European Food Safety Authority

EFSA Journal 2015;13(6):4128

SCIENTIFIC OPINION

Guidance on the agronomic and phenotypic characterisation 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 agronomic and phenotypic characterisation of genetically modified (GM) plants and clarifies the EFSA GMO Panel's view on how agronomic and phenotypic data support the risk assessment of GM plants. Specific recommendations are given on (1) the selection of sites and test materials; (2) the quality and design of field trials; (3) the selection of relevant agronomic and phenotypic endpoints; and (4) data analysis. The guidance proposes a comprehensive and harmonised approach for the agronomic and phenotypic characterisation of GM plants, which should ensure the best use of agronomic and phenotypic data for the comparative analysis of GM plants and derived food and feed products, and for their food and feed and environmental risk assessment.

KEY WORDS

comparative analysis, field trials design, invasiveness, persistence, receiving environments, representativeness, unintended effects

[1] On request from EFSA, Question No EFSA-Q-2013-00606 adopted on 27 May 2015.

[2] Panel members: Salvatore Arpaia, Andrew Nicholas Edmund Birch, Andrew Chesson, Patrick du Jardin, Achim Gathmann, Jürgen Gropp, Lieve Herman, Hilde-Gunn Hoen-Sorteberg, Huw Jones, Jozsef Kiss, Gijs Kleter, Martinus Lovik, Antoine Messéan, Hanspeter Naegeli, Kaare Magne Nielsen, Jaroslava Ovesna, Joe Perry, Nils Rostoks, Christoph Tebbe. Correspondence: gmo@efsa.europa.eu.

[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on the agronomic and phenotypic characterisation of genetically modified plants, Salvatore Arpaia, Hans Christer Andersson, Paolo Bàrberi, Lammert Bastiaans, Tom de Jong, Thomas Frenzel, Jürgen Gropp, Huw Jones, Antoine Messéan, Joe Perry, Angelo Porta Puglia and Geoffrey Squire, and the hearing expert Jeremy Sweet, for the preparatory work on this scientific opinion, and EFSA staff, Hermann Broll, Andrea Gennaro and Claudia Paoletti, for the support provided to this scientific opinion.

Suggested citation: EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2015. Guidance on the agronomic and phenotypic characterisation of genetically modified plants. EFSA Journal 2015;13(6):4128, 44 pp. doi:10.2903/j.efsa.2015.4128

Available online: www.efsa.europa.eu/efsajournal

SUMMARY

The risk assessment of genetically modified (GM) plants starts with hazard identification, during which any characteristic of the GM plant potentially having adverse effects (hazards) on human and animal health and the environment is identified. A comparative approach, based on data derived from the molecular, compositional and agronomic/phenotypic characterisation of GM plants, is typically followed internationally to identify differences between the GM plant and its conventional counterpart. This approach enables an evaluation of the relevance of potential risks posed by a GM plant and derived food and feed products, by assessing whether intended and unintended differences alter the level of risk or give rise to additional risks.

This document provides guidance for the agronomic and phenotypic characterisation of GM plants, and is designed to assist applicants in the generation, analysis and interpretation of the agronomic and phenotypic dataset submitted as part of their GM plant applications under Directive 2001/18/EC or Regulation (EC) No 1829/2003. It supplements general recommendations on the agronomic and phenotypic characterisation of GM plants outlined in previous EFSA GMO Panel guidelines and Implementing Regulation (EU) No 503/2013.

The guidance clarifies the EFSA GMO Panel's view on how agronomic and phenotypic data support the risk assessment of GM plants. The agronomic and phenotypic characterisation aims (1) to detect and measure agronomic and phenotypic differences between the GM plant and its conventional counterpart; (2) to assess to what extent the environmental and agricultural conditions under which the GM plant can be grown affect these differences; (3) to confirm that good agricultural practices have been followed for the generation of the compositional data and to identify possible factors that might bias the outcomes of the comparative analysis; and (4) to support the risk characterisation of GM plants.

Specific recommendations are given on (1) the selection of test materials and sites; (2) the quality and design of field trials; (3) the selection of relevant agronomic and phenotypic endpoints; and (4) data analysis.

1. For the selection of the test material and sites, the EFSA GMO Panel advocates selecting suitable genetic test materials that are adapted to the range of typical receiving environments into which the GM event may be introduced, and the selection of representative site locations and management systems to carry out field trials in order to capture enough variability within the set of possible receiving environments in which the test materials can be grown. Applicants should supply information demonstrating the suitability of test materials and representativeness of sites.
2. To guarantee the reliability of the agronomic and phenotypic dataset, the use of high-quality test materials produced under similar environmental and management conditions is required. Data requirements are specified for the production conditions of the seeds sown, the identity of the starting materials, and seed health and viability.

   A comprehensive and accurate description of various aspects of the receiving environments (such as geographical location, agrometeorological data, soil characteristics, cropping history, post-harvest conditions and crop management practices) is requested for all GM plant applications, irrespective of their scope. This information enables an evaluation of the representativeness of the selected receiving environments and an assessment of the extent to which environmental and agricultural conditions affect potential differences between the GM crop and its conventional counterpart.

   Field trial designs should be described graphically in terms of the spatial distribution and size of blocks and plots within sites, the distances between them and the presence of buffer rows and outside border rows. Choices made should be justified based on biological characteristics and common agricultural practices for the crop species.

3. The selection of relevant agronomic and phenotypic endpoints to be considered should consider the objectives of the agronomic and phenotypic characterisation; the biology of the crop species of concern; the novel trait intentionally introduced into the GM plant; and the scope of the GM plant application. The final choice of which endpoints to examine should take into account the ability of the endpoint to identify biologically relevant differences between the GM plant and its comparator. The guidance makes a distinction between generic endpoints and case-specific endpoints. The generic endpoints contribute to several objectives of the agronomic and phenotypic studies and shall always be measured in accordance with the scope of the GM plant application. The case-specific endpoints are crop or trait related, and, for these endpoints, applicants can decide on a case-by-case basis whether those are to be considered further and shall then provide a scientific rationale justifying their inclusion or exclusion. For all observed endpoints, applicants shall document sampling representativeness and recording methods, where relevant.
4. Clarifications, providing specific requirements for the analysis of comparative data outlined in previous EFSA GMO Panel guidance documents, are offered. These clarifications focus on (a) the submission and presentation of data (including those gathered from discarded sites and/or replicates); (b) the statistical analysis of data when the test of equivalence cannot be carried out owing to zero or limited variance between the non-GM reference varieties; (c) the analysis of genotype × environment (G × E) interactions, which is mandatory in the case of significant differences and/or lack of equivalence; and (d) the analysis of the relationship between correlated endpoints when significant differences are observed in an endpoint that is biologically related to others. If significant G × E interactions are identified, then a per-site analysis should be carried out.

