events. Case-specific monitoring should take into account the results of the ERA, plus any monitoring already proposed or established for single events previously assessed. Consideration should be given to any additional environmental exposure or other effect due to the combination of events identified in the ERA. General surveillance should proceed as for any other GM

plant and take account of any general surveillance plans already proposed or established for single events previously assessed.

3. Specific areas of risk to be addressed in the ERA

The EFSA GMO Panel groups the environmental risks outlined in Annex II of Directive 2001/18/EC (EC, 2001) and Decision 623/2002/EC (EC, 2002) into seven specific areas of risk. For each specific area of risk, applicants are requested to provide information in a clear and concise way, following systematically the first 5 steps of the ERA as described below and in chapter 2.2.

- Step 1: problem formulation (chapter 2.2.1);
- Step 2: hazard characterisation (chapter 2.2.2);
- Step 3: exposure characterisation (chapter 2.2.3);
- Step 4: risk characterisation (chapter 2.2.4);
- Step 5: risk management strategies (chapter 2.2.5);
- Step 6: conclusions (chapter 2.2.6).

For each specific area of risk (step 1 to 5), applicants should conclude by summarising the assessment, the assumptions taken, the available information and identified gaps, the data produced, the estimated uncertainty, the characterisation of the risk(s) and the need, or not, for risk management strategies.
At step 6, applicants are requested to consider the overall evaluation performed and to provide overall conclusions and recommendations of the ERA. The overall conclusions and recommendations should provide the frame for the risk management strategies including the PMEM and therefore, a link to chapter 4 should be made.

3.1. Persistence and invasiveness including plant-to-plant gene flow

3.1.1. Step 1: Problem formulation

Some environmental concerns about GM plants relate to the potential persistence or invasiveness of the plant itself, or of its compatible relatives, as a result of vertical gene flow within either agricultural or other production systems, or semi-natural and natural habitats. The potential adverse effects are of two main types. First, enhanced fitness[18] of the GM plant or of transgenic (introgressed) wild relatives within production systems may make them more persistent, exacerbating weed problems that may need to be controlled by more complex weed control strategies, which themselves might cause environmental harm. Second, enhanced fitness of transgenic feral plants, or of transgenic (introgressed) wild relatives in semi-natural or natural habitats may reduce the diversity/abundance of valued flora and fauna. For instance, native plant species may be displaced, which in turn might affect species that use those plants as food, shelter, etc. Alternatively, and depending on which plant and which transgenes are involved, gene flow to wild relatives may decrease the fitness of hybrid offspring. If rates of gene flow are high, this may cause wild relatives to decline locally, or to become extinct (e.g. swarm effect, outbreeding depression). Therefore, problem formulation should focus on the potential of a GM plant to be more persistent or invasive than conventional counterparts, and on

[18] Fitness is defined as the number of seeds (or propagules) produced per seed sown, and includes the whole life cycle of the plant (Crawley et al., 1993). In some studies, only components of fitness are measured – frequently this is fecundity (Snow et al., 2003). If other vital rates are unchanged (which is an assumption that should be substantiated), an increase in fecundity will lead to an increase in fitness. Enhanced fitness can be defined as a characteristic of an individual or subpopulation of individuals that consistently contribute more offspring to the subsequent generation (Wilkinson and Tepfer, 2009). Fitness will vary depending upon the environmental context (including anthropogenic influences like mowing), particularly upon the presence of inter and intra-specific competitors, herbivores and pathogens, and the abiotic conditions. The variation in fitness according to biotic and abiotic conditions is often referred to as the 'genotype by environment interaction'. It is therefore appropriate that an appropriate range of environmental conditions are considered.

the potential for gene flow to compatible relatives whose hybrid offspring may become more weedy or invasive, or may suffer from outbreeding depression. To cover all relevant receiving environments of the GM plant and its compatible relatives, problem formulation should address not only the conditions of the production system under which the GM plant will be grown, but also relevant semi-natural and natural habitats. It should also consider viable GM plant seeds or propagules spilled during import, transportation, storage, handling and processing that can lead to feral plants that colonize and invade ruderal, semi-natural and natural habitats.

A staged approach describing how the presence of an introduced trait may exacerbate weed problems in a production system, or cause environmental harm within the wider environment is proposed as outlined in Figure 4. The purpose of the staged approach is to ensure that relevant case-specific information is supplied to test hypotheses formulated in the problem formulation process, and that information requirements remain proportionate to the potential risk. Questions 1 to 10 in Figure 4 outline the issues to be addressed to estimate the likelihood of occurrence of adverse effects in ruderal, semi-natural and natural environments. These questions are divided into different stages. Whether information is required for all stages or only for specific stages will depend upon the trait(s), plant species, the intended use, receiving environments under consideration, and the conclusions drawn from lower stages.

Information required for testing the hypotheses formulated in the problem formulation process can be *species-*, *trait-* or *event-specific*. This information can be extracted from data generated by applicants, from the scientific literature, or from any other relevant sources. Some GM plants with the same traits or similar events may have been grown for a number of years at a large scale outside the EU such that field-generated data on fitness, persistence or invasiveness are available. If applicants use data from outside the EU, they should justify why these data are relevant for the range of receiving environments where the plant will be grown in the EU.

- *Species-specific* background information is required at the outset, describing the biology of the parental species including reproductive biology, survival, dispersal and cultivation characteristics in different environments. In addition, sexual compatibility with other cultivated or wild plants occurring in the EU, and the biology and ecology of these relatives should also be considered.

- **Stage 1** consists of providing *event-specific* information that enables the GM plant to be characterised, identifying intended and potential unintended differences between it and conventional counterparts. Information provided should be used to establish whether (1) the GM plant can grow, reproduce and overwinter under EU conditions, and if so (2) how its growth, reproduction and overwintering characteristics compare to its conventional counterpart. It is possible that GM traits may move to wild relatives through hybridisation within one growing season, even if the GM plant is unable to overwinter – consequently, it is important that the hybridisation potential described in the background information is considered before concluding on stage 1 information requirements. It should thus be considered whether sexual compatibility with any relative species is altered since this may result in differences in the rate of gene flow and the establishment of transgenes in other species.

- For plants that can either reproduce or overwinter in the EU, **stage 2** should explore whether the GM trait will enhance the potential for the GM plant to contribute to volunteer populations and persist in production systems, and if so, assess the potential environmental consequences. Stage 2 will also establish whether the GM plant will be capable of forming feral populations outside production systems, or whether the transgene can be transmitted to any relatives independently of the existence of volunteers or ferals. Together, these considerations allow an assessment of whether the transgene is likely to remain confined to production systems.

- If feral populations are likely and/or if hybridisation is plausible, then **stage 3** requires information to establish if GM traits will alter the fitness of feral plants, or of transgenic (introgressed) wild relatives. Since feral plants, or transgenic (introgressed) wild relatives may exhibit fitness differences across a wider range of environmental settings, stage 3 also consists of providing information that enables assessing the ability of these plants to occupy larger ecological niches than their conventional counterparts. It is possible that certain GM traits may enable the GM plant to expand its geographical range, and to grow in new areas close to wild relatives from which it was previously isolated, so the potential for this should be considered.

- Finally, if altered fitness or the ability to occupy new niches are demonstrated, **stage 4** information is needed to establish whether this will allow populations to increase and invade new communities or, alternatively if this will lead to populations of wild relatives to decline or become extinct. In both cases, the potential environmental consequences should be assessed.

- *Trait-specific* information will be appropriate to address questions of changed fitness in stages 2 to 4, provided that potential unintended effects, resulting from the transformation process, have been shown not to alter the fitness of the GM plant compared to its conventional counterpart in stage 1.

In considering the questions in Figure 4, the mechanisms and routes by which plants are exposed to the introduced trait should be taken into account. For GM plant applications for cultivation, the principle route will be through the sowing of seeds/propagules in fields, and the consequent movement of pollen and distribution of seed or propagules to other fields and the wider environment. For GM plant applications for food and feed uses, import and processing, the ERA on persistence and invasiveness is concerned mainly with the environmental consequences of accidental release of viable GM seeds or propagating material during import, transportation, storage, handling and processing. Therefore, the ERA needs to consider the scale of environmental exposure, and if this could ultimately lead to GM plants being established in receiving environments. In the latter case, the risk assessment described above and in Figure 4 is applicable.

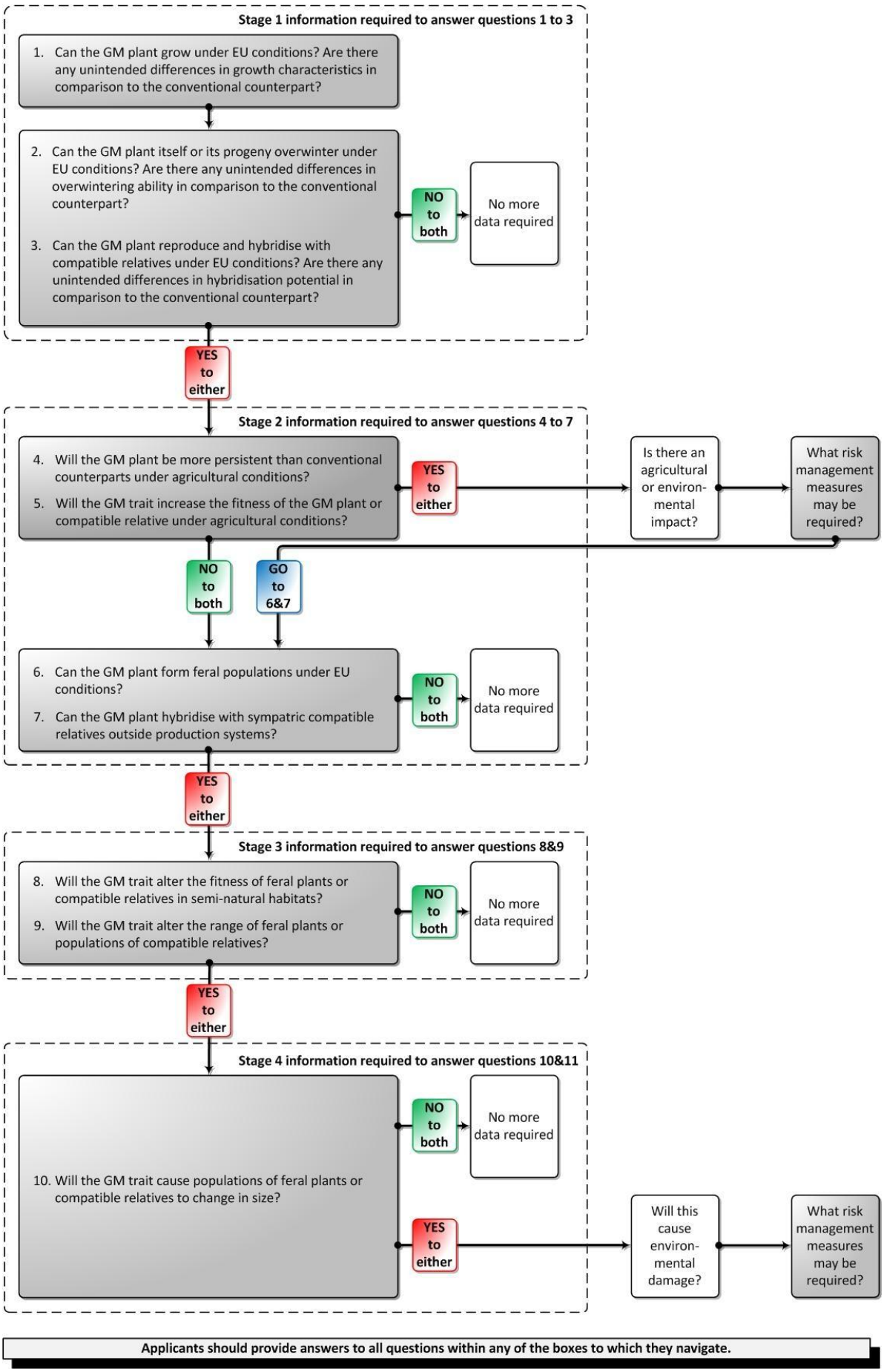
Stage 1 information required to answer questions 1 to 3
1. Can the GM plant grow under EU conditions? Are there any unintended differences in growth characteristics in comparison to the conventional counterpart?
2. Can the GM plant itself or its progeny overwinter under EU conditions? Are there any unintended differences in overwintering ability in comparison to the conventional counterpart?
3. Can the GM plant reproduce and hybridise with compatible relatives under EU conditions? Are there any unintended differences in hybridisation potential in comparison to the conventional counterpart?
NO to both
No more data required
YES to either
Stage 2 information required to answer questions 4 to 7
4. Will the GM plant be more persistent than conventional counterparts under agricultural conditions?
5. Will the GM trait increase the fitness of the GM plant or compatible relative under agricultural conditions?
YES to either
Is there an agricultural or environ-mental impact?
What risk management measures may be required?
NO to both
GO to 6&7
6. Can the GM plant form feral populations under EU conditions?
7. Can the GM plant hybridise with sympatric compatible relatives outside production systems?
NO to both
No more data required
YES to either
Stage 3 information required to answer questions 8&9
8. Will the GM trait alter the fitness of feral plants or compatible relatives in semi-natural habitats?
9. Will the GM trait alter the range of feral plants or populations of compatible relatives?
NO to both
No more data required
YES to either
Stage 4 information required to answer questions 10&11
10. Will the GM trait cause populations of feral plants or compatible relatives to change in size?
NO to both
No more data required
YES to either
Will this cause environ-mental damage?
What risk management measures may be required?
Applicants should provide answers to all questions within any of the boxes to which they navigate.

Figure 4: Questions defining the different stages of information required to test formulated hypotheses concerning persistence and invasiveness of a GM plant itself, or any of its introgressed relatives, as a result of vertical gene flow.

3.1.2. Step 2: Hazard characterisation

Step 2 of ERA consists of characterising any hazards, identified during the problem formulation process, which might lead to adverse effects as a consequence of altered persistence and invasiveness at the production site or in the wider environment.

3.1.2.1. Background information requirements:

All GM plant applications, including those for import and processing of viable propagating plant material, should provide general background information describing the parental species. *Species-specific* information on the following characteristics should be given in order to summarise existing knowledge of that species.

a) *Reproductive biology*. The reproductive biology of the parental species, including their mode(s) of reproduction, dissemination and survivability are important, as plants have different reproduction strategies. Since genetic material can move spatially and temporally via the transfer of pollen, seeds, or vegetative propagules, this description should consider relevant avenues and vectors for gene flow, together with factors that affect the probability of these processes.

b) *Characteristics associated with weediness and invasiveness.* Characteristics associated with weediness or invasiveness have been bred out of many crops during domestication (Warwick and Stewart, 2005), though the degree of domestication varies by crop. While most crops share a similar suite of domestication characteristics, some species may still contain weedy or invasive characteristics (such as seed dormancy, discontinuous germination, rapid seedling growth, phenotypic plasticity, asynchronous flowering, propagule shattering, seed dispersal mechanisms, strong competitive ability) (Warwick et al., 2009). It is therefore considered useful to describe characteristics of the parental plant species that may favour weediness or invasiveness. In this respect, the history of cultivation of the parental species can be examined for confirmatory evidence of whether these plants have become a weed or invasive elsewhere. Historic data from a region may be a valuable indicator of the potential for persistence or invasiveness of the GM plant itself.

c) *Factors limiting persistence and invasiveness*. Many abiotic and biotic factors limit the ability of plants to form self-sustaining populations under either cultivated or uncultivated conditions. It is therefore relevant to describe factors that may restrict or limit the niche of the plant to certain habitats, or that may control its population size, according to the current state of knowledge.

d) *Hybridisation and introgression potential with any sympatric compatible relatives.* Sexual compatibility with other cultivated or wild plants occurring in the EU is to be considered in general terms. The potential for a plant to hybridise with a wild relative is highly dependent on their sexual compatibility and relatedness (Eastham and Sweet, 2002, Ellstrand, 2003, Jenczewski et al., 2003, FitzJohn et al., 2007, Jorgensen et al., 2009). Some level of genetic and structural relatedness between genomes of both species is needed to produce viable and fertile plant x wild relative hybrids that stably express the transgene. Also, both species must occur in their respective distribution range of viable pollen, which requires at least partial overlap in flowering in time and space, and common pollinators (if insect-pollinated). For the stabilisation of the transgene into the genome of the recipient (introgression), genes must be transmitted through successive backcross generations or selfing. Therefore, the risk characterisation should consider features such as the proximity of and flowering synchrony of wild relatives, and the viability, fertility, genetic compatibility and fitness of hybrid and backcross plants.

3.1.2.2. Stage 1 information requirements:

All GM plant applications, including those for import and processing of viable propagating plant material, should provide information to answer all questions in stage 1 of Figure 4. The purpose of this information is to answer whether the GM plant and its progeny can grow, overwinter, reproduce and hybridise under EU conditions, and if so, how the phenotypic growth and reproduction characteristics compare to conventional counterparts. Stage 1 information should include whether there are any unintended differences between the GM plant and its conventional counterpart in growth, reproduction or hybridisation. To answer these questions, *event-specific* information on the following characteristics should be collated and assessed, and compared with those of the conventional counterparts.

a) *Seed germination characteristics.* Growth chamber experiments or information collected during field trials enable assessment of seed germination characteristics of the GM plant under various conditions. The comparison of germination characteristics between the GM plant and its conventional counterpart might identify potential unintended changes, resulting from the transformation process, in the GM plant that require further analysis.

b) *Phenotype under agronomic conditions.* The general phenotypic and agronomic characteristics of the GM plant should be assessed in multi-location field trials representative of the different environments where the GM plant may be grown in order to establish intended or potential unintended differences between the GM plant and its conventional counterpart (see *e.g.* Horak et al., 2007, Garcia-Alonso, 2009, Raybould et al., 2009). Characteristics under consideration include plant establishment and vigour, time to flowering and maturity, growth, plant height and dry matter production, seed and yield characteristics, vernalisation requirement, attractiveness to pollinators, and pollen shed, viability, compatibility and morphology.

In addition to plant growth, development and reproduction observations, any visually observable response to naturally occurring insects, diseases and/or abiotic stressors (such as heat, drought, and excess of water) should be recorded during the growing season, as these observations provide indications of biotic and abiotic stress responses and thus susceptibility/adaption to stresses.

The comparison of phenotypic and agronomic characteristics between the GM plant and its conventional counterpart might identify potential unintended changes, resulting from the transformation process, in the GM plant that require further analysis.

c) *Reproductive biology*. When considering the potential impact of gene transfer from GM plants, it is important to assess whether the GM plant has any different capacity for gene transfer than its conventional counterpart. The gene(s) inserted may modify the potential for plant to plant gene transfer due to altered flower biology (e.g. altered flowering period), attractiveness to pollinators, fertility, or changed pollen viability and compatibility.

d) *Seed persistence leading to volunteer occurrence.* Measurements or observations such as volunteer number in subsequent crops/plantations indicate the potential for seeds and vegetative propagules from a GM plant to give rise to volunteer populations. Post-harvest field inspection data in which volunteer numbers are reported can serve as an information source and provide indications on the overwintering potential of the GM plant seeds. Seed burial experiments can also give indications of changes in dormancy and seed persistence (*e.g.* Hails et al., 1997).

3.1.2.3. Stage 2 information requirements

Stage 2 information will be required for plants that could overwinter in some parts of the EU under production system (e.g. agricultural) conditions, and/or transmit genes to compatible relatives that could overwinter. The risk assessment should consider whether the GM trait (or unexpected

phenotypic trait)[19] could cause the plant to become a more serious weed within the production site. In GM plants with more than a single event (e.g. stacked events), applicants should consider whether the combination of events may lead to enhanced persistence or invasiveness that is more than the simple product of the single traits.

Data on relative persistence and fitness of the GM plant under production conditions may be available in the scientific literature, or new data may be required in the form of (1) monitoring of existing GM plants in comparable climatic conditions[20]; (2) manipulative field experiments comparing GM and conventional plant fitness under a range of environmental conditions representative of EU production receiving environments; and/or (3) population models parameterised by appropriate field data to explore the long-term persistence of GM traits in relevant crop rotations. The most direct way to measure fitness is by conducting experiments in production sites in representative regions over a minimum of two years. Relative fitness is dependent upon the environmental context. Glasshouse, growth chamber and microcosm experiments can reveal differences under specific, possibly ideal conditions (e.g. Snow et al., 1999), and such experiments can be more highly replicated and therefore more powerful then field experiments. However, observed differences in controlled conditions do not necessarily translate into field conditions and may require further data or population modelling to allow a complete interpretation (Birch et al., 2007).

Persistence or enhanced fitness of volunteers or hybrids should be considered in the context of typical crop rotations. For example, herbicide tolerant *Brassica napus*, may be used as a break crop one year in four and could transmit herbicide tolerance genes to weedy *Brassica rapa*. The presence of herbicide tolerant *B. rapa* in years 2-4 may be relatively inconsequential as this weed, and crop volunteers, may be controlled by alternative herbicides. However, persistence of transgenic weedy *B. rapa* x *B. napus* hybrids in year 5 could have consequences for the following *B. napus* crop.

Crops vary considerably in their ability to form feral populations and this is extensively recorded in the scientific literature (e.g. Bagavathiannan and Van Acker, 2008). If the conventional crop forms feral populations, then this will allow the GM trait to persist outside production systems, and the consequences of this will need to be assessed (stage 3). Similarly, there is extensive literature available on the sexual compatibility of crops with their wild relatives, and this was discussed earlier (see background information). The assessment should also consider whether the GM trait has the potential to move beyond production sites through hybridisation and introgression into wild relatives. If the GM trait is unlikely to move beyond production sites via either of these routes, then the characterisation should stop at stage 2.

3.1.2.4. Stage 3 information requirements:

Stage 3 information will be required for plants that can form feral populations in semi-natural habitats, or for which there are sexually compatible wild relatives that are likely to be recipients of transgenes. The risk assessment will need to evaluate whether feral plants, or compatible relatives containing the GM trait, will exhibit changed fitness in semi-natural habitats. If fitness is enhanced, populations may increase; if fitness is reduced, outbreeding depression may occur. The potential for changes in fitness may be estimated through: (1) observations from regions growing the GM plant; (2) manipulative field experiments (Crawley et al., 1993, Crawley et al., 2001); (3) greenhouse, microcosm or growth chamber experiments with additional field data and/or models to aid interpretation; or through (iv) knowledge of the ecology of feral crops and wild relatives and the phenotypic consequences of the

[19] From this point forwards, the term 'GM trait' includes any event-specific unintended trait, identified in stage 1.

[20] For example, monitoring of herbicide tolerant (HT) genes in commercial *B. napus* fields, which also contained weedy *B. rapa*, has confirmed the ability of the HT genes to persist over time in the absence of selection pressure (Warwick et al., 2008).

presence of the GM trait. Fitness will vary depending upon the environmental context (including anthropogenic influences like mowing), particularly upon the presence of inter and intra-specific competitors, the presence of herbivores and pathogens, and the abiotic conditions. The variation in fitness according to biotic and abiotic conditions is often referred to as a genotype-by-environment interaction. It is therefore important that an appropriate range of environmental conditions is considered.

Detailed knowledge of the ecology of feral crops and wild relatives and the phenotypic consequences of carrying the GM trait may lead to the conclusion that the GM trait is extremely unlikely to confer a fitness advantage in semi-natural habitats. This may be supported by information from other events of the same GM trait. For example, it is unlikely that herbicide tolerant genes will influence fitness unless in the presence of the herbicide. There is now a body of evidence to support this conclusion (Crawley et al., 1993, Crawley et al., 2001, Warwick et al., 2008).

However, in some cases, the existing evidence may be insufficient to draw firm conclusions, and further experiments may be required. The most direct way to measure relative fitness is via manipulative field trials in a range of suitable habitats and over a minimum of two years. In designing such experiments, field sites should be representative of the receiving environments. The timescale should be sufficient to ensure a range of abiotic conditions are experienced by the experimental plants. The number of seasons should also be sufficient to ensure that a range of biotic pressures (pathogen and herbivore pressure for example) are experienced, although this may also be enhanced by experimental treatments (see below). Treatments should always include disturbance, in which perennial vegetation is removed before experimental seed is sown, as many crops are not strong competitors with species in semi-natural habitats, but may be able to exploit disturbed areas in the manner of ruderal species. Other treatments should be guided by the GM trait being considered. For example, enhancing the densities of herbivores within limits not infrequently experienced in the field could simulate years of high herbivore. This would allow the hypothesis to be tested that insect resistant GM crops may have enhanced fitness under these conditions. The experimental design should allow the treatment-by-disturbance interaction to be tested. Fitness advantages in response to certain selection pressures may only be manifest under disturbed or undisturbed conditions. Plot size should be sufficient to allow the subsequent generation to be monitored, following seed dispersal and recruitment. The parameters measured should include survival in the seed bank as well as survival and fecundity of adult plants, to allow the lifetime fitness to be estimated.

Greenhouse, microcosm or growth chamber experiments can be used to manipulate the relevant ecological factors to determine the potential impact on the fitness of feral plants or wild relatives (e.g. Vacher et al., 2004). However the detection of fitness differences from controlled greenhouse experiments requires further information for accurate interpretation. For example, the frequency and intensity of herbivore and pathogen attack under field conditions would be needed to interpret the consequences of the possession of herbivore or pathogen resistance traits in the field. Furthermore, competition is likely to modulate the rate at which individual plants recover from herbivore (Weis et al., 2000) or pathogen attack, and so possession of resistance genes may be more valuable when competition is high. Population models, parameterised by greenhouse and/or field data can be used to explore the conditions under which GM plants may invade and establish (e.g. Damgaard and Kjaer, 2009). This allows worse-case scenarios to be explored, and the consequences of any uncertainty in parameter estimates to be explicitly defined.

A form of outbreeding depression may occur if (1) there are high rates of hybridisation with a wild relative, and if (2) the GM trait decreases hybrid fitness. The methods outlined above, specifically manipulative field experiments and/or parameterised population models, could be used to estimate the conditions under which this is likely to occur.

For some GM traits, for example some of the stress tolerance genes (Damude and Kinney, 2008a,b, Newell-McGloughlin, 2008, Roelofs et al., 2008, Ufaz and Galili, 2008, Warwick et al., 2009), it is possible that the GM plant, or any introgressed compatible relative would be able to grow beyond the

geographical range of the conventional crop. The methods outlined above, particularly manipulative field experiments, knowledge of the ecology of the feral plant and its compatible relatives, microcosm experiments and modelling approaches, are tools that can address this issue.

For those crops for which no significant changes in fitness can be detected, or are thought likely, for either GM plants or their compatible relatives, then exposure characterisation should stop at stage 3. However, if fitness differences are detected, then further assessment is required to interpret the potential consequences (stage 4).

3.1.2.5. Stage 4 information requirements:

Stage 4 information would be required for those GM crops for which the presence of the GM trait in either feral crop plant or a compatible relative causes an alteration in fitness, or increases the range of habitats in which the plant may survive and reproduce.

Enhanced fitness may or may not result in population increase of the transgenic plant compared to its appropriate comparator, depending upon the factors limiting or regulating the population. A combination of field experiments, growth chamber data, population models and knowledge of the ecology of the potential recipients of the GM trait would then be required to interpret the potential consequences of enhanced fitness.

Detailed knowledge of the ecology of feral crops and compatible relatives including knowledge of the habitats in which these relatives have established populations, and the factors that limit and regulate populations will facilitate an interpretation of the likely impact of a GM plant. For example, if specific herbivores are known to have an impact on the fecundity of a particular plant species, and these herbivores are susceptible to insect resistance GM traits, then introgression of those insect resistant GM traits could lead to ecological release – but only when those plant populations are seed limited.

Manipulative field experiments may be required to determine if a plant species is seed or microsite-limited. For example, seed addition experiments, in which seeds are added as a supplement to undisturbed habitats, followed by monitoring of subsequent generations (and appropriate controls) can determine the degree to which a species may be seed limited, and may be carried out with conventional counterparts. A reasoned argument may then be presented to assess whether the GM plant would be expected to behave in a similar manner, and whether enhanced fecundity would alter dynamics. Similar experiments may be used to deduce other limiting or regulating factors.

Population models (e.g. stochastic models), parameterised with field data, may be required to interpret the long-term impacts of GM trait presence on field populations. For example, it is likely that more than one biotic or abiotic factor is influential in determining population levels of a plant species over a number of seasons. Parameterised models may allow the impact of the presence of a GM trait to be modelled over several seasons, in which putatively important biotic factors (such as herbivores and pathogens) fluctuate in abundance. The range of conditions under which population increase may occur could then be estimated, in order to determine the occurrence and extent of environmental damage.

Finally, the consequences of an increase in abundance or increased range of the transgenic species or of outbreeding depression could be the decline or even extinction of desirable species, or another form of habitat alteration that is undesirable.

3.1.3. Step 3: Exposure characterisation

An exposure characterisation should be conducted for any hazards identified in the ten questions and four stages of Figure 4. Exposure characterisation should be carried out for all applications, including those for import and processing of viable propagating plant material.

3.1.4. Step 4: Risk characterisation

The answers to the questions posed in Figure 4 lead to the characterisation of possible risks – that of an adverse effect in the production area, in which the GM trait causes the plant and/or its wild relatives to become a more persistent weed in subsequent rotations; and that in the wider environment, where the presence of the GM trait affects plant populations and species, leading to, for example, a decline in biodiversity. Applicants should characterise these risks e.g. by determination whether any expected change falls within the range defined as being acceptable during problem formulation.

3.1.5. Step 5: Application of risk management strategies

If the ERA identifies risks related to persistence and invasiveness, strategies to manage these risks may be required and should be defined by applicants. These strategies might focus on reducing transgene movement by lowering sexual fertility, or be directed at controlling the progeny of GM plants resulting from gene flow. If measures for controlling volunteers, ferals or wild relatives are proposed, the associated impacts should be considered by reference to chapter 3.5. Applicants should evaluate the efficacy and reliability of any risk mitigation measures and conclude on the final level of risk resulting from their application. Remaining identified risks and risk management measures should be considered when formulating post-market environmental monitoring plans.

3.1.6. Conclusions

The risk assessment should conclude on (1) the impact of the GM plant and/or hybridising relatives in the production system, particularly through increased weediness and more intense weed control; (2) the impact of the GM plant and/or hybridising relatives in semi-natural and natural habitats, through change in invasiveness or reduction of biodiversity or ecological function; (3) why any anticipated harm may be considered acceptable; and (iv) what risk management measures may be required to mitigate any harm.

3.2. Plant to micro-organisms gene transfer

In the context of cultivation and use, recombinant DNA will be released from GM plants into the environment, e.g. into soil, or inside the gut of animals feeding on plant material, therefore it is necessary to consider the likelihood of gene transfer into micro-organisms and its stabilisation e.g. by integration into their genomes. Horizontal gene transfer (HGT) is here defined as any process in which an organism incorporates genetic material from another organism without being the offspring of that organism. The evaluation of the impact of this HGT includes analysis of the transfer of recombinant plant DNA to initially receiving micro-organisms and potential transfer to other organisms (micro-organisms, plants) and the potential consequences of such a gene transfer for human and animal health and the environment. Although the extent of environmental exposure is likely to differ between applications for import and processing and for cultivation, the issues to be considered in the ERA are expected to be similar.

3.2.1. Step 1: Problem formulation

Micro-organisms, especially bacteria, are capable of exchanging genetic material directly between each other and even across species boundaries using different mechanisms i.e. conjugation, transduction or transformation. HGT can be initiated by uptake of cell free DNA from the environment, which may also include DNA derived from GM plants. After initial HGT from plant to micro-organism, the horizontally transferred genes may be further spread to other micro-organisms. Although HGT from plant to micro-organisms is regarded as a rare event, there may be consequences for human and animal health and the environment and therefore they should be considered in the ERA. This ERA will depend on the potentially acquired character and the prevalence of similar traits in microbial communities[21] (EFSA, 2009g). The problem formulation also needs to consider the routes of exposure in the receiving environment(s) as well as the assessment endpoints being representative of the aspects/parts of the environment(s) that need to be protected from adverse effects.

Therefore the problem formulation should focus on:

- Detailed molecular characterisation of the DNA sequences inserted in the plant[22] including information on the potential of the promoter elements that could drive expression in micro-organisms;
- Presence of antibiotic resistance marker genes;
- Presence of inserted plant DNA sequences showing similarities with DNA sequences from relevant microbial recipients enhancing the probability of recombination and subsequent stabilisation, or mobile elements[23];
- Presence of recipient micro-organisms for transgenic DNA in the receiving environment(s);
- Selective conditions (including co-selection) enhancing the probability of dissemination and maintenance of the genetic material from GM plants in natural microbial communities (*e. g.* the presence of antibiotics in the receiving environment(s)[24];
- Persistence of GM plant material after harvesting, until degradation of the material has occurred;
- Potential for long-term establishment of the genetic material from GM plants in natural microbial communities (see chapter 3.2.4);
- Ecological or human and animal health consequences of a potential HGT from GM plant to micro-organisms[25].

3.2.2. Step 2: Hazard characterisation

If a hazard has been identified in step 1 of the ERA (chapter 3.2.1), the hazard should be further characterised (e.g. the potential spread of antibiotic resistance genes and potentially reduced efficiency

[21] The current state of knowledge (EFSA, 2009g) indicates that the HGT from GM plants to micro-organisms with subsequent expression of the transgene is regarded as a rare event under natural conditions.

[22] Integration of DNA fragment in micro-organisms occurs mainly by homologous recombination. For this reason, the presence in the plant DNA of sequences with high similarity to microbial DNA would increase the probability of transfer. Available data indicate that integration of genes from plants into bacteria in the absence of DNA sequence identity is, at most, a rare event (EFSA, 2009g).

[23] Mobile genetic elements present in the vicinity of the insertion site could enhance the potential for gene transfer.

[24] Selection pressure would enhance the likelihood for the dissemination and maintenance of horizontally transferred genes.

[25] For instance, the contribution of antibiotic resistance marker genes to the development and dissemination of antibiotic resistance in pathogenic micro-organisms of clinical relevance should be evaluated (EFSA, 2009g).

of antibiotic treatment). Hazard characterisation should consider information on the prevalence and distribution of genes (similar to the transgene(s) in natural environment(s)) and try to establish potential consequences (e.g. for a gene or trait that is already widespread in the environment).

3.2.3. Step 3: Exposure characterisation

Exposure characterisation should consider the sub-cellular location and copy number of the recombinant DNA, the environmental routes of exposure of the GM plant and the recombinant DNA, and the stability of the DNA in the relevant environment(s). After GM plant degradation, cell free DNA may persist in the environment for up to weeks or even years influenced by a number of biotic and abiotic factors (Nielsen et al., 2007, Pontiroli et al., 2007).

It is recognised that the experimental acquisition of data on DNA exposure levels in complex microbial communities is severely limited by methodological constraints under natural conditions. In most cases, the frequency of HGT will be below the detection threshold of particular experiments. Other limitations are related to sampling, detection, challenges in estimating exposure levels and the inability to assign transferable genes to a defined source (EFSA, 2009g). In light of such technical limitations, however, applicants Ares requested to provide an exposure characterisation (of the hazards characterised under step 2) considering the various routes of exposure in the receiving environment(s):

- Plant production (e.g. DNA from GM plants might be released into the environment during cultivation and after harvest as a result of degradation of plant material and might persist in the field and move to aquatic environment(s));

- Food and feed chain (e.g. GM plant intended for food and feed use is often subject to a variety of processing and storage regimes and might be stable/degrade during processing and storage as in silage);

- Gastro-intestinal system (e.g. DNA of GM plant might be consumed as food and feed and might be in contact with micro-organisms, mainly bacteria present in the gastrointestinal tract, and subsequent routes of environmental exposure. These exposure scenarios should include both vertebrates and invertebrates that feed on plants or processed plants and plant ingredients above or below ground, pollinators and human (Gay and Gillespie, 2005, Keese, 2008).

3.2.4. Step 4: Risk characterisation

It is important to focus the risk characterisation on potential impacts on indigenous microbial communities that occur in the various receiving environment(s) (as outlined above in step 3). Environmental microbial communities may include certain human or animal pathogens (e.g. *Pseudomonas aeruginosa*, some *Enterobacteriaceae*), or non-pathogenic bacteria, which could serve as first recipients of genes derived from GM plants (e.g. ARMGs) and the transgenes could be then transferred to other micro-organisms including pathogens (EFSA, 2009g). Any risk identified should be characterised by estimating the probability of occurrence, any positive selection conferred by the horizontally transferred trait and the magnitude of the consequences of the adverse effect(s).

3.2.5. Step 5: Application of risk management strategies

Based on the outcome of the risk characterisation, applicants may need to determine and evaluate targeted risk management strategies. Potential strategies may be related to the avoidance of conditions allowing positive selective.

3.2.6. Conclusions

A conclusion is required of the overall risk *i.e.* a clear rationale on the potential for plant to micro-organism gene transfer and its consequences, taking into account any risk management strategies. The potential impact (consequences) of such an event should be evaluated also for indirect effects on biogeochemical cycles (see chapter 3.7), in particular in the light of possible long-term maintenance of the genetic material from GM plants in natural microbial communities.

3.3. Interactions of the GM plant with target organisms

Target organisms (TO) are organisms on which specifically designed characteristics of a GM plant are intended to act[26] and are generally pests or pathogens of the plant. These target organisms should be defined by applicants. All other organisms should be considered as non-target organisms. Because of the levels of exposure, resistance development is only relevant for applications with scope cultivation of GM plants and not for applications restricted to import and processing of GM plants and their products.

3.3.1. Step 1: Problem formulation

The focus in the problem formulation for herbivore or pathogen resistant plants is to determine the likelihood that the TO will develop resistance, and to design strategies to delay or prevent the occurrence of resistance or to prevent undesired changes in the interaction between the TO and GM plants. Resistance is defined as occurrence of a phenotype of an individual of the TO that can survive on the GM plant and produce viable offspring (Andow, 2008). In case of herbivore resistance, and in line with Annex II of Directive 2001/18/EC, the development of resistance in target pests is considered an environmental as well as an agronomic concern. Adverse effects from resistance development may compromise other pest control products and can destabilise pest control strategies, and may lead to increased pesticide use. As consequence, it might lead to changes of cultivation management and might result in an increased environmental impact.

3.3.1.1. Herbivore resistance development

Various strategies are being used to make plants resistant to herbivores. Currently, most herbivore resistant plants express insecticidal substances (e.g. Bt proteins). The potential future design of GM plants may use other mechanisms e.g. expression of repellent substances, anti-feedants, morphological changes or altered volatiles to influence the host finding process.

However, a potential hazard is the development of resistance to toxic substances in herbivore pests, which is already a well-known phenomenon in plant protection (Whalon et al., 2008) and it is likely that resistance to GM plants expressing certain pesticidal toxins can also occur (Andow, 2008). For example, laboratory studies have shown the widespread potential for development of resistance in the European corn borer (*Ostrinia nubilalis*) to different Cry proteins (Bolin et al., 1999, Chaufaux et al., 2001, Huang et al., 2002, Siqueira et al., 2004, Li et al., 2005, Alves et al., 2006) and two instances of field-evolved resistance of Bt maize were recently reported in the scientific literature: *Busseola fusca* in South Africa on maize MON810 (van Rensburg, 2007, Kruger et al., 2009) and *Spdoptera*

[26] Weeds are not considered to be TO of GM HT plants. Indirect impacts of GM HT plants on weeds are considered in chapter 3.5. Potential risks due to effects on non target organisms *e.g.* other diseases, predators and parasitoids of the target organism or secondary pests are addressed in chapters 3.4 and 3.5 respectively.

frugiperda on Vry1F expressing maize in Puerto Rico (USA) (Matten et al., 2008, Moar, 2008, Tabashnik, 2008, Tabashnik et al., 2008, Storer, 2010). Therefore, applicants should consider in problem formulation the potential for resistance development.

3.3.1.2. Plant pathogen interaction

Various strategies are used to make plants resistant or tolerant to plant pathogens[27] by: (1) expressing proteins, peptides or antimicrobial compounds that are directly toxic to pathogens or influence their growth *in situ*; (2) producing products that destroy or neutralise a component of the pathogen, (3) expressing gene products releasing signals that can regulate plant defence; (4) expressing resistance gene products involved in hypersensitive response and interaction with avirulence; or (5) expressing recombinant antibodies that inactivate pathogens or pathogen proteins (Fischer et al., 2001, Punja, 2001, Tepfer, 2002, Grover and Gowthaman, 2003, Joersbo, 2007, Prins et al., 2008).

However, plant pathogens have the potential to develop resistance to a wide range of plant defence systems (Georghiou et al., 1986) which may be identified as a potential hazard. Potential mechanisms for evolving resistance could be based on (1) phenotypic effects such as complementation, heterologous encapsidation and synergy or (2) genotypic changes in the plant pathogen leading to the development of new virulence determinants (e.g. for viruses according to Tepfer, 2002). Co-evolution may result in adaptive functional modifications of an enzyme active site (*e.g.* Bishop et al., 2000). Hence, there is an expectation that pathogens will evolve resistance to GM plant resistance traits. Applicants should consider the mechanisms used to protect plants and their interactions with pathogens. The resistance mechanisms that evolve in pathogens should be considered taking into account their genetic control and heritability. Linkages to pathogen virulence and selective advantage should also be considered in the assessment of the potential for resistance development.

Furthermore, the possibility of development of new pathogen strains with resistance to the transgenic trait is an additional hazard in relation to plant pathogen.