The guidance proposes a comprehensive and harmonised approach for the agronomic and phenotypic characterisation of GM plants, which should ensure the best use of agronomic and phenotypic data for the comparative analysis of GM plants and derived food and feed products, as well as for their food and feed and environmental risk assessment.

TABLE OF CONTENTS

BACKGROUND AS PROVIDED BY EFSA

Genetically modified organisms (GMOs) and derived food and feed products are subject to a risk assessment and regulatory approval before they can enter the market in the European Union (EU). In this process, the role of the European Food Safety Authority (EFSA) is to independently assess and scientifically advice risk managers on any possible risk that the use of GMOs may pose to human and animal health and the environment. EFSA's scientific advice is elaborated by its GMO Panel (referred to hereafter as EFSA GMO Panel) with the scientific support of specific working groups and EFSA scientists.

The main focus of EFSA in the field of GMOs lies in the evaluation of authorisation applications for the marketing of GMOs (referred to hereafter as GMO applications) and the development of risk assessment guidelines.

The EFSA GMO Panel developed several guidelines for the risk assessment of GMOs, as well as on specific aspects of their risk assessment (see Devos et al., 2014 for a comprehensive overview). These guidelines assist applicants in the preparation and presentation of their applications by describing elements and data requirements for the risk assessment of GMOs.

Key guidelines developed by the EFSA GMO Panel, and used for the evaluation of risk assessments supplied by applicants as part of their GM plant applications, are those on the risk assessment of food and feed from GM plants (EFSA GMO Panel, 2011a) and on the environmental risk assessment of GM plants (EFSA GMO Panel, 2010a).

- The EFSA GMO Panel guidelines on the risk assessment of food and feed from GM plants (EFSA GMO Panel, 2011a) outline the principles of the risk assessment of food and feed containing, consisting or produced from GM plants, and provide definitions of the different steps and objectives of the risk assessment process. These guidelines incorporate previously issued guidelines on specific aspects, such as: (1) the selection of comparators for the risk assessment of GM plants and derived food and feed (EFSA GMO Panel, 2011b); and (2) statistical considerations for the safety evaluation of GMOs (EFSA GMO Panel, 2010b).
- The EFSA GMO Panel guidelines on the environmental risk assessment of GM plants (EFSA GMO Panel, 2010a) describe the principles to follow when assessing potential effects of GM plants on the environment, and provide the scientific rationale underpinning the necessary data requirements for a comprehensive environmental risk assessment.

Although the abovementioned guidelines address the generation, analysis and interpretation of agronomic and phenotypic data used in support of the comparative assessments of GM plants, the EFSA GMO Panel and national risk assessment bodies have identified the need for further guidance, in order to provide a more comprehensive and harmonised approach for the agronomic and phenotypic characterisation of GM plants. This activity is in accordance with EFSA's mission and also with the Council decision (EU, 2008). Therefore, the EFSA GMO Panel decided to develop its guidelines on the agronomic and phenotypic characterisation of GM plants further.

EFSA established a new working group as part of a self-task activity, in order to supplement and specify the EFSA GMO Panel requirements for the agronomic and phenotypic characterisation of GM plants. During the development of these specific guidelines, EFSA consulted EU Member States and all relevant stakeholders via an online public consultation[4] as well as a technical workshop[5]. Relevant scientific comments received during these consultations have been considered when finalising the

[4] The draft guidance was released for public consultation on EFSA's website between 25 September 2014 and 6 November 2014.

[5] 18–19 December 2014 (Parma, Italy).

guidelines. Given the complexity of the topic and the large number of public comments received, the duration of the self-task mandate was extended from 19 March 2015 till the end of June 2015.

TERMS OF REFERENCE AS PROVIDED BY EFSA

The purpose of this assignment is to create a Working Group responsible for the development of detailed, harmonised guidance on the generation, analysis and interpretation of agronomic and phenotypic data to support the comparative analysis of GM plants.

ASSESSMENT

The EFSA GMO Panel regularly reviews its risk assessment guidelines in the light of experience gained, technological progress and scientific development, and it has identified the need to further develop its guidelines on the agronomic and phenotypic characterisation of genetically modified (GM) plants.

1. Introduction

GM plants and derived food and feed products are subject to a risk assessment and regulatory approval before entering the market in the European Union (EU). In this process, the European Food Safety Authority (EFSA) evaluates any risks that GM plants may pose to human and animal health and the environment. The safety assessment is performed in accordance with guidance documents developed by the EFSA Panel on Genetically Modified Organisms (EFSA GMO Panel) and with Directive 2001/18/EC[6], Regulation (EC) No 1829/2003[7] and the requirements laid down in Implementing Regulation (EU) No 503/2013[8].

The risk assessment of GM plants starts with hazard identification, during which any characteristic of the GM plant potentially causing adverse effects (hazards) on human and animal health and the environment is identified. These hazards can arise from the novel trait intentionally introduced in the GM plant. However, genetic engineering can also lead to unintended effects, which are not intended by the genetic modification and which could give rise to safety concerns.

A comparative approach, based on data derived from the molecular, compositional and agronomic and phenotypic characterisation of GM plants, is typically followed internationally to establish the occurrence of intended and unintended differences (OECD, 1993; USDA, 1996; FAO/WHO, 2000; Kok and Kuiper, 2003; MoE, 2004; Codex Alimentarius, 2009; OGTR, 2009; EFSA GMO Panel, 2010a, 2011a; Macdonald, 2012; Garcia-Alonso, 2013; Schnell et al., 2015). Thereby, the composition and phenotypic characteristics of the GM plant are compared with those of a conventional counterpart and other comparators cultivated under similar conditions[9]. If significant differences between the GM plant and its conventional counterpart are detected, then they are assessed for their potential to cause adverse effects on human and animal health and the environment. The underlying assumption of the comparative approach is that traditionally cultivated non-GM plants have a history of safe use for consumers and animals and familiarity for the environment.