3.3.2. Step 2: Hazard characterisation

It is important to identify the TO of the GM plant in the receiving environment(s) where the GM plant is likely to be grown. The potential of these target species to develop resistance to GM plants should be evaluated based on their history of development resistance to conventional pesticides and resistant host plants. Data should be provided by applicants to characterise the potential of resistance development depending on the TO and the genetic modification including:

- Data on biology, life cycle, ecology and/or behaviour of the TO. Data on resistance mechanisms that develop in TO and their genetic control, heritability and linkages to virulence, fitness and selective advantage. In most cases, these data can be taken from literature or from the experience of breeders and plant protection services;
- Distribution of the TO and its resistant populations in the European environments;
- Host range of the TO;
- Information on the population genetics, and epidemiology of susceptible and resistant TOs (e.g. de la Poza et al., 2008);

[27] Plant pathogens include viruses, fungi, bacteria and nematodes.

- Frequency of resistant individuals or resistance alleles. Relevant data for Europe can be taken from available scientific literature (e.g. for Cry1Ab and ECB, (Bourguet et al., 2003) or could be generated e.g. for insects by F_1(Gould et al., 1997, Yue et al., 2008) or F_2 (Andow and Alstad, 1999) (Andow and Alstad, 1998) screening or by other screening methods. Data generated outside Europe with the GM plant itself, or other plant species might be used by applicants, only if its relevance for the European environment(s) has been justified;

- Mode of action of the active GM plant product towards the TO and GM plant characteristics related to this trait;

- Data on baseline susceptibility of the TO to transgenic products either from the literature (Gonzalez-Nunez et al., 2000, Gonzalez-Cabrera et al., 2006, Saeglitz et al., 2006) elaborated for *Ostrinia nubilalis* and *Sesamia nonagroides* or from laboratory tests according to testing protocols e.g. used by the above cited publications.

In some cases, the data might be obtained from the literature, but in other cases, data sets might be incomplete. Therefore applicants should consider various scenarios, including a worst-case scenario, to estimate the potential of resistance development in Europe (see chapter 3.3.4).

3.3.3. Step 3: Exposure characterisation

By definition, TOs are exposed to the GM plant. Data characterising the exposure of TOs should include:

- Expression level of the transgenic products in the plant tissues consumed by TO;

- Estimation of the levels of intake of the transgenic product(s) by various developmental stages of the TO;

- Influence of the expression level and its variability on the interaction between GM plant and TO;

- Proportion of population of the TO exposed to the GM plant in the receiving environment(s);

- Baseline frequency of resistant individuals or resistance/virulence alleles. Relevant data for Europe can be taken from available scientific literature (e.g. for cry1Ab and ECB, (Bourguet et al., 2003) or could be generated e.g. for insects by F_1(Gould et al., 1997, Yue et al., 2008) or F_2 (Andow and Alstad, 1998, Andow and Alstad, 1999) screening or other screening methods. Data from outside the EU could be considered if they can be shown to be relevant to European conditions;

- Deployment of other GM plants expressing similar trait(s).

3.3.4. Step 4: Risk characterisation

After assessing all data, the risk should be characterised for a) evolving resistance or b) developing undesired changes in the interaction between the target plant pathogens and plants in the receiving environment(s).

3.3.5. Step 5: Risk management strategies

Based on the outcome of the risk characterisation, applicants should propose resistance management strategies[28]. Applicants should evaluate the effectiveness of targeted risk management strategies which could minimise undesired interactions between GM plants and target organisms[29] in the European receiving environment(s). Applicants should indicate the efficacy, reliability and expected reductions in risk associated with the strategies. In addition, the risk of resistance may change when taking into account newly available information or changes in production systems. Therefore, management measures need to be able to respond to these changes and appropriate resistance monitoring measures are likely to be required as part of case-specific monitoring within PMEM (see chapter 4).

3.3.6. Conclusions

A conclusion is required of the overall risk considering resistance development of TO or undesired changes in the interaction between the GM plants and the TO. The risk characterisation and conclusions will determine the resistance management measures and requirements for the PMEM plan (see chapter 4).

3.4. Interactions of the GM plant with non-target organisms

According to Annex II of Directive 2001/18/EC, ERA should consider the possible immediate and/or delayed environmental impact resulting from direct and indirect interactions of GM plants with non-target organisms (NTOs). The ERA as described in this EFSA GD should address the potential environmental impact on population levels of herbivores, natural enemies, symbionts (where applicable), parasites, and pathogens.

The scientific opinion of the EFSA GMO Panel on the assessment of potential impacts of genetically modified plants on non-target organisms (EFSA, 2010e) provides guidance to risk assessors for assessing potential effects of GM plants on NTOs, together with rationale for data requirements in order to complete a comprehensive ERA for NTOs. Guidance to applicants as outlined in that scientific opinion has been inserted in the present GD.

3.4.1. Step 1: Problem formulation

3.4.1.1. Environmental concerns and hazard identification

One environmental concern is that GM plants may have an adverse effect on biodiversity and its functioning at several levels, through interactions with populations of other species associated with or sympatric with the GM plant, and referred to as non-target organisms (NTOs)[30]. In this chapter

[28] It is conceivable that risk management measures for risks associated with plant pathogens as TOs might be very limited.

[29] In the context of lepidopteran pests and Bt maize, the high dose refuge strategy is a good example for the introduction of a successful risk management. The consequent use of refuge areas prolonged the expected development of resistance for most lepidopteran pest species so far (Tabashnik et al., 2008). However, the possible resistance management strategies are dependent on the biology of the target organism, the genetic transformation and interactions between the target organism and the GM plant and the receiving environment. In cases where high dose refuge strategy failed other management option are possible (see Bt cotton example: (Tabashnik et al., 2008). This might be the additional use of insecticides or the use of 'pyramided' GM plants producing more than one Bt protein.

30 Potential NTOs are defined as all those species directly and/or indirectly exposed to the GM plant, and which are not targets of the newly expressed metabolite(s) in these plants.

biodiversity is interpreted broadly and covers both species richness and agro-eco functions providing ecosystem services[31]. Since the environment (including biodiversity) is to be protected from harm according to protection goals set out by EU legislation, the protection of species richness and ecological functions should be considered in the ERA.

Specifically when considering NTOs, the receiving environment consists of the managed terrestrial ecosystem (e.g. agro-ecosystem) including the GM cultivated fields, orchards and plantations and their margins and the wider environment (e.g. other adjacent GM or non-GM cultivations and non-cultivated habitats) and, where relevant, aquatic ecosystems.

In a human managed context, sustainable land use (e.g. for agriculture and forestry) is considered a primary environmental protection goal. For the benefit of sustainable production, the scope is to maintain a certain level of biodiversity, providing essential ecological services, including biological control of pests and diseases, nutrient fixing and cycling, decomposition of plant materials, maintenance of soil quality and fertility, and structural stability. Therefore the criterion of functional biodiversity is deemed important in this context, since preserving the functional biodiversity may guarantee the quality of production systems (e.g. agro-ecosystems) and ensure their sustainability. Applicants shall consider whether a GM plant and its use are directly and/or indirectly (e.g. through food web interactions, scale of adoption) potentially harmful to species guilds involved in ecosystem functions. Problem formulation starts with the identification of potential hazards through a comparison of the GM plant with its conventional counterpart. The different features of the GM plant are considered the novel stressor[32] since environmental impacts can be a consequence of changes to the GM plant, to its management as well as the effects of the introduced traits.

These differences are initially assessed theoretically in the problem formulation process in order to identify the potential environmental consequences of these differences. While some differences may be deemed irrelevant to the assessment, others will need to be practically evaluated for their potential to cause harm.

3.4.1.2. Definition of assessment endpoints

Because protection goals are general concepts, they need to be translated into measurable assessment endpoints. Thus the assessment endpoint is an explicit expression of the environmental value that is to be protected. This necessitates defining (a) *species* and (b) *ecosystem functions* that could be adversely affected by the GM plant, and that require protection from harm.

In any ecosystem, there is usually a high number of NTO *species* that may be exposed to GM plants. Considering that not each of these species can be tested, a representative subset of NTO species (referred to as 'focal species') shall be selected, on a case-by-case basis, for consideration in the risk assessment of each GM plant. To lead applicants to a decision on which focal NTO species are to be used as assessment endpoints, species selection shall be performed according to the following four steps outlined also in Figure 5:

Step 1 - Identification of functional groups:

As a first step in species selection, it is necessary to identify the ecosystem functions and services (including maintenance of herbivores as part of food web, pollination, regulation of arthropod pest

31 Ecosystem services are distinct from ecosystem functions by virtue of the fact that humans as well as other species, benefit directly from these natural assets and processes.

32 A GM plant introduces additional potential stressors into the environment: the transgene in an organismal context, its products and the GM plant itself.

populations by natural enemies and decomposition of plant material) provided by the production system (e.g. agro-ecosystem) and the functional groups of species involved, in the environment(s) where the GM plant is likely to be grown.

Step 2 - Categorisation of NTO species from identified functional groups:

In the second step, the main species linked to the functional groups identified in the previous step should be listed, considering the GM plant and the organisms associated to in its receiving environment(s) (Birch et al., 2004, Hilbeck et al., 2006). An indicative list detailing the ecological role for common invertebrates in agro-ecosystems is provided in Table 3. Some taxonomically related species and/or life stages of the same species may have different ecological roles (e.g. different feeding habits) and this aspect should be considered.

Table 3: Examples of functional groups (exposure through trophic interactions)

Functional group		**Examples of taxonomic groups**
Herbivores		Phloem-feeders: aphids (*Hemiptera: Aphididae*), leafhoppers (e.g. *Hemiptera: Cicadellidae*), certain *Heteroptera*
		Cell-content feeders: thrips (*Thysanoptera: Thripidae*), spider mites (*Acarina*) and *Nematoda* (*Tylenchida: Meloidogynidae*)
		Chewing: leaf beetles (*Coleoptera: Chrysomelidae*), *Lepidoptera* larvae, *Diptera* larvae, grasshoppers (*Orthoptera Ensifera)*, gastropods (*Mollusca, Gastropoda*)
Natural enemies	Predators	Beetles: *Coleoptera* (e.g. *Coccinellidae, Carabidae, Staphilinidae*)
		Predatory bugs: *Heteroptera* (e.g. *Nabidae, Anthocoridae*)
		Predatory flies: *Diptera* (e.g. *Syrphidae*)
		Lacewings: *Neuroptera* (e.g. *Chrysopidae, Hemerobidae*)
		Thrips: *Thysanoptera* (e.g. *Aeolothrips*)
		Spiders & harvestmen: *Araneae* and *Opiliones*
		Mites: Acarina (e.g. *Phytoseiidae*)
		Nematoda (e.g. *Mononchus* sp)
	Parasitoids	Hymenoptera (e.g. *Ichneumonidae, Braconidae, Aphelinidae*)
	Parasites & Pathogens	Bacteria, fungi, viruses
	Entomopathogenic organisms	*Nematoda* (e.g. *Heterorhabditidae, Steinernematidae*), pathogenic microorganisms
Pollinators		Solitary and social bees (*Hymenoptera: Apidae*), hover flies (*Diptera: Syrphidae*); Coleoptera (e.g. *Melyridae, Curculionidae, Scarabaeidae*)
Decomposers		Diptera larvae (e.g. *Phoridae, Sciaridae*), Nematoda (e.g. *Rhabditidae, Dorylaimidae*), springtails (*Collembola*), mites (*Acarina*), earthworms (*Haplotaxida: Lumbricidae), Isopoda*, microorganisms
Plant symbionts		rhizobacteria, mycorrhiza

In the categorisation of relevant NTO species, additional species of economic or aesthetic or cultural value, or species of conservational importance considered as threatened or endangered may also need to be included.

Step 3 - Ranking species based on the ecological criteria:

From the list built in step 2 of species selection, applicants shall prioritise NTO species from each relevant functional group (Birch et al., 2004, Hilbeck et al., 2006).

The main criteria to be considered in this prioritisation process are:

- Species exposure to the GM plant under field conditions, specifically considering life stages present during the period of exposure;
- Known sensitivity of the species to the product(s) expressed in the GM plant;
- Linkage to the production system (e.g. agro-ecosystem), and presence of alternative food source;
- Abundance;
- Interactions with target species (trophic and plant-mediated);
- Species vulnerability (i.e. are certain populations already threatened and thus more vulnerable to additional pressures?);
- Relevance to adjacent habitats, including natural and semi-natural habitats.

Step 4 - Final selection of focal species:

Based on the considerations addressed in the previous steps of species selection, a restricted number of focal species needs to be selected from each functional group. A theoretical framework for focal species selection is presented in Figure 3. At this stage, some practical criteria may be considered in the final selection of focal species. It may be that, among the prioritised species, some can be tested more effectively under laboratory conditions, or are more likely to be available in sufficient numbers in the field to give statistically meaningful results (Gathmann et al., 2006a, Gathmann et al., 2006b, Todd et al., 2008). Legal constraints may limit testing of certain NTOs (e.g. protected species), so this aspect may also influence the final choice of focal species.

It is expected that, at the end of the selection process, the applicants have selected at least one focal species from each relevant functional group identified in the problem formulation for further consideration in the ERA. Different possible sources of exposure for each focal species (in the most relevant developmental stages) to be tested should be considered in the focal species selection process.

For field trials, estimation of ecosystem functions and services could complement or replace data on focal species. Ecological functions (such as pollination, biological control, soil functions[33]) depend on the number of species, their abundances and different types of assemblages. In a particular assemblage, the abundance of any species naturally fluctuates and the decline of a certain population might be compensated by another species within the same guild without adversely affecting functionality (Naranjo, 2005b,a). For example, the overall predation rate of a guild of predatory species could be selected as an assessment endpoint in field trials (Arpaia et al., 2009). Likewise, evaluating the earthworm community as a whole might provide data that are more ecologically relevant than measuring the effects on a single (focal) earthworm species.

A theoretical framework for focal species selection is presented in Figure 5.

[33] *E.g.* soil respiration, biomass decomposition, and nutrient dynamics.

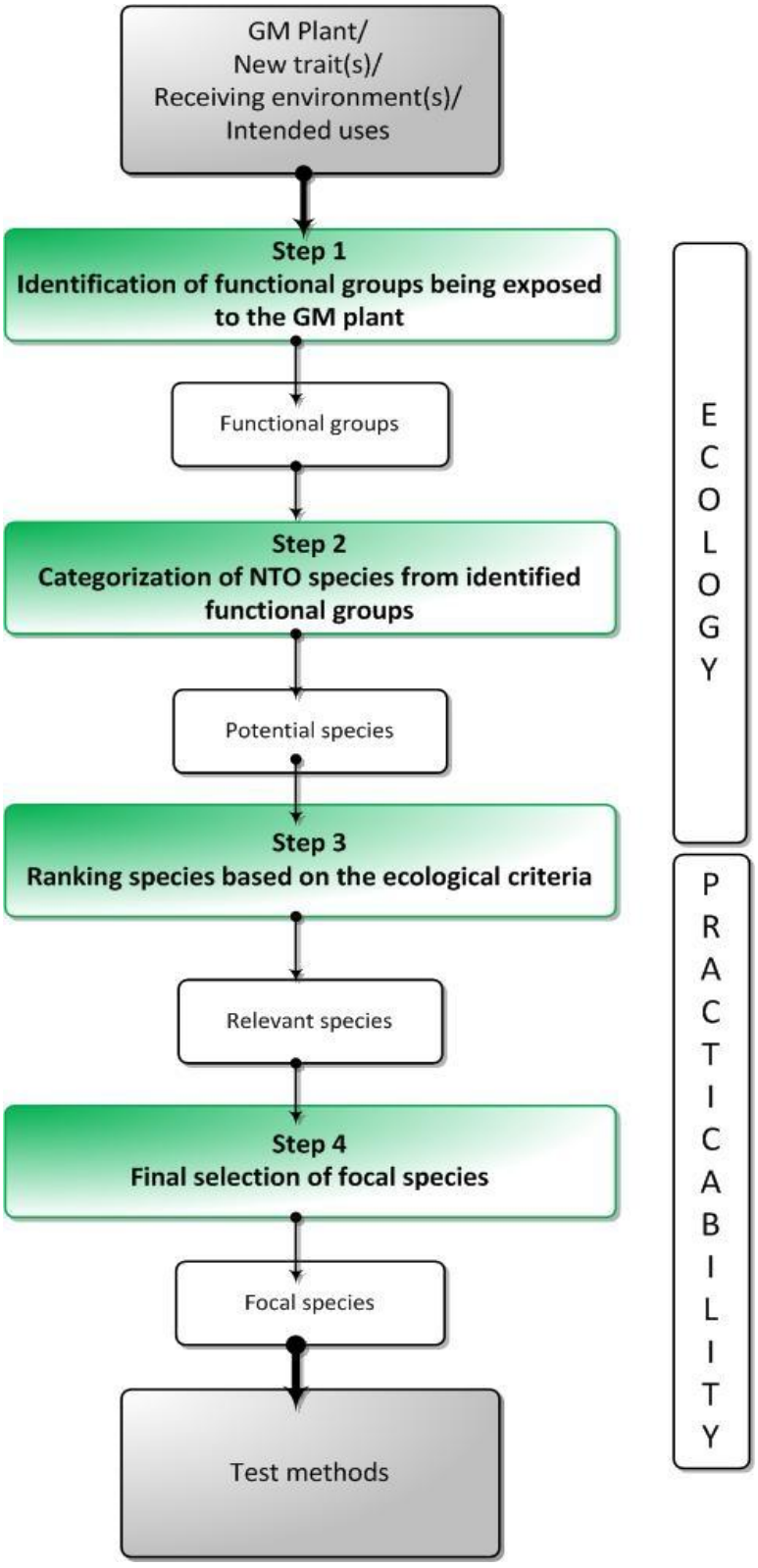

Figure 5: Four steps for selecting focal NTO species to be tested (modified after Birch et al., 2004, Hilbeck et al., 2006).

3.4.1.3. Considering the exposure patterns to NTOs

The overlap of the life cycle and developmental stages of the focal species and the phenology of the GM plants needs to be evaluated. Exposure may also happen after the transgene has moved via dispersal of pollen and grain/seed in and away from the cultivation site of the GM plant (e.g. pollen deposited on leaves of host plants for non-target Lepidoptera and Coleoptera). Moreover, gene flow via outcrossing may result in gene expression in related species and result in additional levels of exposure to other NTO species.

The level of exposure of NTOs to the GM plant will depend on the intended uses of a GM plant:

- In cases where the application does not include cultivation in the EU, direct environmental exposure of NTOs to the GM plant is via the accidental release into the environment of seeds or propagules of the GM plant during transportation and processing. This may result in sporadic occurrence of feral GM plants and therefore exposure of NTO populations is likely to be negligible. The ERA will then focus on indirect exposure to products of the GM plant (e.g. through manure and faeces from the animals fed the GM plant; and other by-products of industrial processes);

- In cases where the application includes cultivation in the EU, the level of environmental exposure is estimated on a case-by-case basis depending upon several factors. These include the biological and ecological characteristics of the GM plant and its transgene(s), the range of expected scales and frequencies of GM plant use, the receiving environment(s) where the GM plant is likely to be cultivated, and the interactions among these factors.

If gene flow to cross-compatible wild/weedy relatives and feral plants inside or outside the areas of cultivation is likely to occur then exposure of NTOs to these GM plants and their products over life cycles and seasons should be assessed.

3.4.1.4. Definition of measurement endpoints

Through the formulated hypotheses, assessment endpoints are made operational into quantitatively measurable endpoints, termed measurement endpoints. Indicators of change, that will be recorded as part of the comparative risk assessment, need to be defined and established by applicants through measurement endpoints. These measurement endpoints should constitute measures to characterise both exposure and/or hazard, and shall be selected when there is an univocal interpretation of the biological data, i.e. how to relate the results to the assessment endpoint.

An alteration in plant metabolism could substantially affect components of the life history of organisms associated with these plants and consequently alter the growth of NTO populations (Charleston and Dicke, 2008). Both lethal and sub-lethal effects are relevant in the assessment of a possible hazard for a given NTO species. Testing for sub-lethal effects is important since it can also give indications of possible long-term effects. An appropriate measurement endpoint for NTO testing is relative fitness (or some component of relative fitness), which is the relative lifetime survival and reproduction of the exposed versus unexposed non-target species (Birch et al., 2004). It is therefore required that NTO tests consider both toxic effects (short-term mortality, longevity) and sub-lethal effects. The latest can be assessed through growth pattern, development rate, reproduction parameters (e.g. number and size of offspring, percentage of eggs hatching, sex ratio of progeny, age of sexual maturity), and, when appropriate, behavioural characteristics (e.g. searching efficiency, predation rates, food choice).

In field conditions, the abundance and species diversity of certain groups of NTOs at a relevant life-stage are typical measurement endpoints. The choice of specific measurement endpoints shall be done according to the problem formulation on a case-by-case basis.

Long-term effects on NTO populations or functional guilds are a substantial element of the ERA, meaning that, in the context of NTO testing, reproduction parameters and testing over multiple generations are considered as appropriate endpoints. In addition modelling and/or post-market environmental monitoring can also be suitable methods for addressing potential long-term effects.

Measures of hazard: Measures of hazard represent the measurable change of the measurement endpoint(s) in response to the GM plant and/or its products to which it is exposed (Storkey et al., 2008). Measures of hazard may be an acute lethal concentration resulting in the death of, e.g. 50% of the organisms tested or the effective response concentration for chronic effects measured or altered reproduction (e.g. fecundity), growth, development and behaviour in a receptor population (Wolt et al., 2010). These measurements can be expressed as effective concentration affecting a x percentage of individuals (ECx). In addition, it is necessary to consider reproduction parameters (e.g. number and size of offspring, percentage of eggs hatching, age of sexual maturity), growth pattern, development rate and behavioural characteristics (e.g. searching efficiency, predation rates, food choice) may also be appropriate measures of hazard for long-term effects. At population level, an important predictor is the intrinsic rate of increase (rm) that integrates measures of survivorship and fecundity (Romanow et al., 1991, Stark and Wennergren, 1995). Moreover, the calculation of the instantaneous rate of increase (ri) allows a good estimate of rm for the study of insect populations at lower tiers (Walthall and Stark, 1997a,b).

Measures of exposure: Measures of exposure shall describe the contact or co-occurrence of the GM plant with the valued entity, and can be expressed as predicted (or estimated) environmental concentrations (PEC or EEC). The description of the novel attribute of the GM plant (e.g. transgenic protein) in terms of the route, frequency, duration, and intensity of exposure for the change relative to the valued entity is considered relevant information (Wolt et al., 2010). Both plant and NTO features assume an important role here, for instance overlapping of the NTO biology (e.g. life cycle stages) with the spatio-temporal concentration of the transgenic product(s) are to be considered to quantify exposure. If a non-target species is not directly exposed to the transgene and/or its product(s) from the plant but indirectly via other target or non-target species, these pathways of exposure need to be evaluated.

3.4.1.5. Hypotheses testing & Tiered approach

A case study approach describing how the GM plant may adversely affect NTOs or their ecological functions is proposed as outlined in Table 4. Based on plant-trait-NTO interactions, five possible cases can be foreseen. On one hand, GM plants may express new proteins/metabolites that have (Ia) toxic properties; (Ib) non-toxic properties; or (Ic) unknown toxicity. On the other hand, GM plants may have an altered composition, in which metabolic pathways known to affect NTO-plant relationships (e.g. glucosinolates in *Brassicaceae*, alkaloids in *Solanaceae*, lignin in trees) are altered (IIa), or not altered (IIb).

In all of those five cases, the metabolism and/or the composition of the GM plants may in addition be unintentionally altered as a consequence of the genetic modification in a way that could affect NTO-plant relationships ('unintended effects'). The presence of unintended effects in GM plants can be due to different reasons (e.g. pleiotropic effects) and it is well documented in the scientific literature (BEETLE_report, 2009).

Only in some of the five identified cases (i.e. Ia, Ic and IIa), can a **specific hypothesis** be formulated to assess plausible intended effects (e.g. a GM plant intentionally altered to produce biologically active compounds may produce the same effects on non-target species).

To test these hypotheses and thus assess possible adverse effects on NTOs, relevant data need to be supplied and considered by applicants.

For the two remaining classes of GM Plants, only the absence of possible unintended effects on NTOs needs to be demonstrated according to the principle described below.

Table 4: Identified cases and hypotheses testing

	GM plants expressing new proteins/metabolites with:			**GM plants with intentionally altered composition**	
	Toxic properties	Non-toxic properties	Unknown toxicity	Alteration of metabolic pathways known to affect NTO-plant relationships	No alteration of metabolic pathways known to affect NTO-plant relationships
	Ia	**Ib**	**Ic**	**IIa**	**IIb**
Possible effects of the transformation process	Intended and unintended	Unintended	Intended and unintended	Intended and unintended	Unintended
Could specific hypotheses be defined?	yes	No, but see chapter 3.4.1.7	yes	yes	No, but see chapter 3.4.1.7

3.4.1.6. Specific hypothesis-driven investigation

For the case studies Ia, Ic, and IIa, specific hypotheses can be formulated and assessed (e.g. the new metabolite can be toxic to some non-target species, or the change in the metabolic pathway will possibly influence the plant's interactions with other organisms on various trophic levels) according to the flow chart illustrated in Figure 6.

Based on specific hypotheses, NTO risk assessment can be performed in a tiered manner; whereby, hazards are evaluated within different tiers that progress from worst-case scenario conditions framed in highly controlled laboratory environments to more realistic conditions in the field. Three main tiers can be used, which comprise experimental tests under controlled conditions (e.g. laboratory tests under tier 1a and 1b[34] and semi-field[35] tests under tier 2), and field tests (tier 3). Within a tier, all relevant data shall be gathered to assess whether there is sufficient information to conclude on the risk at that tier. In case no reliable risk conclusions can be drawn, further data might be needed. Decision of moving between tiers needs to be driven by trigger values. These values shall be set for the species under consideration taking into account the intrinsic toxicity (e.g. estimated by effective concentration (ECx) of the newly expressed products and the expected concentration in the plant), and the sensitivity of the NTO developmental stages (examples of trigger values for NTOs are provided in EPPO[36] guidelines).

Based on the experience with Cry toxins, tier 1 tests generally seem to represent useful predictors for results at higher tier tests (Duan et al., 2009) provided that designs include all ecologically relevant ways of exposure. When laboratory studies are performed, both *in vitro* and in planta tests (tiers 1a

[34] Tier 1a refers to *in vitro* tests carried out with purified metabolites, whereas Tier 1b refers to *in-planta* testing using bi- or multi-trophic experiments according to the focal species selected.

[35] Semi-field tests: outdoors tests carried out with some containment that controls for variability, with manipulation treatments on relatively small experimental units (e.g. caged plants, screen houses).

[36] http://archives.eppo.org/EPPOStandards/era.htm

and 1b) should be done to reach a reliable risk conclusion after tier 1. Tier 1a testing is of crucial importance for the ERA if no or little data on the metabolites expressed by similar GM traits are available (e.g. Table 4: case Ic). Tier 1a tests require purified metabolites in the same form as expressed in the GM plant. Tier 1b complements the results obtained with purified metabolites as they give indications on possible interactions between plant compounds and reflect realistic exposure conditions through bioavailability. In fact, Duan et al. (2008) demonstrated that laboratory studies incorporating tri-trophic interactions of Cry1-expressing plants, herbivores and parasitoids were better correlated with the decreased field abundance of parasitoids than were direct exposure assays. Where purified metabolites are not available, only tier 1b studies shall be conducted using GM plant material that guarantees exposure to both transgene products and the plant. Likewise, it is possible that for some NTO focal species no reliable protocols for performing such experiments exist, in this case applicants may perform this type of test on some focal species only. In all justified cases where testing on a lower tier is not appropriate (e.g. test organisms cannot be reared in the laboratory), applicants can perform tests at the next tier.

The diet regime for each focal species (in the most relevant developmental stages) to be tested must reflect the different possible sources of exposure in nature.

Some impacts on multi-trophic interactions and ecosystem functions may not be observed in tier 1 tests. Higher tier testing may therefore be needed on a case-by-case basis before decisions on the level of risks can be made. In particular, field testing is essential to investigate trait versus environment interactions when laboratory tests give reason to assume a possible adverse effect.

The NTO testing phase can be finalized when sufficient information is compiled to reject the tested hypotheses. Applicants, who conclude that further tests are not required, based on available information, are required to explain the rationale for this conclusion. If at any tier adverse effects are detected, a hazard characterisation is required to determine the biological relevance of these effects. Also, the use of more NTO species in the same functional group might help to clarify how common these adverse effects might be for the specific agro-ecosytstem. In some cases it might necessary to go back to the problem formulation phase, to redefine a hypothesis and to design additional experiments to generate the data needed.

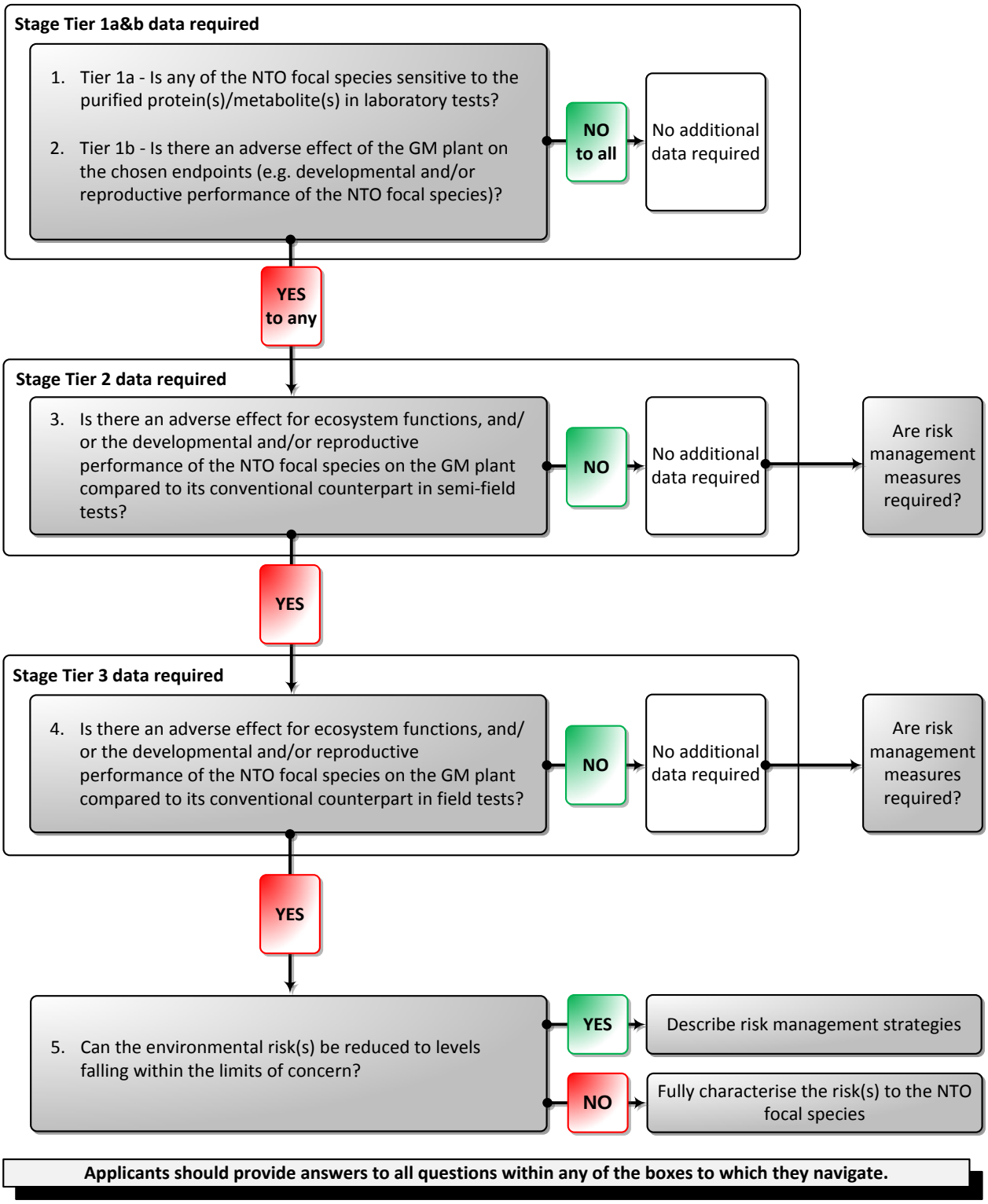

Figure 6: Decision tree for carrying out a specific-hypothesis driven investigation. Applicants shall provide answers to all questions within any of the boxes to which they navigate. The questions are divided into three stages (tiers 1→2→3). Only if all the questions of a stage are answered negatively (answer: NO), are no additional data required. If at least one question of a stage is answered positively (answer: YES), applicants shall move to the next stage and address all the questions of that stage.

3.4.1.7. Data requirement for the evaluation of possible unintended effects

GM plants may have unintended adverse effects on biodiversity through interactions with populations of other species associated or sympatric with the GM plant. It is important that species richness and ecological functions, especially considering guilds that provide ecosystem services, are not disrupted to the extent that populations decline and/or vital functions are impaired. Unintended impacts of GM plants on species richness and ecological functions shall be considered in the ERA.

Problem formulation thus seeks to collect all available information to decrease uncertainty of unintended effects to an acceptable level. The evidence to exclude the likelihood of unintended effects on NTOs can come from numerous sources including data already collected for other parts of the risk assessment, collating all the appropriate information from these data sources to provide a weight-of-evidence approach. Data sources relative to plant-environment interactions are always necessary to support the possible exclusion of unintended effects.

The sources of data, which should be properly justified, are described under chapter 2.1.

The applicants are requested to consider all the information available from these different data sources and to ensure that some field generated data are included. The use of field-generated data from outside the EU may be informative in this context, but applicants must justify why these data are relevant to the ecological functionality of receiving environments in the EU where the GM plant will be grown. Since unintended effects are to a large extent event specific, data from other events or from similar events in other plant species will carry little weight in supporting an application.

3.4.2. Step 2: Hazard characterisation

Once specific measurement endpoints are chosen, appropriate methods and criteria of measurement should be selected and described. This includes information on studies to be conducted, the appropriate tier for analysis, the design of experimental protocols with the definition of the appropriate statistical power (Marvier, 2002, Lövei and Arpaia, 2005, Perry et al., 2009) (see chapter 2.3.3).

3.4.2.1. Laboratory studies

Two kinds of methodologies are relevant for laboratory studies. First, existing conventional eco-toxicology methodologies (e.g. OECD, ISO, EPPO, IOBC standardized methods) can be used and adapted in order to assess the sensitivity of the NTO to different levels of exposure to the GM plant-produced proteins. The methodologies must be adapted to fulfil the measurement endpoint requirements. Secondly, an *in planta* experimental protocol is required in which the GM plant-NTO interactions are evaluated at exposure levels likely to occur in the field. For *in planta* studies, the testing scheme should ensure that the food used is ecologically relevant for the chosen NTO life stage to be tested (e.g. mimicking the trophic interactions existing in nature), and that specimens are exposed to the expected concentration throughout the study duration.

In addition to the above examples, several first tier studies that have been published in scientific literature can be considered by applicants.

All laboratory tests shall satisfy the following requirements:

- The endpoint and species are unequivocally identified;
- The rationale for the selection of the species and endpoint is given;
- Variability is sufficiently low for precise effect level estimation;

- Exposure to known quantities of testing material is maintained throughout the study;
- The experiment is conducted for a time span adequate to reliably estimate measurement endpoints.

When reproduction is an endpoint, the following requirements shall also be fulfilled:

- The processes of the reproductive biology must be included in the testing phase;
- The life-history must be known: age at maturation, duration of egg development, and instars subjected to exposure;
- Optimization of conditions for growth and reproduction must be provided by the test substrate and food supply.

Applicants can develop their own protocols for particular NTO species that are considered in the ERA. In this case, it is requested that, among others, the following aspects of the experimental protocols are correctly addressed:

- Organisms used during tests shall be healthy and of similar age;
- The biological performance of organisms used as controls shall be within acceptable limits (control mortality less than e.g. 20% depending on the testing system and organism);
- Environmental conditions in growth chambers, mesocosms and greenhouses shall be described explicitly and justified;
- Plant material shall be checked for transgene expression;
- Direct and indirect exposure pathways shall be clearly identified in the experimental setup.

When designing experiments with natural enemies, the following additional requirements shall be considered:

- The suitability of artificial diet or surrogate host/prey species vs. natural food (e.g. some species do not grow well or do not reproduce when reared on artificial diet);
- Host/prey herbivores have to be properly exposed (possibly from hatching) to the right treatments;
- A uniform supply of prey/host quality, age, etc;
- The availability of additional food sources for species with mixed feeding habits (e.g. availability of pollen, honey or sugar solution, possibility for sucking from plants, etc.);
- The availability of an appropriate oviposition surface for predators;
- The provision of particular microhabitats (e.g. providing additional sources of water-saturated surfaces).

For tier 1a, it is assumed that the test substance can be dosed and conventional testing approaches of chemicals can be followed. The sensitivity of the endpoint must be presented as EC10 and EC50 with confidence intervals. Laboratory practices (e.g. environmental conditions, specimen handling) should be according to standardised and published testing procedures. Limitations of some laboratory protocols should be considered (Lövei and Arpaia, 2005) when designing tests and concluding test results. When novel or non-standardised testing procedures are used, it shall be demonstrated that the method is appropriate, reproducible, reliable and of correct sensitivity.

The *in planta* testing required for tier 1b needs particular considerations concerning modifications of the standard procedures to accommodate for plant material. NTOs in tier 1b tests could be exposed to plant material through whole plants, plant parts (e.g. leaves, pollen) or ground plant material in diets or soil.

For *in planta* tests where feeding is an important route of exposure, it will not normally be possible to produce doses of the GM product that exceed the concentrations in plant tissues. Thus the normal level will act as the maximal exposure concentration in a test. Doses lower than the maximal dose can be made by dilution with a near-isogenic non-GM variety and EC10 and EC50 effect levels may be obtained. Different levels of exposure can also be achieved by mixing levels of GM plant material into the test substrates, e.g. soil, and a true dose-response relationship can be established delivering EC10 and EC50 effect levels. Appropriate controls for the effects of these diet regimes can be made by making similar mixtures with near isogenic non-GM materials.

In order to provide an optimal nutrition in soil ecotoxicological tests, a food source may be added. The amount of additional food source may need to be adjusted in order to ensure worst-case exposure.

When the aim is to demonstrate equivalence of the GM plant to the appropriate comparator, the standard tests should include the appropriate comparator as a negative control at an exposure level identical to the GM plant, as well as a positive chemical control to prove the functionality of the experimental setup, as advised in the pesticide test guideline.

3.4.2.2. Field trials

Experimental complexity and variability increases from tier 1 (e.g. toxicological studies), to bi- and tritrophic studies with plant parts, bi- and tritrophic studies with whole plants, to field assemblage studies. Laboratory testing provides the best way to control and manipulate experimental conditions (environmental factors, set-up) and to limit complexity and variability. In contrast, field tests allow the evaluation of trait x environment interactions, but they exhibit the highest experimental complexity and provide the lowest ability to control experimental conditions due to large natural variability.

The objectives of field trials are:

- To identify and study exposure routes (including trophic relationships) and confirm observed effects in lower tier experiments;
- To discover potential unintended effects not anticipated in lower tier tests;
- To provide feedback for further testing hypotheses;
- To study food chain and indirect effects;
- To determine effects of scale on NTO populations, including effects on generations and other spatio/temporal interactions;
- To study effects of interactions between several NTOs species in natural environment(s).

Field testing for NTOs is of special importance for certain species that can not be tested in laboratory (e.g. rearing methods and experiences are not available). Field testing provides a very broad range of arthropods in terms of species number, life stages, exposure to abiotic and biotic stress, complexity of trophic interactions, etc. that can not be reproduced in the laboratory. Hence, attention should be paid to the trade-off between standardised laboratory tests in lower tiers and the testing of NTO species in field experiments. Moreover, field studies offer the opportunity to estimate the functioning of whole ecological functions in natural conditions (*e.g.* Naranjo, 2005b,a).

Design and analysis of field trials for NTOs should be performed according to the criteria explained in chapter 2.3.3.

3.4.3. Step 3: Exposure characterisation

A major factor in evaluating the likelihood or probability of adverse effects occurring to the NTO is the characteristics of the environment into which the GM plant is intended to be released, and the manner of release. Several ecological characteristics specific to the crop-trait-receiving environment interactions need to be taken into account to characterise NTO exposure.

The introduction of a GM plant into a productive system will indeed introduce two new stressors, the transgene and its products and the genetically modified organism itself. In addition to this, new management practices may be associated with the cultivation of the GM plant. If hazards are identified (step 1) and hazard characterisation gives sufficient evidence for potential environmental damage (step 2) an exposure characterisation is conducted (step 3) to determine whether and to what degree the NTO species comes into contact with the GM plant and the transgene product. This assessment requires information on the phenotypic pattern of transgene expression in the various parts of the plant over the growing season. This exposure can be bitrophic via exposure to the GM plant (or plant parts, e.g. pollen) or can occur in higher trophic level organisms exposed to prey or host feeding on GM plant (Andow et al., 2006). Organisms at higher trophic levels can be exposed in different ways to the plant and/or its products, therefore direct, indirect or mixed exposure models needs to be evaluated according to the NTO and the GM plant characteristics. For example, a carnivore in an agro-ecosystem including GM plants will be faced with the presence in its diet of the transgene product and/or its metabolites, combined with the constitutive compounds of the prey/host species and the combination of both might interfere with the normal development of the natural enemy.

Based on the specific biological characteristics, the likelihood of exposure needs to be estimated. For this purpose, the highest mean protein expression level in any plant tissue is often taken as the worst-case environmental exposure concentration (EEC) in regulatory risk assessments (e.g. Raybould, 2007).