The choice of which components and agronomic and phenotypic characteristics to examine is, in part, determined by the nature of the novel trait intentionally introduced into the GM plant, but it is generally broader to allow the identification of possible unintended effects of the genetic modification (OECD, 1993; Macdonald, 2012; Schnell et al., 2015).

[6] 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. OJ L 106, 17.4.2001, p. 1-38.

[7] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, 1-23.

[8] Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L 157, 8.6.2013, p. 1-48.

[9] In the European Union, the statistical evaluation of the comparative assessment requires the simultaneous application of two complementary tests: a test of difference, to identify possible differences between the GM plant and its appropriately selected non-GM conventional counterpart with a history of safe use (OECD, 1993); and a test of equivalence to assess whether the characteristics of the GM plant fall within the range of natural variation estimated from a set of conventional non-GM reference varieties (referred to hereafter as non-GM reference varieties) with a history of safe use (EFSA GMO Panel, 2010b, 2011a; van der Voet et al., 2011). Additional optional test materials can be included in the field trial design (i.e. null-segregant, conventional GM reference varieties) to support the interpretation of possible differences and or lack of equivalence.

Compositional and agronomic and phenotypic data are typically obtained from field trials performed at multiple sites under conditions that are representative of the receiving environments (REs) in which the GM plant can be grown (EFSA GMO Panel, 2011a; Implementing Regulation (EU) No 503/2013). The field trials can be conducted in a single year, or spread over multiple years (Implementing Regulation (EU) No 503/2013), and the conventional counterpart should be a suitable line with a genetic background similar to that of the specific GM line[10] assessed in the application (EFSA GMO Panel, 2011b). To put observed differences into the context of natural variation in plant characteristics, non-GM reference varieties must be included in the field trials, along with the GM line(s) and its conventional counterpart(s) (EFSA GMO Panel, 2011a; Implementing Regulation (EU) No 503/2013). This approach enables an evaluation of the relevance of potential risks posed by a GM plant and derived food and feed products, by assessing whether intended and unintended differences alter the level of risk or give rise to additional risks.

1.1. Relevance of agronomic and phenotypic data for the risk assessment of GM plants

During the public consultation, it became apparent that stakeholders have different views on the role that agronomic and phenotypic characterisation plays in the risk assessment of the GM plants. The current guidelines therefore clarify the EFSA GMO Panel's view on how agronomic and phenotypic data support the risk assessment of GM plants.

In line with internationally agreed standards for the risk assessment of GM plants, Implementing Regulation (EU) No 503/2013 states that "*the comparative analysis of composition and agronomic as well as phenotypic characteristics shall constitute, together with the molecular characterisation, the starting point to structure and conduct the risk assessment of a new genetically modified food. It shall aim at identifying similarities and differences: (a) in composition, agronomic performance and phenotypic characteristics (intended and unintended alterations) between the genetically modified plant and its conventional counterpart; (b) in composition between the genetically modified food and feed and its conventional counterpart*."

The main objectives of the agronomic and phenotypic characterisation are:

- to detect and measure agronomic and phenotypic differences between the GM plant and its conventional counterpart (whether intended or unintended);
- to assess to what extent the environmental and agricultural conditions under which the GM plant can be grown affect these differences.

Moreover, the agronomic and phenotypic dataset can contribute:

- to confirming that good agricultural practices have been followed in the generation of the compositional data and to identifying possible factors that might bias the outcomes of the comparative analysis;
- to the risk characterisation of GM plants.

1.1.1. Detecting intended and unintended differences

The agronomic and phenotypic characterisation is used internationally in the comparative analysis of GM plants to identify intended and unintended differences between the GM plant and its conventional counterpart. This requires a proper characterisation and description of the plant's biology and performance under representative environmental conditions.

[10] In some cases, in order to cover the range of receiving environments, more than one genetic background is used by applicants. For the definition of GM line see Section 2.1.

Typically, a range of plant characteristics (endpoints) are measured in the agronomic and phenotypic field trials, in order to characterise the plant's biology and performance and to allow for the identification of intended and unintended differences (Schnell et al., 2015). The agronomic and phenotypic characteristics examined cover plant vigour, growth and development, morphology, yield, crop characteristics, pest and disease susceptibility and fertility (e.g. Horak et al., 2007; Nickson, 2008; Garcia-Alonso, 2010; Raybould et al., 2010, 2012; Gray, 2012; Macdonald, 2012). Seed and pollen characteristics of the GM plant are also assessed but typically under controlled rather than open field conditions.

The selection of relevant agronomic and phenotypic endpoints to be considered should consider the objectives of the agronomic and phenotypic characterisation; the biology of the crop species of concern; the novel trait intentionally introduced into the GM plant; and the scope of the GM plant application. The final choice of which endpoints to examine should take into account the ability of the endpoint to identify biologically relevant differences between the GM plant and its comparator (OECD, 1993).

As there is often no generic hypothesis concerning the differences that can occur during the transformation process, the use of a range of standard characteristics, as typically measured by plant breeders and agronomists, can capture changes in the plant's biology and performance, which might be indicative of possible alterations related to safety (see Section 1.1.4 below) and is therefore recommended to allow for the identification of such differences.

The abovementioned rationale applies equally to GM plant applications for import/processing for food and feed uses, as well as for applications for cultivation. Imported plant products may also include viable plant propagules (e.g. seed, tubers). As the accidental release of viable plant parts in the EU may lead (in specific cases) to the establishment of feral plants, a characterisation of the whole GM plant, and not only of the imported parts, is required.

1.1.2. Assess to what extent differences depend on receiving environments

Another objective of the agronomic and phenotypic characterisation is to assess to what extent the environmental and agricultural conditions under which the GM plant can be grown affect the differences observed between the GM plant and its conventional counterpart. Indeed, as the GM plant is likely to be grown in REs differing from those tested to support the application, it is paramount to assess the extent to which different REs affect the characteristics of the GM plant. This is achieved by testing whether interactions between the GM plant and the selected REs for a minimum set of endpoints exists (see Section 5.3).