3.4.4. Step 4: Risk characterisation

Based on the conclusions reached in steps 3.4.2 and 3.4.3, applicants should estimate each identified risk that a GM plant will cause to NTOs considering the magnitude of the effects detected and the likelihood of their occurrence. Applicants should summarise the outcomes of the ERA considering intended and unintended effects as outlined in step 3.4.1. Hence applicants should conclude on risk for intended and unintended effects on NTOs taking into account focal species as well as the overall functionality of the agro-ecosystem. Applicants should provide an assessment of the range of effects likely to occur in different receiving environments based on the collected data and other relevant information (see chapter 2.3.2).

Considering receiving environment-plant-trait combinations, applicants are also required to characterise the risk (a) in the production site of the GM plant and (b) outside the production site in different habitats (e.g. adjacent crops and other non-crop habitats) where relevant exposure of sensitive NTO may occur. Quantification of risks and its relative uncertainties shall be provided in relation to each selected assessment endpoint and upscaling of data from lab, semi-field and field trials to landscapes considering the expected adoption rate of GM plants. The conclusions of risk characterisation should assess the consequences of each identified risk to NTOs and applicants should propose appropriate risk management measures where levels of risk exceed threshold levels (see step 3.4.5).

3.4.5. Step 5: Risk management strategies

In situations where risk due to the GM plant and/or its product(s) on NTOs and related ecosystem services has been identified and characterised, applicants should propose appropriate risk management strategies. These strategies should be designed, under assumptions of high exposure scenarios, to reduce the risk to a level considered acceptable (criteria defining this acceptability should be explicitly discussed). The implementation of measures should fit to common principles e.g. the principles of good agricultural practice and Integrated Pest Management that are being introduced by Member States under the Framework Directive on the sustainable use of pesticides in the EU.

These mitigation measures may include measures to reduce exposure in order to reduce risk to NTOs and ecosystem services. Examples might be the planting of non-Bt plants as border rows (EFSA, 2009c) or, where feasible, detasseling of GM maize plants in border rows in order to limit Bt maize pollen dispersal outside of the maize field. Also, the establishment and maintenance of habitats (ecological compensation areas) that provide *refugia*, feeding source, etc. for NTO populations over larger area and time might also be considered.

Applicants should also consider the implications of the introduction of the GM plant on present cultivation and farming practices. Applicants should describe how the GM plant will be introduced into Integrated Pest Management and farming systems so that present pest management strategies and practices contribute to sustainability of pest management. These practices that should be in line with general IPM principles (EC, 2009a) may cover rotation of crops and crop varieties, use of pesticides with different modes of action in order to maintain and support natural regulating mechanisms, including beneficial NTOs.

These mitigation measures and strategies should be devised in the light of a long-term management and maintenance of NTOs and ecosystem services in rural landscapes.

3.4.6. Conclusions

Applicants should conclude on the risk of intended and unintended effects on NTOs taking into account focal species considering all relevant ecosystem services. Applicants should provide an assessment of the range of effects likely to occur in relevant EU receiving environments based on the collected data and other relevant information. Applicants are also required to characterise the risk (1) in the production site of the GM plant and (2) outside the production site in different habitats considering relevant exposure routes. Quantification of risks and its relative uncertainties shall be provided in relation to each selected assessment endpoint in comparison to relevant baselines. The consequences of these risks for all relevant protection goals, including the overall functionality of the ecosystems, integrated pest management and the sustainability of production systems, should be considered.

The conclusions of risk characterisation should assess the consequences of each identified risk to NTOs and applicants should propose appropriate risk management measures where levels of risk exceed acceptable threshold levels.

3.5. Impacts of the specific cultivation, management and harvesting techniques

A GM plant for cultivation will be introduced into various receiving environment(s) (see chapter 2.3.2) and will be managed according to the requirements of the plant and the production systems into which it is introduced. There is a requirement in Directive 2001/18/EC to assess the environmental impact of the specific management and production systems (e.g. agriculture, forest tree or others)

associated with the GM plant, including how the plant will be cultivated, managed, harvested and processed (EC, 2001).

The introduction of GM plants for cultivation may require specific management practices and cultivation techniques and, may lead to additional changes in management and production systems. In Europe, current agricultural management and production systems are diverse (intensive, integrated, organic, etc) and already cover a wide range of management practices and cultivation techniques which, in addition, are continuously evolving under external drivers (e.g. regulation on pesticides, common agricultural policies, market requirements or agricultural innovations). Changes in management practices and cultivation techniques due to the introduction of GM plants and their potential environmental impacts shall therefore be seen in the context of this already existing and evolving range of current management and production systems and their environmental impacts. In this chapter, the ERA shall aim at comparing the range of different systems likely to occur in the practical management of GM crops, with the continuously evolving management in non-GM systems, using scenario analysis (see below). The comparative environmental impacts of different management systems will vary according to the receiving environment(s), intensity of crop production, rotational systems and a range of other factors. Thus, the ERA shall consider under what circumstances the specific GM management and production systems adopted may lead to greater, similar or lower adverse environmental effects than the current systems they are likely to replace.

Due to the high diversity of management and production systems across multiple receiving environments, the ERA is based on a scenario analysis which shall consider scenarios representative of the diversity of situations that may occur and assess their potential implications. The assessment of potential consequences is carried out by reviewing scientific literature from both peer-reviewed and technical publications, performing meta-analyses, conducting field experiments, studying commercial uses in non-EU countries, and/or modelling studies.

The cultivation of GM plants in non-EU countries where imports come from could change management practices and cultivation techniques in these countries and, in turn, may have adverse environmental impacts. The assessment of the environmental impacts of cultivation in such countries is out of the scope of this present ERA. Therefore, for GM plants for import and processing that are not intended for cultivation in the EU, there is no need for an ERA for altered cultivation, management and harvesting techniques[37]. However, information from cultivation in non-EU countries can provide useful information relevant to the management practices and cultivation techniques of the GM plant in Europe.

Stacking of GM events by conventional crossing may lead to additional and/or specific management practices and cultivation techniques not necessarily associated with the single events, which, in turn, may affect the environment. Therefore, GM plants with stacked events shall be fully risk assessed for the potential impacts of their cultivation, management and harvesting techniques. Applicant shall therefore describe the specific cultivation, management and harvesting techniques of the GM plant containing stacked events and of each of the cultivated sub-combinations covered by the application, taking into consideration the various receiving environments, and shall assess their potential environmental impacts with respect to the parental lines or conventional counterparts. In addition, the ERA of a GM plant containing three or more single events combined by conventional crossing shall include a consideration of the management of all other sub-combinations of these events that may occur by natural segregation (e.g. volunteers).

Effects of management practices and cultivation techniques are more related to the GM trait than to the specific transformation event. Nevertheless, whenever studies based on other events expressing the same trait are used, applicants shall provide evidence that the assessment of environmental impacts of

[37] The assessment of the consequences of accidental loss and spillage of a GM plant is covered under chapter 3.1.

management techniques of the GM plant can be derived from such studies, as variation among transformation events (e.g. expression level) may alter the conclusions of the ERA.

Applicants shall also consider potential adventitious stacked events (resulting from outcrosses between GM plants and existing GM volunteers or neighbouring GM crops or from commingling of seeds of different GM events) and the environmental impact of their cultivation and management.

3.5.1. Step 1: Problem formulation

The production system is defined by the specific use of the GM plant, the context in which the GM plant is grown, its cultivation (including crop rotation), harvesting and management and the crop type in which the transgenic trait(s) has/have been introduced. For example, grain maize, forage maize and sweet maize have different production systems with different environmental impacts in similar receiving environment(s). All may receive the same GM event but the subsequent changes in management and production systems and, consequently, the resulting environmental impacts may differ. Similarly GM plants introduced for amenity, forestry, land reclamation and other uses may also possess traits or other characteristics which require different management practices and cultivation techniques and the impact of these must also be assessed. Consequently, the problem formulation shall take into consideration receiving environment(s), which include the various agricultural production systems where the GM plant might be grown and any potential subsequent changes in the cultivation, management, harvesting and processing techniques associated with the GM plant compared to its conventional counterpart.

Examples of GM plants that can cause significant changes in production systems and, in turn, affect the environment, are provided below:

- GM herbicide tolerant (GM HT) plants will change herbicide regimes (e.g. type of herbicides and application timing) and may induce additional weed control changes to minimize weed shifts and manage weeds that have evolved resistance to the broad-spectrum herbicide. In addition, crop rotations and cultivation of other plants in a rotation may change in response to enhanced weed control or the presence of GM HT plant volunteers; additional environmental harm and greater adverse effects on biodiversity may result from these altered weed control systems (fewer weeds and/or weed shift);

- GM HT plants facilitate the adoption of minimum tillage or no-till cultivation techniques which may lead to beneficial or detrimental environmental effects. Potential changes in soil tillage resulting from adoption of GM plants are likely to affect soil structure, moisture retention, greenhouse gasses emission and the overall energy balance. These changes may also have impacts on soil biodiversity or flora and their importance may be higher than the direct effect of the GM plant (Krogh and Griffiths, 2007); in addition, some GM HT plants (e.g. soybean) management practices might have an effect on nitrogen-fixing symbiotic partners and might therefore induce a change in nitrogen fertilizer use, and subsequently affect biogeochemical cycles functioning;

- GM insect resistant (GM IR) plants will reduce the use of some insecticides and may cause changes in crop rotations in response to reduced pest pressure. GM IR plants may require establishment of non-IR refuges with specific cultivation requirements. The efficient control of target pests by adoption of GM IR plants may result in situations where the niche of the suppressed target pests will be occupied by other herbivores. This may lead to changes in pest management which can have further environmental implications (Wang et al., 2008); GM drought tolerant or salt tolerant plants could change irrigation regimes and other management practices and cultivation techniques as well as expand the receiving environment into which the GM plant might be grown; depending on the crops which might be substituted, this may lead to additional impacts on biodiversity;

- GM plants with a high potential for gene flow may require specific management techniques to minimize flowering or seeding (e.g. coppicing of trees); more generally, adoption of pest, disease and herbicide tolerant GM plants will alter requirements for Integrated Pest Management in these plants and in other plants in rotation or proximity and may affect the spatial organisation of cropping systems (e.g. to reduce selection pressure on weeds, mitigate insect resistance);

- Stacked events, combining various GM events (e.g. several HT traits combined with several IR traits) may lead to changes in crop management and/or allow changes in cropping systems causing novel impacts compared to those anticipated from the combination of traits. For example, GM plant containing both IR and HT traits may result in changes in weed control practices that affect host plant densities for non-target insect species and, therefore, alter potential mortality of such species. Stacking several HT traits into one variety will allow the use of novel combinations of herbicide treatments which may lead to additional alteration of biodiversity.

An assessment is required of the possible immediate and/or delayed, direct and indirect environmental impacts of the specific cultivation, management and harvesting techniques used for the different receiving environment(s) in which the GM plant may be grown where these techniques are different from those used for non-GM plants. This shall include the impact on biogeochemical processes (see chapter 3.6) as well as on biodiversity in the receiving environment(s).

Current agricultural production systems are diverse and their environmental impacts display a huge variability. Changes in management practices and cultivation techniques due to the introduction of GM plants and their potential environmental impacts shall therefore be seen in the context of the already existing and evolving range of current management and production systems and of their environmental impacts. The ERA shall

- describe the potential range of GM-based management and production systems likely to occur across receiving environments and how they differ from current management systems;

- identify the potential adverse environmental impacts associated with these systems;

- assess to what extent the environmental impacts overlap those of the range of non-GM systems;

- determine which conditions (receiving environments, management and production systems) are related to potential higher adverse effects than current systems;

- assess to what extent the range of GM management and production systems would meet the assessment endpoints identified in the other chapters.

When addressing these steps, applicants shall discuss to what extent its conclusions depend on the deployment scale of the GM plant (see scenario analysis below).

The problem formulation shall first identify, through relevant assessment endpoints (see chapter 2.2.1), the aspects of the environment(s) that need to be protected from adverse effects due to changes in cultivation, management and harvesting techniques (biodiversity, water and air quality, etc).

Second, the problem formulation shall consider potential changes of receiving environment(s) and management and production systems (e.g. crop rotations and cropping systems, rate of adoption of the

GM plant, introduction of other GM crops, pest pressure evolution[38]) which are foreseeable in the near future (e.g. consequences of the implementation of IPM by 2014 under the framework of Directive 2009/128/CE On Sustainable Use of Pesticides) (EC, 2009a).

Third, the problem formulation shall identify the potential adverse effects that may result from the changes in management and production systems (see examples above) in a range of different environments, taking account of anticipated future changes in agriculture associated with other drivers (e.g. market forces, legislation etc.).

3.5.2. Step 2: Hazard Characterisation

Based on the hazards identified in step 1, applicants are requested to further characterise hazards associated with the change in specific cultivation, management and harvesting techniques in the receiving environment(s). In assessing the information on the receiving environment(s), applicants shall identify the various representative management and production systems (e.g. use of the plant, crop rotation, cultivation techniques and crop type) in which the GM plant may be introduced, and then consider how the GM plant is likely to alter the existing management and production systems, taking into consideration both direct and indirect effects as illustrated in the problem formulation chapter. Applicants shall also consider via relevant hypotheses, potential changes to receiving environment(s) within the timeframe of the authorisation as detailed earlier in the problem formulation (see chapter 3.5.1).

For each of the representative management and production system, applicants shall identify the possible adverse effects due to changes in management practices and cultivation techniques. The introduction of the GM plant may induce changes in applications of plant protection products (e.g. pesticides and/or biocontrol agents), rotations and other plant management measures. These changes may result from the characteristics of the GM plant itself or from the implementation of management measures aiming to mitigate potential adverse effects (e.g. insect resistance or weed resistance).

Information is required for all foreseen potential changes in management practices and cultivation techniques and an assessment shall be made of likely adverse environmental impacts of these changes. In addition the impact of the GM plant on the cultivation of other plants (e.g. change of weed control regimes in subsequent crops) shall be considered and the consequences of any changes in the management practices and cultivation techniques of these plants shall also be risk assessed.

The application of risk management measures identified in chapter 3 (e.g. to limit gene flow to weeds, feral plants and crop volunteers) may result in new cultivation, management and harvesting techniques and the consequences of these for the environment shall be assessed.

Where cultivation and management techniques of GM plants and their associated production and management systems have different effects on species or both increase and decrease biodiversity throughout cropping seasons or rotations then an assessment shall be made of any overall long-term harmful effects of these changes on biodiversity. Examples of indicators of such changes can be changes in weed seed bank populations or higher species in the food web.

Longer-term and indirect effects due to changes in cultivation, management and harvesting techniques might be difficult to evaluate through small-scale and short-term field experiments (see chapter 2.3.4). Applicants are requested to analyse this information and any information on potential environmental

[38] Unrelated changes due to external drivers such as climate change, regulation on pesticides, common agricultural policies, market requirements or agricultural innovations may alter the relationship between GM plants and farming systems and may inform the potential evolution of receiving environments and help applicants to identify which relevant scenarios might be considered.

impacts of the management and production systems in those countries where the GM plant has been/is currently grown.

In addition, as far as they have been validated, models may be used to complement applicant's statements (Heard et al., 2005, Gibbons et al., 2006, Butler et al., 2007, Pidgeon et al., 2007, Storkey et al., 2008). Applicants may provide simulations, carried out under representative receiving environment(s) and various GM adoption scenarios, to assess to what extent the changes in management and production systems may have adverse effects on the environment.

3.5.3. Step 3: Exposure characterisation

Applicants shall assess the magnitude of changes in cultivation, management and harvesting techniques for each selected representative receiving environment(s) and also consider whether the changes in practices are likely to change the range of environments in which the GM plant is cultivated (see chapter 2.3.2). Changes in management practices and cultivation techniques in the EU cannot always be anticipated but data on cultivation of GM plants outside EU can provide some indications. Applicants shall consider various scenarios which might occur in representative receiving environment(s) and assess, via scenario analysis, the consequences in relation to different level of adoption of GM plants (in term of exposure). Due to the high diversity of management practices and cultivation techniques across EU, applicants shall consider possible scenarios by combining selected receiving environments and representative management and production systems.

At least three kinds of scenarios shall be considered:

- A ''field level' or 'substitution' scenario which describes the foreseen introduction of GM plants and their recommended management practices and cultivation techniques into most common current management and production systems and receiving environments (e.g. at field level, over a rotation); this scenario considers the substitution of the non-GM plant (and its specific cultivation techniques) by the GM plant and its specific management without any other changes in other management practices (only direct effects, field and its immediate surroundings considered here);

- A 'landscape level' or 'typical' scenario which considers the likely rate of adoption of the GM plant in production systems, the indirect effects in management which are foreseen as well as the upscaling effects (e.g. at field, farm and landscape level, over a rotation); in this scenario, management systems are adapted to take advantage of the GM plant (indirect changes in cultivation techniques occur, management of other crops may be affected); the likely uptake at the landscape level is considered and mitigation measures are adopted;

- A "worst-case" scenario which describes the effects of repeated, large-scale, and intensive management of production systems on receiving environments, where additional impacts are likely to occur (e.g. at field, farm and landscape level, over a rotation). This scenario considers the effect of large-scale cultivation of the GM plant with its adapted management practices (temporal and spatial scales) and of high selection pressure factors.

These scenarios shall be elaborated by considering the factors which may drive the environmental effects in terms of exposure (crop acreage, GM adoption rate) and hazard (selection pressure, etc).

Whenever relevant, a fourth scenario shall consider the potential adoption of other GM plants, the potential changes in the management and production systems which may result from adoption of such other GM plants within the receiving environments as well as their potential additional adverse environmental effects.

Applicants shall justify that the selected scenarios cover the range of receiving environments and management and production systems which may occur.

As far as they have been validated, models may be used to support that scenario analysis and complement applicant's statements on exposure characterisation e.g. exposure assessment models (Perry et al., 2010) or gene flow models (Colbach et al., 2005, Angevin et al., 2008, Colbach, 2009, Colbach et al., 2009).

3.5.4. Step 4: Risk characterisation

Applicants shall characterise the identified risks related to changes in management and production systems. The scenario approach, covering representative situations that may be encountered, shall indicate the circumstances that may lead to specific GM management practices causing greater, similar or lower adverse environmental effects than the current management and production systems they are likely to replace. Even if the scenario analysis can cover representative situations, it may be difficult to predict the whole range of impacts that the changes in management practices and cultivation techniques may have. The conclusions for risk characterisation shall take into account the consequences of this unpredictability of management and relate them (in chapter 3.5.5) to proposed mitigation measures to ensure that adverse environmental impact is maintained at or below current levels found in comparable non-GM management and production systems.

In addition, as far as validated, models may be used to complement applicant's statements and clarify uncertainties, applicants may provide simulations, carried out under representative receiving environment(s) and various GM adoption scenarios, to assess the level of risk (Heard et al., 2005, Gibbons et al., 2006, Butler et al., 2007, Pidgeon et al., 2007, Storkey et al., 2008).

3.5.5. Step 5: Risk management strategies

In situations where the ERA concludes that changes in management and production systems may cause adverse environmental impacts compared with the comparable non-GM management and production systems, applicants shall present and assess risk management strategies to mitigate adverse effects.

The efficacy of each proposed management strategy in the relevant receiving environment(s) shall be presented and discussed by applicants.

Applicants shall assess to what extent the proposed management strategies or options do not induce more harm than non-GM management and production systems and are consistent with the environmental protection goals.

Validated models, e.g. models used for assessing the efficacy of the high dose/refuge strategy for Bt crops, may be used to complement applicant's statements. Applicants may provide simulations, carried out under representative receiving environment(s) and GM adoption scenarios, to assess to what extent the proposed risk management strategies may prevent adverse effects on the environment. This would help with the establishment of monitoring schemes since their design may depend on adoption scenarios and other factors.

3.5.6. Conclusions

Applicants shall conclude on the overall risk considering immediate and delayed effects on the environment, both in-field and wider, resulting from potential direct and indirect effects of changes in

management and cultivation practices. The applicant shall also consider effects of further potential changes in receiving environment(s) and farming systems.

Where specific risks associated with the cultivation of a GM plant are identified during the ERA, risk management strategies shall be proposed to mitigate these risks and applicants shall indicate how these measures will be introduced and enforced. Furthermore, monitoring is required either to confirm any assumptions regarding the occurrence of adverse effects or to verify the efficacy of mitigation measures (chapter 4).

Specific considerations for GM HT Plants

Under current EU legislations for the introduction of GM plants (Directive 2001/18/EC, Regulation (EC) No. 1829/2003), there is a requirement to assess the environmental impacts of GM plants and also to assess the environmental impacts of the specific cultivation and management of GM plants. In the case of GM HT plants, this means evaluating the overall environmental impact of the specific cultivation practices due to the change in herbicide use associated with these GMHT plants, as well as the environmental impacts directly associated with the GM plant itself.

In the current regulations governing pesticide registration in Europe (Directive 91/414/EC (EC, 1991) and recent Regulation (EC) No 1107/2009), the ERA of pesticides includes an assessment of impacts on certain NTOs and studies of residual activities in soil and water. The ERA of plant protection products does not currently include studies of impacts on biodiversity within crops or changes in agricultural management practices (which are required in relation to GM plants under (EC, 2001). However the new pesticide regulations in the EU are under review and consideration of impacts on biodiversity is included in the new revised Regulation (EC) No 1107/2009, adopted on October 2009[39].