In some cases the differences between the test materials observed across sites in the compositional and agronomic and phenotypic characteristics could be interpreted by different expression levels of the newly expressed protein(s). Therefore, in those cases in which protein expression data could be informative for the interpretation of compositional data and/or agronomic and phenotypic characterisation of the GM plant, it is advisable to generate such data at appropriate time points and for appropriate tissues.

1.1.3. Confirm that field trials were conducted following good agricultural practices

The agronomic and phenotypic characterisation provides information on the quality of the field trials used to produce material for the compositional analyses when agronomic and phenotypic and compositional data are generated in the same field trials. This helps to identify potential confounding factors which may affect the results of the comparative analysis. Indeed, the underlying assumption of the comparative assessment is that observed differences should indicate changes linked to the genetic modification and not to other factors, such as heterogeneity of starting test material and/or bias in the conduct of trials. Therefore, the EFSA GMO Panel recommends that applicants generate compositional data in the same field trials as those performed for agronomic and phenotypic characterisation of GM plants.

1.1.4. Support to the risk characterisation of GM plants

According to Implementing Regulation (EU) No 503/2013, "*the comparative analysis of composition and agronomic as well as phenotypic characteristics shall constitute, together with the molecular characterisation, the starting point to structure and conduct the risk assessment of a new genetically modified food*". In that respect, differences and/or lack of equivalences in the agronomic and phenotypic characteristics of the GM plant may also be relevant to help assess food and feed safety. For example, changes in agronomic and phenotypic characteristics resulting from modifications of an endogenous metabolic pathway may require additional comprehensive comparative analyses and subsequent food and feed safety assessments[11,12].

Agronomic and phenotypic data may provide information relevant to the assessment of the persistence and invasiveness of some GM plants, as some intended and unintended differences may be associated with changes in the plant's biology and/or life cycle characteristics. Therefore, where considered relevant, agronomic and phenotypic data can be part of the weight of evidence that is used in the environmental risk assessment to evaluate whether or not the GM plant is likely to have significantly altered characteristics indicative of a change in persistence and invasiveness[13].

1.2. Objectives of the guidelines

This document provides guidance for the agronomic and phenotypic characterisation of GM plants, and is designed to assist applicants in the generation, analysis and interpretation of the agronomic and phenotypic dataset submitted as part of their GM plant applications in the framework of Directive 2001/18/EC and Regulation (EC) No 1829/2003. It supplements the general recommendations on the agronomic and phenotypic characterisation of GM plants outlined in the EFSA GMO Panel guidelines on the risk assessment of food and feed from GM plants (EFSA GMO Panel, 2011a), the environmental risk assessment of GM plants (EFSA GMO Panel, 2010a) and Implementing Regulation (EU) No 503/2013.

Based on an in-house analysis of the agronomic and phenotypic data recently supplied to EFSA by applicants as part of their GM plant applications, the EFSA GMO Panel identified aspects of the agronomic and phenotypic characterisation of GM plants that require attention. These include:

- the representativeness of the selected sites of field trials in order to ensure that "*the different sites selected for the field trials reflect the different meteorological and agronomic conditions under which the plant is to be grown*" (Implementing Regulation (EU) No 503/2013);
- the suitability of the selected test materials and their quality in order to support the identification of differences between the GM plant and other test materials;
- the experimental design of field trials and their description to allow a proper interpretation of the outcomes of the comparative assessment;
- the selection and description of relevant endpoints for the agronomic and phenotypic characterisation of GM plants;
- the analysis of the data gathered.

For the abovementioned aspects, specific recommendations are developed here in order to (1) provide a more comprehensive and harmonised approach for the agronomic and phenotypic characterisation of

[11] For example, a modified seed colour may result from complex changes in the GM plant's secondary metabolism.
[12] For example, a GM plant exhibiting altered pest resistance might have an altered level of an anti-nutrient—e.g. glucosinolates in oilseed rape—or toxicant (reviewed by Hopkins et al., 2009; Bjorkman et al., 2011), or another type of bioactive constituent (see also Section 4.2).
[13] For example, in the case of a GM oilseed rape, an increase in seed number (yield/seed weight) can have consequences on its persistence.

GM plants; and (2) ensure that the gathered agronomic and phenotypic data are useful for the food and feed and environmental risk assessments.

1.3. Scope of the guidance on the agronomic and phenotypic characterisation of GM plants

The guidelines apply to the most common plant species and trait combinations for which GM plant applications have been submitted so far in the frame of Directive 2001/18/EC and Regulation (EC) No 1829/2003. GM plant applications mostly cover maize, cotton, soybean and—to a lesser extent—oilseed rape, potato and sugar beet. The guidelines address both GM plants containing single events and those containing stacked events.

Although the scope of most GM plant applications submitted in the framework of Regulation (EC) No 1829/2003 is limited to import/processing for food and feed uses or for industrial uses and does not include cultivation in the EU, both scopes are covered here.

In the next decade, EFSA may be faced with a number of challenges that may require the adjustment and revision of its current risk assessment guidelines or the development of new risk assessment strategies. Therefore, the EFSA GMO Panel will continuously consider the possibility of complementing its guidelines with specific recommendations on additional plant species (e.g. perennials, trees), topics (e.g. sampling, with the aim of harmonising sampling approaches for generation of data in support of the risk assessment of GM plants[14]) and technological developments (e.g. phenotyping platforms), as needed.

1.4. Transitional period of the guidance on the agronomic and phenotypic characterisation of GM plants

The requirements laid down in the current guidance affect several steps in the implementation of field trials, including the planning of the experiment, the identification of sites, the characterisation of the test materials and the data collection. Those steps are distributed over an extended period of time. For this reason, and to allow the conduct of studies complying with all the requirements in this guidance document, the EFSA GMO Panel considers a transition period of 24 months appropriate[15]. Therefore, the requirements will be fully applicable for all applications submitted 24 months or more after the publication date of this guidance document.

The transitional period does not apply to requirements for which only the provision of a scientific rationale based on available information is necessary, such as:

- a rationale for the selection of test materials;
- a description of seed production conditions;
- the specification of the location of field trials (including a geographical map);
- a description of applied crop management practices in all plots;
- a description of the field trial design (size, plot shape, inter-plot distances);
- a rationale for the selection of additional agronomic and phenotypic endpoints not included in the list of the generic ones (see Section 4.2);
- a discussion of the correlated endpoints (see Section 5.4).