Whatever the new provisions of the Plant Protection Product Regulation (e.g. on biodiversity), it is necessary to assess the possible adverse effects on the environment (biodiversity and NTOs) which any particular individual GM HT plant may cause due to changes in management practices, including those due to different herbicide practices.

GM HT plants may lead to changes in management which may have environmental impacts:

- The effects of the changes in weed management may affect biodiversity (flora and fauna) in and around fields (Firbank et al., 2003).

- GM HT crop volunteers and hybridising relatives may require additional measures for control in other crops and therefore lead to additional environmental impacts.

- Related HT plants already in the environment may hybridise with the new GM HT plant and thus the management of GM plants containing unintended stacked HT genes also needs to be considered.

- Weed shift and resistance may occur, either through selection pressure (repeated application of the same herbicide over the rotation (Heap, 2008) or through outcrossing gene flow to weed relatives (Jorgensen, 1999, Chèvre, 2003, Jorgensen, 2007, Heap, 2008). The consequences of this may be further changes in weed management and potential additional environmental impacts which require assessment;

- Some specific GM HT crop (e.g. soybean) herbicide management might have an effect on nitrogen-fixing symbiotic partners and might therefore induce a change in nitrogen fertiliser use. The potential environmental consequences of such changes (e.g. on biogeochemical processes) need to be considered (see chapter 3.6).

Depending on the receiving environments and the management systems likely to be adopted, GM HT crops may therefore have (1) greater adverse effects on biodiversity resulting from altered weed control (fewer weeds and/or weed shift) and (2) environmental harm resulting from the management of weeds that have evolved resistance to the broad-spectrum herbicide.

[39] Regulation (EC) No 1107/2009 – adopted October 2009; in force from June 2011, repealing Directives 79/117/EEC and 91/414/EEC
http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:309:0001:0050:EN:PDF

The ERA shall discuss the potential environmental impact of the management of GM HT plants, considering the range of receiving environment(s) and agricultural production systems which might be concerned. This shall include consideration of likely effects on the environmental protection goals and farmland Biodiversity Action Plans of Member States and comparison with the management of non-GM plants. For example, the published results of the UK's Farm Scale Assessments of GM HT plants (Squire et al., 2003) may give information relevant to other HT plants. However, it will be necessary to compare the relative efficacy of different herbicides and their management programmes on e.g. weed species in order to assess the impact of herbicide regimes on biodiversity.

The EFSA GMO Panel considers that the novel use of herbicides on GM HT plants will change agricultural practices and that this requires an ERA. The applicant is therefore requested to:

- Describe the potential herbicide regimes to be applied to the GM HT crop under consideration across receiving environments in Europe as well as the other changes in management practices it might induce and how they differ from current weed control systems. Typical dose(s) of the active ingredient and timing recommendations, their respective ranges for GM HT plant, and possible use in conjunction with other active ingredients are to be specified;

- Assess the implications of these herbicide regimes on the selection pressure exerted by the non-selective herbicide and which can be estimated by considering the overall amount of active ingredient, the frequency of applications, the level of dependence of weed control on this non-selective herbicide.

- Assess the potential environmental effects due to the cultivation of the GM HT crop in the receiving environment(s) where the GM HT plant is likely to be cultivated, considering in particular (1) the evolution of less desirable weed assemblages leading to reductions in farmland biodiversity, (2) evolution of weed resistance, (3) potential effects on soil microbial communities. Reference to scientific publications in support of each statement/observed/non observed effects is expected;

- Specify under what circumstances the potential herbicide regimes likely to be adopted for the GM plant may lead to greater, similar or lower adverse environmental effects than the current management systems they are likely to replace. Special attention shall be paid to changes in efficacy of weed control techniques and selection pressure on weeds which may affect (1) changes to weed communities that lead to reductions in farmland biodiversity; (2) evolution of weed resistance and (3) effects on soil functions (including plant-associated microbial communities).

- Consider the consequences of the assessment on the impact of the herbicide treatments on biodiversity in fields and the implications of this for wider biodiversity within farming regions, integrated pest and disease management and the functioning of agricultural ecosystems;

- Indicate the specific management strategies that will be put in place to restrict adverse environmental impacts of the GM HT plant to the levels currently found in equivalent non-HT crops or to meet specific protection goals, as well as to manage situations where herbicide resistant weeds and volunteers might develop. The applicant shall describe the recommended herbicide regimes and mitigation measures that will be put in place to maintain biodiversity at current levels. The ERA shall consider the stewardship recommendations (or proposals for stewardship recommendations) in the range of production and management systems of the GM HT plant likely to be applied in various Member States in Europe. These management systems shall include measures to control HT volunteers and HT weeds, and to manage interactions with other HT plants including gene stacking. The potential environmental impacts of these recommended herbicide management measures shall be compared with those currently observed in equivalent non-HT plants and non-GM HT plants;

- Consider in its assessment that other GM HT plants might be grown in the future in Europe, some in rotation or sequence with this GM HT plant and assess the potential environmental implications of any changes in management this may induce. The potential consequences in terms of management of HT volunteers or biodiversity shall be assessed as well as the environmental impact of additional management measures which would be undertaken;

- Estimate the likely rate of evolution of HT weeds considering that other crops tolerant to the same herbicide will be grown in Europe, some in rotation or sequence with this HT plant;

- The risk assessment shall consider whether these uses of the herbicide could result in reductions in biodiversity leading to environmental damage equivalent to, or greater than, non-HT plants and non-GMHT plants, taking into account both the different production systems and different levels of biodiversity found in different European farming regions.

Management plans of GM HT systems shall be constantly reviewed and to consider new scientific information in this area.

Where herbicide regimes and associated management of GMHT crops have different effects on species or both increase and decrease biodiversity throughout cropping seasons or rotations then an assessment shall be made of any overall long-term harmful effects of these changes on biodiversity. Examples of indicators of such changes can be changes in weed seed bank populations or higher species in the food web.

When studies used to support the ERA have been carried out with the another event or construct, the applicant shall provide evidence that the assessment of environmental impacts of management techniques of the GM plant can be derived from studies using this other event; indeed, even if the GM HT plant expresses the same proteins and consequently, confers the same herbicide tolerance, variation among events may alter the conclusions (e.g. expression level).

The above aspects shall be addressed by taking into consideration the potential levels of exposure which may occur across receiving environments and representative management and production systems. The scenario analysis, as described in 3.5.3, shall consider the following scenarios:

- The 'field level' or 'substitution' scenario which considers the substitution at the field level of the non-GM plant (and its specific cultivation techniques) by the corresponding GMHT plant and its specific herbicide without any further changes in management practices;

- The 'landscape level ' or 'typical' scenario which considers the likely rate of adoption of the GMHT plant in production systems as well as indirect effects in management which are foreseen such as minimum soil tillage;

The "worst-case" scenario which describes the effects of repeated, large-scale, and intensive use of GM HT plants (same herbicide tolerance trait) over the rotation and indirect changes with result in higher selection pressure of the herbicide (shorter rotations, dominant use of one herbicide).

The applicant shall justify that the selected scenarios cover the range of receiving environments and production and management systems which may occur.

In situations where the ERA concludes that changes in management and production systems may cause adverse environmental impacts compared with the comparable non-GM management and production system, the applicant shall present and assess risk management strategies to mitigate adverse effects due to the introduction of GMHT plants. The efficacy of each proposed management strategy in the relevant receiving environment(s) shall be presented and discussed by the applicant.

3.6. Effects on biogeochemical processes[40]

3.6.1. Step 1: Problem formulation

Biogeochemical processes underlie the movement, transformation and storage of energy, water, carbon, nitrogen and other elements in ecosystems. Biogeochemical processes include the uptake of carbon dioxide from the atmosphere by plants, degradation of plant material, formation of soil organic matter, evaporation of water from fields and transformation of nitrogenous compounds. Biogeochemical processes can build soil fertility, but they may also bring about mobilization and loss of materials, for example in the form of greenhouse gases (CO_2, CH_4, N_2O). Therefore applicants should assess whether GM plants and their associated management have potential adverse effects on biogeochemical processes compared to the effects of a range of current production systems (see *3.6.4, Step 4 Risk characterisation*). Problem formulation should cover principally two scales: the *production site*, for example a field, in which the GM plant is grown; and the *wider environment* with which the field interacts through exchanges of energy, elements and materials. Indirect impacts due to altered cultivation, management and harvesting techniques could affect both of these scales and should be considered by reference to chapter 3.5.

The production site comprises the soil, plants, animals and microorganisms within the area in which the GM plant is to be grown (e.g. an agricultural field). Soil organisms are the main drivers of biogeochemical processes in the production site, determining soil structure, nutrient cycling, immobilization and mobilization of nutrients, degradation of soil organic matter (SOM) and emission of greenhouse gases. Soil fertility is a key parameter of soil quality and is to a large extent consequent on previous generations of plants and micro-organisms acting on and mediating biogeochemical processes. As plant-associated (e.g. rhizosphere) and soil microbial communities perform the vital biotransformations for sustainable soil fertility, any negative impact(s) on these organisms should be carefully evaluated on a case-by-case basis with particular reference to the characteristics of the introduced trait and the consequences of the genetic modification or alteration of the GM plant.

The wider environment comprises land, water and air outside the production site, with which the GM plant and its management might interact. An assessments of impacts on the wider environment should take account of the import and export of materials (such as fertiliser, fuel, seed, pesticides, carbon amendments, plant matter), and losses to the atmosphere and water as a result of human (e.g. agricultural) operations. When taking account of imports of materials, the manufacture and procurement of fertilisers (organic and inorganic) are included, not only their application or turnover at the production site.

Admittedly, information is limited on many aspects of biogeochemical processes. Accordingly, the level of detail required in the ERA will depend upon the characteristics of the plant and the transgenic trait and the scope of the application. Problem formulation should start with a desk study comparing the cultivation system used for the GM plant with current production systems (see 3.6.4). The desk study would refer to available data and apply published methods of assessing, for instance, greenhouse gas emissions, erosion, soil degradation and the potential to pollute watercourses, backed by more specific experimental data if available.

With respect to biogeochemical processes in the production site, the evaluation should address the potential impact of GM plants through factors such as:

I. Release of recombinant gene products or GM specific metabolites into the plant-soil system, which may directly influence soil fertility, nutrient transformations and food webs;

[40] This chapter of the ERA Guidance Document combines previous chapter on biogeochemical processes and potential interaction with the abiotic environment from the former ERA GD (EFSA, 2006b).

II. Altered movement of other compounds from roots to soil, which may directly influence soil fertility, nutrient transformations and soil food webs;

III. Altered plant litter that decomposes differently from that of non-GM plants due to either the presence of specific compounds (e.g. toxic metabolites) or altered concentration of substances resistant to decomposition;

IV. Altered uptake and cycling of plant nutrients within the plant-soil system (including the fixation of atmospheric nitrogen).

With respect to biogeochemical processes in the wider environment, the evaluation should address the potential impact of GM plants and the associated production (e.g. agricultural) management on:

V. Losses from production sites systems to air, or water e.g. greenhouse gas emissions, including those that result from operations and processes that are essential to plant production but which occur outside the production site (e.g. manufacture and transport of fertiliser);

VI. The capacity of production (e.g. agricultural) systems to store water, carbon, nitrogen, phosphorus and other elements essential for plant growth and ecosystem functioning41.

Any indications in the desk study that the GM plant and its management have potential effects on biogeochemical processes should receive detailed attention in the following steps.

3.6.2. Step 2: Hazard characterisation

Step 2 consists of characterising any hazards identified during consideration of the problems in step 1 that might lead to adverse effects on biogeochemical processes in the production site or in the wider environment.

Hazards should be considered that might result from an intended change in the plant (e.g. change in plant nutrient relations) or an ancillary change related to the GM plant or its method of cultivation. For example, if plant compositional analysis indicates a substantial change in the C/N ratio of plant structures or the lignin composition of plant litter, then the potential effects of these changes on biogeochemical processes should be evaluated. Similarly, with respect to wider biogeochemical processes, if the GM plant and its cultivation are likely to alter fertiliser inputs, tillage or the timing of cultural operations in the receiving environment(s), then effects of these on the wider biogeochemical processes should be evaluated.

Many of the potential impacts, particularly those with respect to wider biogeochemical processes, may result from the interaction of the GM plant and its management with general agricultural practices in the receiving environments. Indeed, variables such as greenhouse gas emissions, pollution of water and reduced carbon sequestration will be strongly affected by general change in the production system (e.g. agricultural), for example in the extent to which inversion tillage is practiced and the type and origin of fertiliser. The aim of step 2 is to assess whether the hazards identified in step 1 would have additional adverse effects relative to current production practice. The applicant should make reference to chapter 3.5 if changes in cultivation (e.g. soil tillage) associated with the GM trait are likely to have a major effect on biogeochemical processes.

[41] In the past, the capacity for storage has been strongly reduced by certain (*e.g.* conventional agricultural) practices such as repeated tillage and over-extraction of primary production.

3.6.3. Step 3: Exposure characterisation

An assessment is required of the likelihood that biogeochemical processes in the receiving environments will be exposed to any hazards arising from the GM plant and its cultivation. Exposure in this instance should be considered in terms of the GM plant and its management affecting biogeochemical processes both in the production site and in the wider environment, as previously defined. The degree of exposure is likely to be high at the production site, e.g. exposure of the plant-soil matrix, since it is the intention to grow the GM plant within that matrix. However, the degree to which the wider environment is exposed to a hazard is more likely to depend also on the local context. For example, if a GM plant and its management are considered under 3.6.2 to cause a potential hazard through an adverse change in production practice (e.g. increased use of mineral nitrogen fertiliser), but that change is not likely to occur in a particular receiving environment because of soil type, climate, local fertiliser practice or any other reason, then the exposure may be low or zero in that receiving environment.

In most cases, there will be little or no exposure of biogeochemical processes to imported GM plants and their products. However, the ERA should consider whether there will be exposure to products of a GM plant through manure or organic plant matter, either imported as a fertiliser or soil amendment, derived from the faeces of animals that are fed an imported GM plant or plant product, or derived from other bioproducts of industrial processes.

3.6.4. Step 4: Risk characterisation

Risk characterisation should aim to establish the degree of risk from the characterisation of hazard in 3.6.2 (step 2) and exposure in 3.6.3 (step 3). Risk characterisation should be carried out for both the production site and the wider environment (as defined) by considering the potential impacts listed in 3.6.1, I to VI. The applicant should make reference to chapter 3.5 if changes of cultivation (e.g. soil tillage) are likely to have a major effect on biogeochemical processes.

Risk characterisation for biogeochemical processes could initially compare existing data from current production systems (e.g. fertiliser and pesticide applications, frequency and depth of tillage) with the practice expected during the growing of the GM plant, possibly supported by with data from GM field trials. For example, if growing the GM plant is unlikely to change the current input of nitrogenous fertiliser, then the risk characterisation should be able to consider the consequences of this without further field experiment.

However, the choice of comparator needs to be considered carefully and justified. It is accepted that (a) most methods and materials used in current production (e.g. agricultural) cause losses from and reduced storage capacity of the production system, (b) there may be several types of production system operating in a receiving environment, and (c) of the systems may change over time (e.g. due to phasing out of pesticides).. Therefore, risk characterisation should ideally make reference to existing information and experiments from a range of production systems, including optimised systems if present. The characterisation should demonstrate that the GM plant and its management do not have more adverse effects on biogeochemical cycles than any present system, and assess whether they will contribute to more sustainable or optimised production. Such comparisons can be conducted initially as part of the desk study, referred to in 3.6.1. If any factors are identified that are likely to alter biogeochemical; processes, then experimental work may be needed to substantiate the risk characterisation.

3.6.5. Step 5: Risk management measures

Based on the outcome of the risk characterisation, the applicant should determine and evaluate targeted risk management strategies (altered production practices, see Chapter 3.7) which could

minimize undesired impacts of the GM plant on biogeochemical processes. Since biogeochemical processes are influenced by many operations in farming, it may be possible to compensate for negative effects associated with the release of the GM plant by modifying other operations in the production system. The assessment should consider the general scope for such modification in the production systems of the receiving environment(s).

3.6.6. Conclusions

A conclusion is required of the overall risk of the GM plant on biogeochemical processes in both the production site and the wider environment ,. The applicant should also consider long-term effects of adverse changes in biogeochemical processes and should address indirect effects on biogeochemical processes as a consequence of altered production practices related to the GM plant in chapter 3.5. The risk characterisation and conclusions will determine the requirements for the post-market environmental monitoring plan (see chapter 4).

3.7. Effects on human and animal health

In the framework of GMO risk assessment under Directive 2001/18/EC, an assessment is required of whether the GM plant and its products presents a new hazard for human and animal health. In particular, if a potential hazard has been identified, the risk to persons working with the GM plant, coming into contact with it or exposed to products such as pollen or dust from processed plants should be assessed (see Annex II D.2.6 of the Directive). This assessment is particularly required for GM plants which are not destined for human or animal consumption and where impacts on human health may not have been so meticulously studied.

For GM plant applications for food and feed purposes, the applicant is requested to refer to the requirements detailed in the EFSA GMO Panel GD "Guidance Document of the Scientific Panel on Genetically Modified Organisms (GMO) for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed" (EFSA, 2009f) and where relevant, the EFSA opinion on "assessing the allergenicity of GM plants and microorganisms and derived food and feed" (EFSA, 2010d).

For GM plant applications for non-food or non-feed purposes, the applicant is requested to refer to the requirements detailed in the EFSA GMO Panel "Guidance for the Risk Assessment of GM plants used for non-Food or non-feed purposes" (EFSA, 2009d).

The applicant should follow the 6 steps approach as outlined in chapter 2.2 on a case-by-case basis. A conclusion is required of the overall risk on human and animal health.

3.8. Overall risk evaluation and conclusions

On the basis of the ERA performed under chapter 3.1 to 3.5, the weight-of-evidence and the conclusions reached under each chapter, the applicant is requested to perform an overall evaluation of the risk(s) of the GM plant in the receiving environment(s). The overall evaluation of the risk(s) of the GM plant should take into account the risk characterisation (step 1 to step 4) and any risk management strategies proposed (step 5).

The overall risk evaluation should be expressed in a form of a summary, in a concise way, of the overall risk(s) from deliberate release or placing on the market of the GM plant, including the overall uncertainties. The quality of existing data and information should be discussed, an explanation on how the body of information has been taken into account and the potential uncertainties. The overall risk

evaluation should result in informed qualitative, and if possible quantitative, guidance to risk managers. The applicant should explain clearly what assumptions have been made during the ERA, and what is the nature and magnitude of uncertainties associated with establishing the(se) risk(s).

The applicant should provide a summary of the overall risk evaluation in a way that conclusions can be drawn up for the PMEM (chapter 4).

4. POST-MARKET ENVIRONMENTAL MONITORING PLAN[42]

4.1. General

The Regulation (EC) No. 1829/2003 introduces the obligation for applicants to implement, if appropriate, a GMO monitoring plan for environmental monitoring according to Annex VII of the Directive 2001/18/EC (Regulation (EC) No. 1829/2003 Art. 5(5)(b) and Art 17(5)(b)) In reference to Directive 2001/18/EC the environmental monitoring is introduced in order to identify any direct or indirect, immediate and/or delayed adverse effects of GMOs, their products and their management to human health or the environment, after the GMO has been placed on the market.

Since the Regulation (EC) No. 1829/2003 explicitly refers to Annex VII of Directive 2001/18/EC the structure and content of this environmental monitoring plan should be designed in accordance with the Council Decision 2002/811/EC supplementing Annex VII (strategy, methodology, analysis, reporting (EC, 2002, Wilhelm, 2003, ACRE, 2004).

An environmental monitoring plan is required for applications for placing on the market of GMOs or food/feed containing or consisting of GMOs conforming with Annex VII to Directive 2001/18/EC. It is explained in the Guidance notes supplementing Annex VII that the extent of the market release should be taken into account. Thus, the monitoring plan should be targeted rather than considering every possible environmental aspect. Applications concerning only food/feed or ingredients (for example, imported into but not cultivated within the EU) will thus not normally be required to describe a detailed environmental monitoring plan if the applicant has clearly shown that environmental exposure is absent or will be at levels or in a form that does not present a risk to other living organisms or the abiotic environment.

Monitoring can be defined as the systematic measurement of variables and processes over time and assumes that there are specific reasons to collect such data, for example, to ensure that certain standards or conditions are being met or to examine potential changes with respect to certain baselines. Against this background, it is essential to identify the type of effects or variables to be monitored, an appropriate time-period for measurements and, importantly, the tools and systems to measure them. Monitoring results, however, may lead to adjustments of certain parts of the original monitoring plan, or may be important in the development of further research. The Council Decision 2002/811/EC (EC, 2002) provides no clear differentiation between the monitoring principles of either case-specific monitoring or general surveillance (den Nijs and Bartsch, 2004). This GD provides further assistance in the following chapters.