[14] See internal mandate proposed by EFSA to the GMO Unit for a procurement on the development of reliable sampling approaches for GM plants (http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2013-00904; Contract number OC/EFSA/GMO/2013/04).

[15] The proposed transitional period of 24 months is in line with the indicative timeline for the applicant to submit new field trials in the event of a request for additional information (EFSA, 2014).

Therefore, the information listed above should be provided with all applications submitted after the publication of this guidance document.

It should be emphasised that the transitional period of the current guidance does not affect in any way requirements already specified in Implementing Regulation (EU) No 503/2013, such as the description of the field trials and the experimental design, the appropriate statistical analysis and the submission of the data collected.

2. Selection of sites and test materials

2.1. Representativeness of the sites and suitability of test materials

In order to ensure that the comparative analysis is appropriate for GM plants that will be imported and/or grown in Europe, Implementing Regulation (EU) No 503/2013 states, in Chapter 1.3.2.1(b) and in other sections, two fundamental conditions: "*the different sites selected for the field trials shall reflect the different meteorological and agronomic conditions under which the plant is to be grown; the choice shall be explicitly justified. [...] The choice of non-GM reference varieties shall be appropriate for the chosen sites and shall be justified explicitly.*"

The GM event covered by the scope of the GM plant application (be it for import/processing for food and feed uses or cultivation in the EU) is likely to be introduced under REs different from those tested to support the application. Therefore it is important that the REs selected for the comparative assessment allow conclusions to be drawn on the safety of the GM plant produced in the other possible REs. The material under assessment in agronomic and phenotypic field trials is the GM event introduced or introgressed into a specific genetic background variety, referred to as the "GM line".

It is not feasible, in practice, to assess such GM lines under all possible REs, as defined in Section 2.3.2 of the EFSA guidance (EFSA GMO Panel, 2010a), because REs are highly diverse and dynamic over time. Therefore, the strategy consists of (1) comparing the GM line(s) with the conventional counterpart and several non-GM reference varieties across a range of REs; and (2) assessing whether interactions exist between the material assessed and the REs (i.e. whether some differences might be consistently linked to specific REs). Therefore, applicants should select localities of field trials so that they sufficiently capture the variability that may exist across potential REs for the GM line.

Two critical choices have to be made and justified during the selection process of test materials and sites:

- *Selection of suitable genetic test materials (GM line, non-GM comparator and non-GM reference varieties*): here, suitability refers (i) to the ability of the GM line to be adapted to a range of typical REs in which the GM event may be introduced; (ii) to the fact that the non-GM comparator should be genetically as close as possible to the GM line; and (iii) to the fact that non-GM reference varieties should be appropriate for the areas in which the GM line can be grown (see EFSA GMO Panel, 2011b).
- *Selection of representative site locations and management systems to carry out field trials*: representativeness refers here to the ability of the selected sites and management systems to capture enough variability within the set of possible REs in which the test materials can be grown.

The selection of test materials and sites are closely related and interdependent, because a given GM line is not generally appropriate to be grown in all the possible REs in which the GM event may be introduced. Particular attention should therefore be paid to the selection of the line used in the transformation or to the variety(ies) in which the GM event is introgressed[16]. A variety with high adaptability will guarantee the potential for testing the materials in a wider range of REs.

There are several factors (dimensions) driving the selection of representative test materials and sites. These include, but are not restricted to, soil characteristics, climatic conditions (average temperature over a particular period, average rainfall during the growing season, etc.), day length, soil moisture and fertility, biotic factors (presence/absence of pests), etc. It is therefore recommended that applicants

[16] The introgression of the event into one genetic background, via backcrossing, can minimise the impact of somaclonal variation occurring in the regeneration process.

provide a scientific rationale justifying the selection of sites and test materials, and that this selection is based on the abovementioned factors using the strategy exemplified in Section 2.3 (see below).