The monitoring results should be presented in accordance with the Cultivation Monitoring Format established by Commission Decision 2009/770/EC43

[42] The chapter has not been revised since 2006 and is similar to the Guidance document of the EFSA GMO Panel issued in 2006.

[43] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:275:0009:0027:EN:PDF

4.2. Interplay between environmental risk assessment and monitoring

4.2.1. Monitoring of effects: Foreseen and unforeseen

The environmental monitoring of the GM plant will have two aims: (1) to study any possible adverse effects of the GM plant identified in the formal risk assessment procedure, and (2) to identify the occurrence of adverse unforeseen effects of the GM plant or its use which were not anticipated in the ERA. Where there is scientific evidence of a potential adverse effect linked to the genetic modification, then case-specific monitoring should be carried out after placing on the market, in order to confirm the assumptions of the ERA. Consequently, case-specific monitoring is not obligatory and is only required to verify the risk assessment, whereas a general surveillance plan must be part of the application. Applicants who are proposing to have no case-specific monitoring are encouraged to provide arguments in support of this position. These arguments should relate to the assumptions applicants have made in the ERA as well as to the lack of any identified adverse effects in steps 1 to 5 (see chapter 2.2).

4.2.2. Monitoring framework

Council Decision (2002/811/EC) (EC, 2002) explicitly suggests that general surveillance should include long-term monitoring, to allow for unexpected effects that may occur after longer periods of environmental exposure.

The environmental monitoring plan should describe in detail the monitoring strategy, methodology, analysis, reporting and review as laid down in Council Decision 2002/811/EC. In this respect, GM plant-based parameters will depend on the particular GM plant, trait and environment combination. Key parameters to be observed may refer to species/ecosystem biodiversity, soil functionality, sustainable agriculture, or plant health. Indicators should be measurable, appropriate, adequate in terms of statistical power, and comparable with existing baseline data. Background and baseline environmental data e.g. soil parameters, climatic conditions, general crop management data e.g. fertilisers, plant protection, crop rotations and previous crop history should be collected, where appropriate, to permit the assessment of the relevant parameters.

4.3. Case-specific GM plant monitoring

The main objective of case-specific monitoring is to determine the significance of any adverse effects identified in the risk assessment (see chapter 3). The assessment of risk should be based on Annex II of the Directive (EC, 2001).

Case-specific monitoring should be targeted at those environmental factors most likely to be adversely affected by the GM plant which were identified in the ERA. The scientific approach should be designed in order to test the specific hypothesis of expected adverse effects derived from the environmental risk assessment. The monitoring programme design should also reflect levels of exposure in different geographical regions and other specific site influences. Such monitoring may be carried out at a limited number of sites (‘local monitoring’), where exposure is greatest and intensive recording and data collection can take place. This would be particularly appropriate when it is envisaged that there will be a phased or gradual introduction of the GM plant into a limited number of regions in various EU Member States. The scale of the monitoring should be increased as the area and range of the GM plant expands, and the plant is grown in more regions. The monitoring should consist of the systematic recording of relevant parameters at representative locations where there is significant and repeated growing of the GM plant. This might also be defined according to the extent of the cultivation of the GM plant, the occurrence of targeted pest species or particular climatic/eco-regions. The methods selected, the duration of the monitoring, the extent or number of areas and the parameters to be monitored will be determined on a case-by-case basis. Whilst the planning and execution of

case-specific monitoring is under the applicant's responsibility, it may be appropriate for the applicant to involve public institutions to contribute to the agreed work.

4.4. General surveillance for unanticipated adverse effects

The objective of general surveillance is to identify the occurrence of unanticipated adverse effects of the GM plants or its use on human health or the environment that were not anticipated in the environmental risk assessment. General surveillance applies where no adverse effect has been identified in the ERA, but is always required in order to detect unanticipated adverse effects (EC, 2002). Monitoring of potential adverse cumulative long-term effects and areas of uncertainty identified in the ERA are important objectives of monitoring (EC, 2002) which should be considered initially within case-specific monitoring. When there is a negligible degree of uncertainty in the environmental risk assessment then no case-specific monitoring is indicated. However, general surveillance is always required for monitoring any unanticipated adverse effects.

An effect can be defined as an alteration that results in values that fall outside the normal range, given the variation due to the constant changes in the agricultural practices, rural environment and associated biota in the EU. A major challenge of general surveillance is determining whether:

- an unusual effect has been observed
- the effect is adverse and
- the adverse effect is associated with the GM plant or its cultivation.

The use of a range of monitoring systems to supply data and the ability to compare data from these different sources will help to indicate whether an effect is unusual and adverse. The identification of an adverse effect which is potentially linked to specific GM plants would trigger the need for a specific study to evaluate harm and determine cause.

An objective of the Directive 2001/18/EC (EC, 2001) is to protect the environment including biodiversity, water and soil. The EFSA GMO Panel is of the opinion that one important task within general surveillance is to link monitoring to these environmental protection goals. Recently, EU Directive 2004/35/EC on environmental liability with regard to the prevention and remedying of environmental damage (EC, 2004) defined environmental damage as a measurable adverse change in a natural resource or measurable impairment of a natural resource service which may occur directly or indirectly.

Within a broader concept of environmental issues, unanticipated adverse effects on human health have also to be addressed in the monitoring plan presented by the applicant. The scope of monitoring for unanticipated adverse effects on human health is defined, according to Directive 2001/18/EC, as monitoring for unanticipated adverse effects that may result from handling of the GM plant.

It might prove very difficult to design monitoring (including general surveillance) for unanticipated adverse effects on human health. However, knowing that the release of GM plants needs to be considered in context of their interaction with other environmental components, monitoring for health effects could be considered in conjunction with human population screening methods currently used by public health organisations (for assessing such elements as incidences of allergic reactions) and as part of the suggested plant production and farm questionnaires.

4.4.1. Approach and principles of general surveillance

Applications concerning food/feed uses and import and processing do not require scientific information on possible environmental effects associated with the cultivation of the plant. The extent of general surveillance for these GM plants will depend on the level of environmental exposure. Therefore the EFSA GMO Panel differentiates between general surveillance plans as part of applications for import/processing and applications for cultivation.

4.4.1.1. Approach and principles for GM plants intended for import and processing only

General surveillance plans as part of applications for import and processing will need to take account of the modified characteristics specific to the GM plants in question, their intended use and the receiving environment(s) (EC, 2002). The extent of the general surveillance plan will depend on the level of environmental exposure, the establishment, persistence and spread of the GM plant and does not require scientific information on possible environmental effects associated with the cultivation of the plant. The applicant has to show that environmental exposure will be at levels or in a form that does not present a risk to other living organisms or the abiotic environment (see chapter 3.6).

In the case of non-viable GM material (e.g. derived products not containing any living GMOs) and according to Directive 2001/18/EC, the applicant does not have to provide any environmental monitoring plan (including general surveillance).

In the case of imported GM products containing viable propagating material, general surveillance plans should consider that if substantial loss, spillage and establishment is possible, appropriate management systems should be in place to restrict environmental exposure.

4.4.1.2. Approach and principles for GM plants intended for cultivation

General surveillance plans as part of applications for cultivation will need to take account of the full environmental effects of the GM plant including its cultivation.

The EFSA GMO Panel is of the opinion that general surveillance is a general overseeing of the geographical regions where GM plants are grown without having any specific hypothesis on adverse effects on human health or the environment. As general surveillance is not hypothesis-driven, it is not conducted using directed experimental approaches (see also ACRE, 2004, Sanvido, 2005). However, robust scientific methodology should be applied wherever possible in order to evaluate empirical knowledge. This especially refers to defining sample sizes, sampling and recording methods, in order to produce statistically valid data for determining causes and effects.

Existing surveillance systems should be used where practical (e.g. routine farm recording systems) and any ‘unusual’ effect, not occurring in similar situations within conventional cropping, should be recorded (e.g. effects on soil).

The establishment, persistence and spread of a GM plant is not an environmental hazard in itself. Similarly, dispersal of pollen and seeds and gene flow per se are not environmental hazards and thus the focus of general surveillance should be on recording any unanticipated consequences of the cultivation of the GM plant, such as unforeseen weediness, invasiveness or changes in plant population dynamics or populations of biota associated with the GM plants. However, an unanticipated adverse effect is most likely to occur where the level of environmental exposure is highest. Thus, an evaluation of how and where the GM plant will be grown and the associated environmental exposure is considered a good starting point in any general surveillance plan.

General surveillance of the impact of GM plant should

- be applicable, in a proportionate and cost-effective manner, for monitoring the GM plant in a range of representative environments, reflecting the range and distribution of farming and environments exposed to the GM plants and its cultivation. If unusual effects on human health or the environment are reported, more focussed in-depth studies should be carried out in order to determine cause and relationship with GM plants. Such additional studies would be case-specific monitoring studies as they would require an experimental approach to confirm the specific hypothesis that an observed effect is associated with the GM plant,

- complement available general environmental monitoring. The higher the ecological integration and scale (from the individual to a population, from single farm to regions) the more difficult it is to distinguish potential effects of the GM plants from other factors. Initially, general surveillance should focus on each event individually. Additionally, when several GM plants have been commercialised, the interactions between these GM plants and their management may need to be considered where appropriate.

4.4.2. Main elements of general surveillance

The applicant should:

- define the methods and approaches that will be used to conduct general surveillance of regions where the GM plant occurs,

- refer to introduction, stewardship and exploitation plans for the GM plant, and

- make proposals for the time period, area covered, and the frequency of monitoring.

4.4.2.1. Existing monitoring systems

Applicants will have developed plans for the introduction, marketing, management and stewardship of the GM plant. The EFSA GMO Panel is of the opinion that applicants should include these into the monitoring plans, where appropriate, as they will contain some data of relevance to the implementation of the monitoring plan.

General surveillance should, when compatible, make use of established routine surveillance practices such as monitoring of agricultural plants, variety/seed registration, plant protection, plant health and soil surveys as well as ecological monitoring and environmental observations (EC, 2002).

Many of the existing monitoring systems and networks collecting environmental data are unlikely to always provide data of relevance that may be used in monitoring impacts of GM plants. The design of the existing monitoring programs, the targets (e.g. birds, plant protection, etc.), the time, frequency and scale of data collection, sampling, analysis and reporting methods may not suit the monitoring of GM plants because they have been designed for other purposes. Moreover, the existing monitoring systems will differ from country to country and it may not be feasible or practicable to modify existing surveillance systems in order to make them suitable for general surveillance of GM plants. Thus applicants may not consider existing networks to be sufficiently useful sources of information for monitoring. There may be a need for additional environmental surveys and to amend the monitoring objectives of existing monitoring systems (see also Sanvido, 2005).

Because existing monitoring systems can be of variable quality and consistency, it is important that the consistency and reliability of surveys utilised in general surveillance is evaluated in order to ensure long-term coherence and reliability of data collection and data quality. In addition, as environmental surveys will differ between networks, methods for integrating data from different origins should be evaluated.

Knowing the limitations of existing monitoring systems, it is important for the applicant to describe the processes and criteria that will be used for selecting and evaluating existing monitoring systems for supplying data related to the unanticipated adverse effects of GM plants in the general surveillance.

Specifically the applicant should

- describe which observations could be monitored through existing monitoring schemes,
- identify the type of existing monitoring systems that would be appropriate for this in the countries where the GM plant will be grown (e.g. monitoring of agricultural cultivars and plant protection surveys),
- describe the criteria and generic approach used to evaluate existing monitoring networks and how appropriate networks will be selected,
- describe how arrangements for collecting, collating and analysing data will be made,
- identify which category of additional surveys could be required to contribute to the general surveillance (e.g. public institutions, farm associations) in selected regions or Member States,
- describe how formal agreements, procedures and communication will be established with the Commission and Member States or other third parties before commercial market introduction, although detailed arrangements may not have been agreed at the time of the application.

According to Council Decision 2002/811/EC the responsibility for each step in the monitoring plan should be clearly assigned by the applicant. Where third parties are employed or contracted to conduct monitoring studies, the nature of their involvement should be detailed.

4.4.2.2. Use of GM plant-focussed monitoring systems

In addition to using existing monitoring systems, applicants are encouraged to develop new and more focused monitoring systems especially at the production level. Questionnaires, directed at farms where GM plants are grown, are considered a useful method to collecting first hand data on the performance and impact of a GM plant and for comparing it with conventional plants (ACRE, 2004, Wilhelm, 2004a,b, Sanvido, 2005). Experience from other established surveillance and monitoring systems (e.g. the approach used for consumer and pharmaceutical surveillance systems) could be used in designing questionnaires. Special emphasis should be given to the statistical design of such questionnaires. Issues of human health (e.g. due to exposure and handling of GM plants) may also be integrated into farm questionnaires.

As appropriate, the applicants should

- inform growers, seed suppliers or other stakeholders about the GM plant and the need to supply data on seed sales, areas sown, plant management, etc.
- be pro-active in developing reporting systems so that farmers (or their agents and advisors) intending to purchase genetically modified seeds will be fully informed about the GM plant, the importance of the monitoring programme and the reporting of unanticipated effects during and after the cultivation of the GM plant,
- describe the number of farmers/growers involved, the area covered, the reporting methods and the suitability of the data collected for statistical analysis,
- establish independent audits to ensure the independence and integrity of all monitoring data,

- indicate the likely frequency of inspections.

Farm questionnaires should

- be designed to ensure the statistical validity and representativeness of the collected data, including the proportion of fields growing the GM plant in a region and the number of questionnaires required to achieve statistical power in the data collected,
- be designed to generate data on the agronomic management of GM plants as well as data on impacts on farming systems and the farm environment,
- use a field or group of fields growing the GM plant as the basic unit for monitoring,
- observe the field/fields in subsequent years for any unusual residual effects,
- be user friendly but also information rich,
- be constructed to encourage independent and objective responses from farmers, land managers and others involved with the GM plant or its products.

Questionnaires adapted to agronomists or other stakeholders working on the farms growing the GM plants may also be useful sources of information. Focussed questionnaires and interviews are generally accepted by respondents. Professional interviewers may be an additional help.

Examples of farm questionnaires have been developed by Wilhelm et al., (Wilhelm, 2004a,b) and some farm questionnaires have already been assessed by the EFSA GMO Panel.

Farm questionnaires should be distributed, completed and collated annually via an arranged reporting system (e.g. farm questionnaire forms or online systems). These should be analysed by the applicant and reports submitted at the agreed time intervals (usually annually) to appropriate Competent Authorities. The results of the farm questionnaires will allow the applicant to record the implementation of recommended management and stewardship of the GM plant (e.g. good agricultural practice, hazard analyses, critical point compliance) and to identify unanticipated adverse effects.

4.4.3. Importance of a baseline

There is a need for general surveillance plans using both existing and novel monitoring systems to be able to compare impacts of GM plants and their cultivation with those of conventional plants. The baseline is the current status quo e.g. current conventional cropping or historical agricultural or environmental data. Direct comparison with non-GM plant reference areas should be used if available, but reference can also be made to the historical knowledge and experiences of the ”observer” (e.g. farmers, inspectors, wildlife surveyors) in relation to the situation prior to the introduction of the GM plant . It will be important to inform observers to report any unusual events and not to attempt to anticipate impacts.

There is also a need to take into account the fact that the GM event will occur in a changing genetic background of new varieties which may have an impact independent of the GM event and thus it is the event that needs to be monitored in any variety.

4.4.4. Data quality, management and statistical analyses

The design of the monitoring programme will influence the quality and usefulness of resulting data, hence efforts should be made to ensure that data from all the monitoring systems used can be

statistically analysed (Wilhelm, 2003, Wilhelm, 2004a,b). Meta-analyses of different datasets might be useful. If relationships between datasets can be identified, it will contribute to the credibility of monitoring.

The general surveillance plan should

- take account of the scale of commercialisation as well as the historical baseline knowledge in different areas to be monitored,
- consider the geographical areas to be studied and which existing environmental monitoring programmes could be useful for inclusion,
- consider national cultivation registers of GM plants (including co-existence measures) as they can provide useful data,
- describe the generic approach used for data collection, management and exploitation within general surveillance (e.g. data from existing networks and questionnaires),
- describe how any unusual adverse effects related to GM plants will be identified, including details of the statistical approach,
- include a comprehensive description of the techniques to be used for data analysis and statistical analysis, including the requirements for statistical significance,
- provide a detailed description of the operational handling of data from different sources into a 'general surveillance database',
- describe the approach to categorise the data (e.g. influencing factor, monitoring character) and the method for pooling the results and matching them with data on GM cultivation in time and space,
- contain data from Case-Specific Monitoring that might complement the general surveillance data.

4.5. Reporting the results of monitoring

Following the placing on the market of a GM plant, the applicant has a legal obligation to ensure that monitoring and reporting are carried out according to the conditions specified in the consent. The applicant is responsible for submitting the monitoring reports to the Commission, the competent authorities of the Member States, and where appropriate to EFSA. Applicants should describe the methods, frequency and timing of reporting in their monitoring plan.

Although no timeframe for reporting is specified in Council Decision 2002/811/EC (EC, 2002), reports, allowing for case-specific adaptations, preferably should be submitted

- annually confirming that monitoring has been carried out according to the given consent together with a summary of major preliminary results that are important for a short-term feedback on the ERA ('annual reports'), and
- periodically (e.g. every third year) covering longer periods in which observations and data collected are reported and analysed in detail and which therefore provide more comprehensive reports that are important for a longer term feedback on the ERA ('comprehensive report').

The comprehensive monitoring report should include in more detail the results of any relevant monitoring by third parties, including the farmers/growers, seed companies, independent surveyors, local, regional and national environmental surveyors. In addition, the applicant should evaluate these results and incorporate full analysis and conclusions in the submitted monitoring report. If appropriate, the applicant should provide access to raw data for stimulating scientific exchange and co-operation.

Flow of information on the cultivation of GM plants:

Where GM plants are grown the following procedures should be complied with:

- All GM seeds must be labelled with the variety, and should also contain information on the construct, the supplier's name and address, full instructions on any specific cultivation requirements, and reporting procedures for any incidents, including the address of the Consent Holder for the marketing of the seeds.

- The farmer/grower is required to declare the variety, sowing date, amount of cultivated plants and exact geographic location to the national cultivation register according to Directive 2001/18/EC - Art 31 (3b).

- The farmer should record all relevant cropping and management data for that GM plant and these data should be available for inspection.

Flow of information in instances where GM plants are thought to have caused unusual or adverse effects:

If adverse effects have been detected in areas where GM plants are grown or where there is a suspicion that the GM plants may be associated with an incident, the following procedures should be complied with:

- Farmers should follow the procedure for reporting established by the applicant at the time of purchase of the GM seeds and provide information to the information point specified therein of any unusual observations without delay.

- The applicant should immediately take the measures necessary to protect human health and the environment, and inform the competent authority thereof. In addition, the applicant should revise the information and conditions specified in the application.

- The applicant may inform external organisations (e.g. public institutions), asking them to immediately communicate any adverse effects they may detect to a specified information point.

- The applicant could carry out a preliminary examination in order to verify whether a GM plant-related effect has really occurred and provide the competent authority with a report on the result of its preliminary investigations, including an assessment of potential harm.

- If information becomes available to the competent authority which could have consequences for the risks of the GM plant(s) to human health or the environment it should immediately forward the information to the Commission and the competent authorities of the Member States.

Where adverse effects on the environment are observed, further assessment should be considered to establish whether they are a consequence of the GM plant or its use, as such effects may be the result of environmental factors other than the placing on the market of the GM plant in question. The competent authority should inform the Commission of the reported observation and, together with the applicant and scientific institutions or experts investigate the causes and consequences of the reported incident. The competent authority should submit a report to the Commission and EFSA on the extent

of any environmental damage, remedial measures taken, liability and recommendations for the future use/management of the GM plant.

4.6. Review and adaptation

Monitoring plans should not be viewed as static. It is fundamental that the monitoring plan and associated methodology are reviewed at appropriate intervals and may need to be modified and adapted depending on the results of the monitoring information collected. The monitoring plan might also be adapted based on an assessment of the appropriateness and cost effectiveness of the monitoring plan. Implementation of the revised monitoring plan remains the responsibility of the applicant unless otherwise determined by the competent authority.

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APPENDICES

A. BACKGROUND INFORMATION FOR THE GEOGRAPHICAL ZONES IN THE RECEIVING ENVIRONMENT(S) IN EUROPE

The EFSA GMO Panel GD on ERA of GM plants gives special emphasis on the receiving environment(s) (see chapter 2.3.4).

With reference to natural diversity, a broad range of various environments in terms of their flora and fauna, climatic conditions, habitat composition and ecosystem functions and human interventions occurs in the EU. Accordingly, GM plants will potentially interact with those differing environments. Here we give a short overview of the geographical regions and zoning concepts defined for various purposes and we discuss how these can be utilized in the framework of ERA of GM plants.