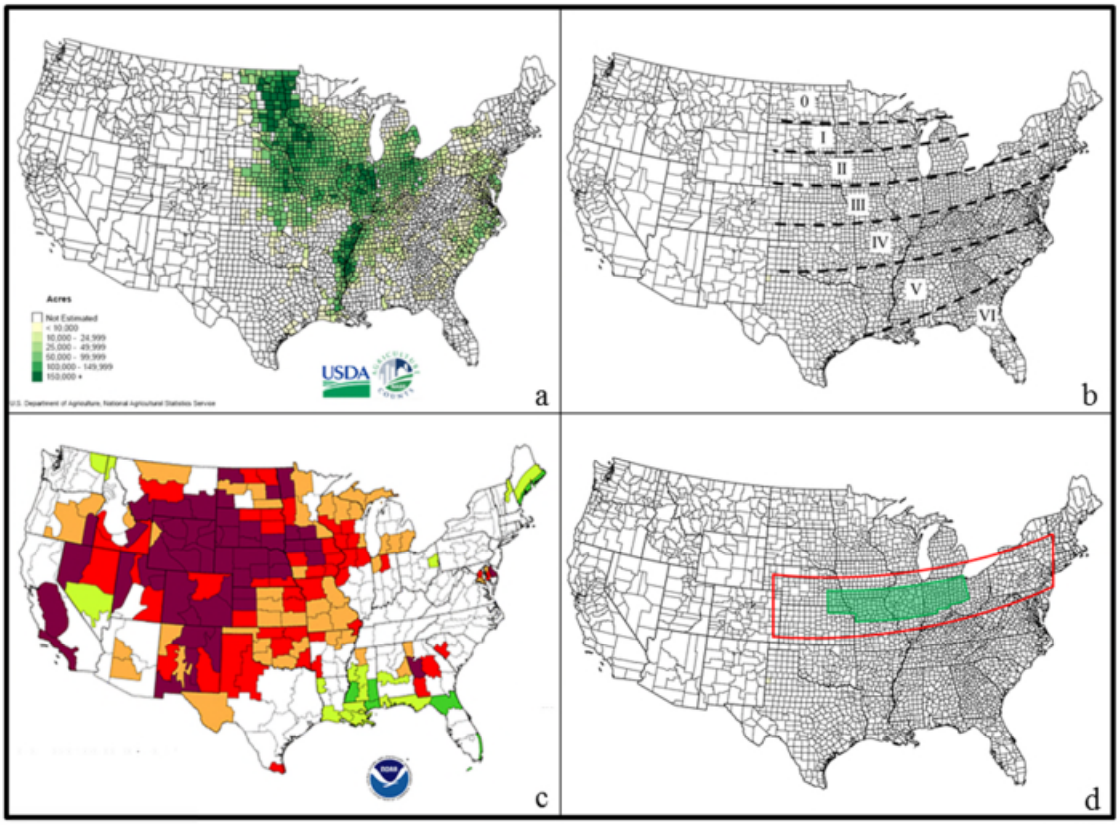
2.2. The need for diversity of sites

In Implementing Regulation (EU) No 503/2013, the concept of representativeness is enshrined in phrases such as "*the [...] sites selected [...] shall reflect the different [...] conditions under which the crop is to be grown*" and "*chosen to be representative of the range of likely REs where the plant is to be grown*", where the terms "different conditions" and "range" emphasise the need for diversity of sites. The finding that underpins this requirement, genotype × environment (G × E) interactions, has been well known to plant breeders for almost a century (Tabery, 2008). As outlined in Implementing Regulation (EU) No 503/2013, the present guidance supports strongly the need to study such interactions, and appropriate forms of analysis are discussed in Section 5.3 below. Such analysis should seek to identify which, if any, of the above factors might be responsible for the variability observed among sites. The need for such analysis requires a full and adequate description of sites in terms of characteristics such as the meteorological, agronomic and biotic factors described in more detail in Section 3.2.

2.3. A strategy to select sites and varieties

The information submitted to demonstrate representativeness of sites and suitability of test materials could take the form of maps and/or a table of the factors listed in a qualitative or, preferably, quantitative format, including relevant information for each site.

As an example, consider a hypothetical GM plant application covering the import/processing for food and feed use of a GM event that is a herbicide-tolerant soybean, introduced or introgressed into a variety in maturity group III, for which it is proposed to conduct the agronomic and phenotypic field trials in North America. The first step should be to delineate the boundary within which sites for the trials could be selected. A map of soybean crop area by county might be used as initial information to delineate the possible area for site selection (Figure 1a). In the second step, other factors (dimensions), such as the maturity group (Figure 1b), might help to further characterise the possible growing area for such a GM line and its conventional counterpart to the southern portion of the mid-western states of the USA, roughly between the latitudes of 38 °N and 42 °N. Although the cultivation of the selected line is optimal in its maturity zone, it would be possible to grow it in adjacent zones. For each of the remaining factors for which appropriate information exists, it is necessary to characterise the values of the factor within this boundary. For example, regarding the factor soil moisture, it may be possible to use maps or tables of drought indices to illustrate the major geographic east–west trend and the range of average values along this axis (Figure 1c). Geographical information on other factors such as weed profile, pests and natural enemies may be similarly characterised and the separate information from each factor overlaid to locate areas within the boundary that reflect the "*different meteorological and agronomic conditions under which the crop is to be grown*", as defined in Implementing Regulation (EU) No 503/2013. After having delineated the boundary within which the GM line(s), the conventional counterpart(s) and the non-GM reference varieties could be grown (Figure 1d, red line), the third step should be the selection of representative sites within this boundary, in accordance with the strategy outlined below.



(a) Soybean crop area per county in the USA during the 2012 growing season. The intensity of the green colour is proportional to the area in each county. Map available at: http://www.nass.usda.gov/Charts_and_Maps/Crops_County/#sb. (b) Schematic representation of different soybean maturity zones in the USA from group 0 to group VI. (c) Map showing the drought index, which might be used as a surrogate indicator of soil moisture. Red shades in the west of the region indicate drought conditions; white indicates mid-range conditions; green indicates moist conditions. Map available at: http://www.noaa.gov. (d) After accounting for those factors described for this example, the red line delineates the area deemed to contain possible site locations for the tested GM line; the smaller green-shaded area delineates the approximate typical sub-area within which the selected GM line would most likely be grown. For representativeness, some sites should be chosen outside of the most typical (green) area, but still within the (red) area that delineates possible site locations.

Figure 1: Maps of soybean factors (dimensions) in the USA used as an example to select sites and varieties.

Considered as a whole, the values of these factors (dimensions) are an example of high-dimensional data, i.e. data that require more than two or three dimensions for representation and, as such, may be difficult to interpret. Gower (1967) referred to the multidimensional nature of the space of these dimensions and outlined multivariate techniques to explore this space. Subsequently, Tufte (1990) and Inselberg (2009) explored graphical solutions to its characterisation.

Applicants should attempt to use the factors (dimensions) to characterise the multidimensional nature of the space of possible suitable REs. Regarding the justifications required by Implementing Regulation (EU) No 503/2013, applicants should aim in their site selection to capture, as far as possible, the diversity of the range of potential suitable values across the set of factors (dimensions). Practically, this should be done by ensuring that some sites are chosen outside the sub-region of this multidimensional space that represents the most typical area (exemplified by the green area in Figure 1d). For example, sites might be chosen in maturity zone II or IV, and/or from those with sub-optimal soil moisture, but still within the area where the GM line can be grown.

The EFSA GMO Panel emphasises that, firstly, while it is neither desirable nor feasible for site selection to be determined by purely mathematical or algorithmic techniques, quantitative information should form the basis of the justifications wherever possible. In addition, while it is not possible or

necessary for risk assessment to select sites from all the possible REs defined by combinations of the factors (dimensions) discussed in Section 2.1, the greater the diversity of REs selected, the greater is the certainty with which conclusions on safety in the particular REs chosen may be drawn for potential REs that were not selected.

3. Quality and design of field trials

This section defines the necessary information that applicants should supply to ensure an accurate description of test materials and organisation of field trials. Here, the EFSA GMO Panel complements its previous guidelines by providing recommendations on how applicants should demonstrate the quality and uniformity of the starting material used as test material in the agronomic and phenotypic comparative studies. This is required so that the observed differences between the test materials can be attributed to real differences between lines and not to differences in the quality or the production of the starting materials. The information on the quality and design of field trials will enable a critical appraisal of the agronomic and phenotypic data provided in support of the comparative analysis, to demonstrate that selected receiving environments are representative and to help interpret the outcomes of the data analysis. Some of them might be needed only when differences across sites and/or G × E interactions are detected but, in all cases, it is advisable to record all of them as they can be requested at any time by the Panel, should it be deemed useful to support the risk assessment of the GM plant. The requirements listed in this section apply to all test materials and sites in field trials. Applicants should ensure that appropriate quality assurance systems (e.g. OECD, 1999; International Standards Organization) are in place for the agronomic and phenotypic characterisation of GM plants, which should be documented in the study reports dedicated to the agronomic and phenotypic characterisation.

3.1. Seed quality

The use of high-quality test materials produced under similar environmental and management conditions is advocated to guarantee the reliability of the agronomic and phenotypic dataset. Agronomic and phenotypic characteristics can be affected by the source of test materials, their methods of production and environmental factors (He et al., 2014). Therefore, particular attention should be paid to the production and selection of test materials. Applicants should use certified seed for the non-GM reference varieties, and they should specify the certification authority and the type of certification. If such information cannot be given, or in the case that non-certified seed is used, then applicants shall verify the quality of the test materials and supply the same information outlined in the sections below, as for the GM line and its conventional counterpart.

3.1.1. Production conditions of the sown seeds

The seed lots of the GM line and its conventional counterpart used in the agronomic and phenotypic studies should be as homogeneous as possible in terms of their origin, year of production and production conditions, and they should be tested for their identity, purity and health. It is requested that seeds of the GM line(s), its conventional counterpart(s) and other comparators used are produced, harvested and stored under similar conditions. Any difference in seed treatments (e.g. coating or pre-treatments) between the GM line and its conventional counterpart should be documented and justified.

Seed treatments applied to the non-GM reference varieties should also be described.

3.1.2. Identity of starting materials

The adventitious presence of any GM events other than the one under test should be limited in the GM line and in the conventional counterpart. Applicants are asked to characterise the identity of the GM line and its conventional counterpart by identifying and quantifying other GM events present in the seed sample. The methods used for sampling, sample preparation (ground seeds, single seed, etc.) and characterising seed purity (such as PCR, real-time PCR, ELISA) of the test materials should be described. Applicants should use validated detection methods recognised by international institutions (e.g. the European Commission's Joint Research Centre) whenever available. The testing for event(s) in addition to the one(s) present in the GM line under assessment should consider event(s) cultivated in proximity to the seed production areas, as well as any potential additional sources of contamination during seed handling (e.g. packaging, storage, labelling).

3.1.3. Seed health and viability

Seeds used as test material in comparative studies should be of adequate phytosanitary quality and viability. The GM line and its conventional counterpart should always be tested for seed health, germination and viability/vigour (in accordance with International Seed Testing Association (ISTA, 2015) rules or other recognised procedures; see above). If incidences of seed-borne pathogens/pests above damaging thresholds are observed in seed health testing of untreated seed, then seed health, germination and viability should also be assessed on treated samples.

3.2. Description of the receiving environments of field trials

Implementing Regulation (EU) No 503/2013 and the EFSA GMO Panel guidelines on the risk assessment of food and feed and derived products (EFSA GMO Panel, 2011a) require applicants to describe several aspects of the RE as part of their GM plant applications. The Implementing Regulation states that "*field trials should be adequately described, giving information on important parameters such as management of the field before sowing, date of sowing, soil type, herbicide use, climatic and other cultivation/environmental conditions during growth and time of harvest, as well as the conditions during storage of the harvested material*". The EFSA GMO Panel therefore requires a comprehensive and accurate description of relevant aspects of REs, as this information will enable an evaluation of the representativeness of the selected REs and an assessment of the extent to which environmental and agricultural conditions affect potential differences between the GM plant and the conventional counterpart.

The field trials should be adequately described by providing detailed information, which is elaborated further in the subsections below, on:

- their geographical location;
- agrometeorological data;
- soil type and soil characteristics;
- cropping history;
- crop management;
- post-harvest storage conditions for harvested materials to be used for further testing.

3.2.1. Location of field trials

Applicants are requested to specify the position of the locations where field trials have been performed, specifying their geographical coordinates in terms of latitude and longitude (degrees and minutes) and altitude (metres above sea level). Moreover, a map of the geographical position of the selected sites is to be supplied (see also Section 2.3).

3.2.2. Agrometeorological data

Agrometeorological data provide fundamental information needed for the description of the REs and of the occurrence of extreme weather. Applicants shall provide the necessary agrometeorological data to describe REs and identify potential differences in meteorological conditions across sites during critical growth stages of the GM plant (e.g. emergence, flowering, harvest).

Applicants are required to report descriptive statistics (such as mean and standard error on a weekly basis) throughout the cropping period for average air temperature and precipitation and to produce a combined thermopluviometric graph for each site, as this facilitates the comparison of trends across sites. Exceptional weather conditions (e.g. drought, frost, hail or wind storms), their time and duration of occurrence (day), and the crop growth stage (BBCH scale; see Meier, 2001) at which they occurred at each locality should also be reported.

On a case-by-case basis (e.g. in the case of G × E interactions), the EFSA GMO Panel may request a more detailed analysis of data to further support the interpretation of the comparative assessment outcome. Applicants shall therefore record the following information in electronic format:

- daily maximum, minimum and average air temperature (°C);
- daily precipitation (mm);
- daily maximum, minimum and average air relative humidity taken at the same time as air temperature (% vol/vol);
- daily global radiation (MJ/m^2);
- daily maximum, minimum and average wind speed (m/s)
- historical thermopluviometric data (if available).

All the recorded meteorological parameters are to be collected at each site throughout the cropping period, i.e. from sowing to harvest, and can be annexed to the GM plant application at the time of submission.

If one or more of the selected sites are not equipped with an on-site weather station, then data generated from a nearby weather station can be used, provided that these are representative. In that case, the geographical coordinates of this station (see Section 3.2.1) and its distance (kilometres) from the field trial site should be given.

3.2.3. Soil characteristics

Applicants shall provide a detailed description of the soil characteristics for each of the field trial sites, covering the aspects below. Internationally agreed standards for soil analyses (e.g. ISRIC, 2002) should be followed[17]:

- soil texture (particle size classes: % clay, % silt and % loam);
- soil organic carbon (wt/vol soil);
- pH;
- soil bulk density.