The EFSA GMO Panel considered the following existing concepts of geographical regions/zoning in Europe:

a. Plant protection product registration-based zoning

According to the new Regulation on Plant Production Product (Regulation (EC) No 1107/2009), concerning the placing of plant protection products on the market (and repealing Council Directives 79/117/EEC and 91/414/EEC), approvals for these products would be granted by geographical zones in the EU. Zones are defined as areas where agricultural, plant health and environmental (including climatic) conditions are comparable. For this purpose, 3 geographical zones have been defined to cover Europe:

Zone A = North: Denmark, Estonia, Latvia, Lithuania, Finland and Sweden;

Zone B = Centre: Belgium, Czech Republic, Germany, Ireland, Luxembourg, Hungary, the Netherlands, Austria, Poland, Romania, Slovenia, Slovakia and the United Kingdom;

Zone C = South: Bulgaria, Greece, Spain, France, Italy, Cyprus, Malta and Portugal.

The zoning for placing plant protection products on the market offers valuable information for applicants on registered pesticides in 3 zones in Europe that could be considered when ERA of GM plants are put in cultivation, production practice context.

b. Phytogeographic zoning

This zoning concept subdivides the Circumboreal region (Eurasia and North America) into a number of floristic provinces. Three provinces (Atlantic, Central European, Illyrian) cover vast majority of the area of Member States within the European Union. However, some parts of several Member States belong to more than one provinces (such as Arctic, Euxinian, Eastern European, Northern European provinces). This zoning defines provinces based on natural distribution of plant species.

However, this zoning focuses on floristic characteristics but does not cover and reflect differences arising from agricultural activities or cultivation.

c. Natura 2000

Natura 2000 is a network of special protected areas within the European Union. It covers the special areas of conservation under the Habitats Directive and special protection areas under the Birds Directive. Natura 2000 areas are areas of importance to the Community that have been designated by MS of the EU.

The Natura 2000 concept proposes 9 biogeographical regions (across 27 Member States) for covering the European ecological diversity. These biogeographical units are differentiated into the following regions: Alpine; Atlantic; Black Sea; Boreal; Continental; Macaronesian; Mediterranean; Pannonian; and Steppic Region.

However, this zoning focuses on habitat and species protection but does not cover and reflect differences arising from agricultural activities or cultivation.

The Indicative Map of European Biogeographical Regions (Natura 2000 or its modified version), was developed with the purpose of defining in practice the biogeographical regions mentioned in Art.1 c) (iii) of Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and wild fauna and flora (ETC/BD, 2006). Consequently this map and the information on 9 biogeographical regions may offer specific information for the ERA but are not related to plants being cultivated in production systems in Europe.

d. Seamless SEAMLESS zoning approach

Since GM plant deployment will primarily (but not exclusively) be linked to agricultural areas and within these to arable fields and any nearby semi-natural habitats, an agriculture land use oriented zoning that also considers the differing regional quality for crop plant cultivation would reflect a more practical approach for GM plant risk assessments. Therefore, a zonation like that recently proposed in the SEAMLESS research project (van Ittersum et al., 2008) could be more relevant for the ERA of GM plant for cultivation purpose. Undoubtedly, geographical zones based on a scientific rationale, taking into account realistic agricultural situations, will be more valuable for GM risk assessment.

In the agriculture-environment oriented zonation generated by the SEAMLESS project, the current EU territory is – based on upscaling of farm-scale and physical data by a statistical approach – differentiated into 12 environmental zones. According to this approach, EU territory is differentiated into the following Zones: Boreal; Nemoral; northern Atlantic; central Atlantic; Lusitanian; Continental; Pannonian; northern Alpine; southern Alpine; mountainous Mediterranea; northern Mediterranea; and southern Mediterranea.

The selected regions according to this zonation are characterised not just in relation to climatic factors, but also to agricultural aspects such as preferred farming types.

Each of the above examples for various zonings reflects the main goal (e.g. flora, habitat and species conservation, economically relevant pesticide approval, more integrated agricultural and environmental modelling system) of the given zonation but can not be automatically transferred to the risk assessment of GM plants. For instance, the continental zone – also according to the SEAMLESS zonation – covers about 20 % of the EU surface, but even within these biogeographical (or agro-ecological) regions, there will be significant differences in NTO species, habitat characteristics, flora and fauna of these habitats.

e. LANMAP

Mücher et al. (2010) have developed a new hierarchical European Landscape Classification that can be used as a framework for, e.g. indicator reporting and environmental sampling. Landscapes are ecological meaningful units where many processes and components interact. And as such, landscapes themselves have resulted from long-term interactions of natural abiotic, biotic and anthropogenic processes. The authors argue that a good understanding of landscapes is essential for its assessment, protection, management and planning. An internationally consistent approach is proposed to be obligatory and the production of landscape classifications and associated maps should be an important tool in this context. Although intuitive maps are available there are no consistent quantitative maps of European landscapes. In Mücher et al (2010), landscapes are regarded as forming recognizable parts of the earth's surface and as showing a characteristic ordering of elements. It is argued that the complex nature of the underlying scientific concepts, which sometimes overlap and conflict, requires an objective and consistent methodology. As there are many regional differences in landscape properties, it is crucial to strike the right balance between reducing the inherent complexity and maintaining an adequate level of detail. Against this background, a European Landscape Map (LANMAP) has been produced, making use of available segmentation and classification techniques on high-resolution spatial data sets. LANMAP is a landscape classification of Pan-Europe with four hierarchical levels; using digital data on climate, altitude, parent (geologic) material and land use as determinant factors; and has as many as 350 landscape types at the most detailed level. According to Mücher et al. (2010) LANMAP is thus far limited to a biophysical approach, since there is a lack of consistent and European-wide data on cultural–historical factors.

B. CONSIDERATIONS FOR LONG-TERM EFFECTS

Changing standards and comparators

The main difficulty in predicting when long-term effects are likely to occur is that the habitat itself is subject to repeated major change over a timescale of several years to several decades due to climatic shifts, soil degradation, plant varietal improvement, agronomic innovation and other factors The choice of comparator is therefore crucial (EFSA, 2006b, 2007, 2009f,e). Moreover, the concept of the familiar comparator, which might be satisfied by a non-GM plant that has already been shown through long practice to be safe as food or animal feed, may be inappropriate for some ecological effects, especially where a prevalent, conventional production system is not environmentally safe or sustainable. Modern systems of intense cultivation are often driving down (for example) soil carbon, plant populations and food webs. A new system that is simply comparable to the existing one might not be judged safe therefore. While criteria for healthy soils and food webs, or for minimal external impacts of agriculture, are currently being developed (e.g. Krogh and Griffiths, 2007), as yet there are no widely accepted ecological criteria that might be used as standards in risk assessment.

Much of the difficulty in defining criteria is that the existing dynamics (the rate and extent of change) operate at a scale not much longer than that required to establish even some short term impacts. Some changes to the system (such as the move from spring to winter cropping in some regions) extend over one to several decades depending on a local response to markets and policy. Oilseed rape had been grown for many centuries in Europe before the breeding of varieties for food brought about the large increases in sown area throughout northern and central Europe in the 1970s. Other changes can be quite abrupt, such as the increase in set aside from zero to 5-10% of the arable surface in one or two years in the mid 1990s and the slightly slower but proportionately greater rise in the general (*i.e.* non-GM) use of the herbicide glyphosate within the 1990s. The effects of these changes have not been documented, and such effects need to be understood before differences due to GM plant cultivation after commercialisation would be considered important or not.

The main feature of any new methodology for assessing long-term effects is that it would concentrate on the system as defined by biophysical and economic criteria. The main aim of assessment would be to define whether the GM plant cultivation is likely to affect any of the main variables in the system above the existing 'noise' and relative to the existing dynamical variation. And if the existing comparator is considered ecologically undesired, then the assessment can consider whether GM plant cultivation will move the system at least in the direction of an ecologically more desired state.

Development of and recovery from long-term effects

One of the aims of problem formulation in chapters 3.1 to 3.6 is to define ranges in which an indicator or process can fluctuate without an adverse effect occurring. If the GM plant displaces the function outside this range, it will deteriorate, in some instances gradually and in others first gradually then suddenly. The most well documented and perhaps most relevant example of decline in function is that caused by severe depletion of the arable seedbank and emerged weed flora, well beyond the point where the weeds that emerge limit crop yield. The positive functions provided by the seedbank are the provision of plant biodiversity in farmland and support of the food web, including pollinators and natural enemies of pests. Primary (simple) and secondary (complex) effects can be envisaged. Sustained, intensive cropping (which GM herbicide tolerant break crops might exacerbate), will cause the primary effect – a gradual decline in the seedbank, eventually after several decades, to the point of zero ecological function. Effects on the flora are likely to be found in the year of cultivation, and might be carried over to the subsequent one or two years for some variables. They might then disappear until the next time the GM herbicide tolerant plant is grown. Over several cultivation sequences, the effects are likely to accumulate. A primary effect of this type should be expected from

existing data. In experiments where re-seeding was reduced to zero, a logarithmic decline in the seedbank was measurable and change detected after only two years (see references to seedbank data from the 1920s and 1950s in Squire et al., 2003). Indeed, the analysis of this previous data informed the seedbank studies in the Farm Scale Evaluations of GM herbicide tolerant crops. If similar effects are expected from a new GM plant, they should be detectable in field plots over several years. Moreover, the downward trend in function can to a large degree be reversed in a further few years, if it has not gone too far, by small changes to the cropping sequence and weed management.

The primary effect will lead to secondary effects through loss of habitat and food for the invertebrates and vertebrates dependent on the plants. Such secondary effects on distributed food web organisms are spatially complex and cannot be determined in small experimental plots, however. Depletion of function might occur gradually at first, but there may come a point when the function ceases, for example if food plants become so low in abundance that the dependent animal populations decline and finally collapse In this case, the loss of function might not be readily reversible. If the decline occurs over a wide area of the landscape, recolonisation might be very slow.

Experience has shown that it is certainly possible to detect effects on biophysical variables such as components of the food web above the background noise through monitoring with adequate intensity. The spread of GM plant cultivation following commercialisation to cover greater parts of the landscape brings additional problems of analysis to those required to assess repeated use of a GM plant in one site. For example agricultural fields each tend to have a different biota drawn from the 'pool' of species or functional types in the total receiving environment. If, a GM plant would tend to reduce the overall abundance of plants or invertebrates or (for whatever reason) reduce the abundance of the least common types or species, then differences between GM and non-GM treatments, though possibly very small at each site, would 'accumulate' over the landscape when assessed through presence or absence of the uncommon species (Squire et al., 2009).

The question is whether GM plant cultivation would cause any such spatial effects to occur more so than existing cultivation. An assessment needs estimates from existing distribution patterns of organisms. If these are not available, modelling of population dynamics and trophic interactions may be used to explore scenarios in GM plant cultivation. In examples to date, the outcome of modelling has been shown to differ, even in direction, depending on the input variables, the characteristics of the model's domain and the questions asked. To gain greater credibility in GM risk assessment, impact models need to be assessed against hard, comparative data. Nevertheless, modelling should continue to be developed and adapted for use in future impact studies.

Examples

Two hypothetical examples are given to illustrate potential approaches to estimating long-term effects. The first is for a GM insect resistant plant, resistant to lepidopteran pests. The assessment of categories 3.1 to 3.6 will probably indicate non-target effects on invertebrates as a potential adverse effect in the problem formulation. Reference can be made to field trials in Europe, mostly on Bt maize including those within the ECOGEN project (Krogh and Griffiths, 2007) and others in the ten years of assessment on commercial fields in Spain (Farinos et al., 2004, de la Poza et al., 2005, de la Poza et al., 2008, Farinos et al., 2008), that found certain effects of Bt maize, some positive, some negative, on various microorganisms and invertebrates, but effects that were much less than those observed to routine influences such as tillage, choice of variety and insecticide usage. The same studies showed that some non-target organisms ingested the Bt protein, generally without apparent adverse effect, and that data for certain insects was lacking or incomplete. The main potential long-term effect that might have been observed was the development of resistance to a pesticide by an arthropod pest. Development of resistance can be anticipated from prior experience with non-GM plants and can be reduced to a degree by measures such as the use of refuges of non-GM plant. The risk assessment might therefore indicate that risks to non-target organisms of the Bt crop designed against lepidopteran

pests are likely to be low, but because of the uncertainties, monitoring should nevertheless be carried out. The monitoring plan might lay down how the possible effects of Bt maize can be judged in relation to contemporaneous effects of other agricultural change in the receiving environments.

The second example is for a GM herbicide tolerant plant. The assessment of specific risk in 3.5 should have indicated where a possible long-term environmental effect might occur in the receiving environments stated. Areas of concern might be indirect effects of further reductions in the weed flora on arable food webs (where these have already been reduced) and changes to the profile of pesticide residues in soil and watercourses. Information on the current state, sensitivity and ecological importance of these indicators can be obtained from the literature. The results of field experiments on HT crops should be scrutinised for any unforeseen effects. They should reveal that not all effects of herbicides were apparent during early, small scale field testing or nor in commercial growing in other parts of the world. In the UK's GM plant trials on winter oilseed rape (Hawes et al., 2003, Bohan et al., 2005, Squire et al., 2009), the cultivation practice was found to increase grass weeds (competitive to cereals) and decrease broadleaf weeds (supporting the food web), a generally negative response. This unforeseen response was most likely due to the increased complexity of the context of the receiving environment – a combination of weather, the agronomy of crop, the timing of operations and the specificity of the herbicide. It might not be detectable above the 'noise' during initial field trials, but the response would be cumulative over time and space, increasing grass weeds and reducing food web annuals every time the crop was grown in a field. On the basis of this evidence, monitoring of any new HT crop should be planned to include assessment of weed shifts and an estimate of the longer term impacts on the weed flora and food webs based on the likely frequency of use of the GM HT plant in the rotation and its occupancy in the landscape. There is also experimental evidence that the specific agronomy of an HT plant can in some cases be altered so as to gain the intended effect of the practice on yield with little detriment to the food web (May et al., 2005). As for the example of Bt maize the monitoring plan might lay down how the possible effects of the HT management might be assessed in relation to other contemporaneous change.

Conclusions

Long-term effects of GM plant cultivation (or any other innovation) are not likely to be revealed in highly constrained experimental systems, while the assessment of long-term impacts in the field are hampered by incomplete knowledge of the dynamical states of arable ecosystems. Notably, criteria for environmentally safe and sustainable production systems have still to be fully defined by scientific consensus and used as a baseline in the assessment of long term effects. While moderate impacts of certain GM plants have been detected above the natural background variation (for example the effects of HT crops on arable plants and food webs), the long-term effects of any one GM plant may be difficult to measure given the comparatively short time-scale over which other large changes habitually occur in crop type, management and weather. In accordance with the BEETLE report (BEETLE_report, 2009), research studies, modelling and monitoring are appropriate tools to investigate long-term environmental effects during GMO cultivation close to practice[44]. To support PMEM, efforts should be raised for the development of indicators and databases for EU wide surveillance of long-term effects on soil and other biodiversity resulting from GM plant cultivation and management. More local potential indicators should be developed over time by risk assessors and risk managers. The indicators for environmental monitoring should be selected in accordance with the GM plant and trait and the receiving environment.

[44] 'Close to practice' means here the initial cultivation phase after first consent for placing on the market is given. It is in many cases a priori (epistemically) not possible to experimentally study long term effects related to large-scale cultivation.

GLOSSARY

Note: The definitions provided in this glossary are to be considered in the context of the EFSA guidance on ERA of GM plants and the scientific opinion on the assessment of potential impacts of GM plants on NTOs.

Assessment endpoint: is defined as a natural resource or natural resource service that needs protection. It is the valued attribute of a natural resource worth of protection (Suter, 2000).

Baseline: is defined as a point of reference against which future changes can be compared (EC, 2002).

Biogeographical region or zone: is defined as spatial scale of Earth's surface containing related biotic (e.g. fauna and flora) and abiotic (e.g. climate, soil, or elevation) conditions.

Case-by-case: is defined as the approach by which the required information may vary depending on the type of the GMOs concerned, their intended use and potential receiving environment, taking into account i.a. GMOs already in the environment (EC, 2001).

Deliberate release: is defined as any intentional introduction into the environment of a GMO or a combination of GMOs for which no specific containment measures are used to limit their contact with and to provide a high level of safety for the general population and the environment (EC, 2001).

Desk study: is defined as an investigation of relevant available information, often before starting practical study of a problem.

Ecosystem services: include all services provided by ecosystems, e.g. production of food, fuel, fibre and medicines, regulation of water, air and climate, maintenance of soil fertility, cycling of nutrients. Ecosystems services are distinct from ecosystem functions by virtue of the fact that humans, rather than other species, benefit directly from these natural assets and processes (Millennium Ecosystem Assessnment, 2005).

Effects:

Adverse effects: are defined as a harmful and undesired effects consisting of measurable changes of protected entities (e.g. change in a natural resource or measurable impairment of a natural resource service) beyond accepted ranges.

Unintended effects: are defined as consistent differences between the GM plant and its conventional counterpart, which go beyond the primary intended effect(s) introducing the target gene(s).

Direct effects: are defined as primary effects on human health or the environment which are a result of the GMO itself and which do not occur through a causal chain of events (EC, 2001).

Indirect effects: are defined as to effects on human health or the environment occurring through a causal chain of events, through mechanisms such as interactions with other organisms, transfer of genetic material, or changes in use or management (EC, 2001).

Immediate effects: are defined as effects on human health or the environment which are observed during the period of the release of the GMO. Immediate effects may be direct or indirect (EC, 2001).

Delayed effects: are defined as effects on human health or the environment which may not be observed during the period of the release of the GMO, but become apparent as a direct or indirect effects either at a later stage or after termination of the release (EC, 2001).

Cumulative long-term effects: are defined as the accumulated effects of consents on human health and the environment, including flora and fauna, soil fertility, soil degradation of organic material, the feed/food chain, biological diversity, animal health and resistance problems in relation to antibiotics (EC, 2001).

Environmental harm: is defined as a measurable adverse change in a natural resource or measurable impairment of a natural resource service which may occur directly or indirectly (EC, 2004).

Environmental risk assessment: is defined as the evaluation of risks to human health and the environment, whether direct or indirect, immediate or delayed, which the deliberate release or the placing on the market of GMOs may pose and carried out in accordance with Annex II (EC, 2001).

Fitness: is defined as the number of seeds (or propagules) produced per seed sown, and includes the whole life cycle of the plant (Crawley et al., 1993). Enhanced fitness can be defined as a characteristic of an individual or sub-population of individuals that consistently contribute more offspring to the subsequent generation (Wilkinson and Tepfer, 2009).

Functional groups: are defined as non-phylogenetic, aggregated units of species sharing an important ecological characteristic and playing an equivalent role in the community (Cummins, 1974, Smith, 1997, Steneck, 2001, Blondel, 2003).

Genetically modified organism (GMO): is defined as an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination (EC, 2001).

Hazard (harmful characteristics)**:** is defined as the potential of an organism to cause harm to or adverse effects on human health and/or the environment (EC, 2002).

Limits of concern: are defined as the minimum ecological effects that are deemed biologically relevant and that are deemed of sufficient magnitude to cause harm. These limit, limits of concern are set for each assessment endpoint in the problem formulation.

Measurement endpoint: is defined as a quantifiable indicator of change in the assessment endpoint, and constitute measures of hazard and exposure. Examples include: fitness, growth, behaviour, development.

Problem formulation: is defined as the process including the identification of characteristics of the GM plant capable of causing potential adverse effects to the environment (hazards) of the nature of these effects, and of pathways of exposure through which the GM plant may adversely affect the environment (hazard identification). It also includes defining the assessment endpoints and setting of specific hypothesis to guide the generation and evaluation of data in the next risk assessment steps (hazard and exposure characterisation).

Production system: is defined as the specific use of the GM plant, the context in which the GM plant is grown, its cultivation (including crop rotation), harvesting and management, and the genetic background in which the transgenic trait has been introduced.

Protection goals: are defined as natural resources (e.g. arthropod natural enemies, bees) or natural resource services (e.g. regulation of arthropod pest populations, pollination) that are to be protected as set out by EU legislations.

Risk: is defined as the combination of the magnitude of the consequences of a hazard, if it occurs, and the likelihood that the consequences occur (EC, 2002).

Receiving environment: is defined as the environment into which the GM plant(s) will be released and into which the transgene(s) may spread.

Stacked events: are GM plants in which two or more single events have been combined by conventional crossing.

Step-by-step approach: is used in this ERA GD to describe the six assessment steps (1. Problem formulation; 2. Hazard characterisation; 3. Exposure characterisation; 4. Risk characterisation; 5. Risk management strategies and 6. Overall risk evaluation and conclusions) for the ERA. This assessment approach is different from the Stepwise approach defined hereunder.

Stepwise Approach: is defined as all the steps (used in the sense of 'containment-level') beginning with experiments in the contained use system through temporarily and spatially restricted deliberate release up to placing on the market, where data should be collected stepwise as early as possible during the procedure (EC, 2002).

Stressor: the GM plant itself, the transgene(s) in this organismal context and its products, are all considered as potential stressor.

Weight-of-evidence approach: is defined as the use of scientific evidence from various data sources to support assessment conclusions.