The soil description should reflect field conditions before sowing. Locally important soil characteristics, such as high heterogeneity, or the occurrence of specific conditions leading to abiotic stress (e.g. soils with low water infiltration capacity, compacted soils), should be analysed and reported.

On a case-by-case basis (for specific crops or traits, e.g. in case of G × E interactions), the applicant might be requested to provide a more accurate soil description. In such a case, data on additional soil characteristics gathered from at least each block per each site (i.e. four values per site) could be useful:

- total nitrogen (wt/wt soil);
- extractable phosphorus (wt/wt soil);
- exchangeable potassium (wt/wt soil).

[17] See, for example, USDA-NCRS, 1999. Surveys available online: http://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_051232.pdf

3.2.4. Cropping history

Applicants should provide information documenting the cropping history of the selected sites during the previous three years and the cultivation management practices applied (e.g. tillage). On a case-by-case basis, any additional information that may be useful to support the representativeness of sites and the interpretation of the comparative analysis, as well as information on the incidence and severity of plant disease epidemics and pest outbreaks at each site, should be supplied.

3.3. Crop management system

3.3.1. Crop management

The following information for each field trial site should be supplied:

- Main form of tillage: (i) type, (ii) depth and (iii) date.
- Seedbed preparation: (i) type, (ii) depth and (iii) dates.
- Sowing or planting: (i) date, (ii) method, (iii) number of seeds or vegetative plant parts per square metre, (iv) number of rows per plot, (v) inter-row distance (cm), (vi) sowing depth and (vii) details of thinning (if any).
- Fertilisers: for each fertiliser application, detailed information is required on (i) type (element), (ii) method (e.g. on soil, on vegetation), (iii) rate (kg element/ha), (iv) date and (v) growth stage of crop (BBCH scale), where relevant.
- Insecticides, fungicides, herbicides, other agrochemicals: for each application, detailed information is required on (i) date, (ii) type, (iii) target, (iv) active ingredient(s), (v) name of commercial product, (vi) rate (g active ingredient/ha), (vii) application method (e.g. soil, on vegetation, other), (viii) water volume (L/ha), (ix) adjuvants (type and rate in g/ha) and (x) growth stage of the crop (BBCH scale), where relevant. These details should also be provided for agrochemicals (e.g. herbicides) applied pre-sowing.
- Irrigation: (i) date(s), (ii) method, (iii) amount (mm).
- Harvest: (i) type, (ii) moisture (%) of harvested plant part, (iii) date(s), and (iv) growth stage of crop (BBCH scale).
- Any other crop-specific management interventions (post-emergence hoeing or cultivation, ridging/hilling, etc.): (i) type, (ii) date and (iii) growth stage of crop (BBCH scale).

To facilitate registration of information related to the application of crop management interventions, applicants are required to provide a table.

It is advisable to follow local pest management practices and consider the principles of Good Agricultural Practice to keep the treatments with pesticides to the minimum required to contain the level of disease/infestation below acceptable levels, since excessive use of plant protection products may impair a thorough evaluation of pathogen/pest–plant interactions. Similarly, the lack of pest management measures in the experimental field may lead to excessive stress on plants, which does not reflect normal agricultural practice.

3.3.1.1. Herbicide regimes in the case of GM herbicide-tolerant plants

Owing to the diversity of current weed management strategies and the numerous additional options that GM herbicide-tolerant (GMHT) plants give to farmers, further guidance is given on which herbicide regimes can be applied in field trials used for the agronomic and phenotypic characterisation of GM plants.

Herbicide regimes

Implementing Regulation (EU) No 503/2013 indicates that three treatments should be compared in field trials with GMHT plants. It states that "*in the case of herbicide tolerant genetically modified plants and in order to assess whether the expected agricultural practices influence the expression of the studied endpoints, three test materials shall be compared: the genetically modified plant exposed to the intended herbicide; the conventional counterpart treated with conventional herbicide management regimes; and the genetically modified plant treated with the same conventional herbicide management regimes*".

All field trials, including those performed for the comparative assessment of GM plants, require maintenance according to local needs (fertilisation, tillage, pesticide treatment, etc.), in order to establish suitable agronomic conditions. This maintenance may also include herbicide treatments. A conventional herbicide regime is the programme of herbicide applications commonly used by farmers at a given site throughout the growing period of a crop, and it may include pre-sowing, pre-emergence and/or post-emergence treatments. It is expected that conventional herbicide regimes differ between locations, reflecting different soil, crop history, weed, environmental and usage conditions. In the case of low weed pressure, favourable climatic conditions and/or alternative weed control options (e.g. mechanical weeding), no herbicide use may be expected at some sites.

Implementing Regulation (EU) No 503/2013 refers to the conventional counterpart (A) and the GMHT plant (B) treated with the same conventional herbicide regime. The comparison of A and B allows a direct comparison of the GM plant and its conventional counterpart under the same conventional herbicide regimes and enables the detection of unintended effects arising from the genetic transformation.

In addition, Implementing Regulation (EU) No 503/2013 refers to the GMHT plant exposed to the intended herbicide (C). Depending on whether the GM plant is tolerant to single or multiple herbicidal active ingredients, the use of the intended herbicide(s) can occur in various ways.

In order to be representative of local REs (crop management, weed pressure and climate), the third treatment (C) may differ across sites.

Recommendations

For each site, applicants shall specify which combinations of herbicides were applied to each of the test materials, including non-chemical weed management interventions (e.g. pre-sowing treatments, pre-emergence cultivation, post-emergence hoeing).

For each site, a rationale justifying the selection of the conventional herbicide regime and of the regime applied to the GMHT crop in treatment C is required. If the agronomic and phenotypic and compositional data are generated in the same field trials, then applicants should also account for the potential implications of herbicide regimes on the compositional analysis when selecting suitable herbicide regimes.

Plausible options for weed management for GM plants with tolerance to single or multiple herbicidal active ingredients (C) are listed below. These represent possible ways of herbicide application that could be chosen, taking into account the potential negative interactions between herbicides that may occur in the case of a tank mix application.

Options for GM plants with tolerance to a single herbicidal active ingredient include:

- GMHT plants treated with the intended herbicide in addition to the full conventional herbicide regime[18] included in the field management (additional treatment);
- GMHT plant treated with the intended herbicide only (full replacement of the conventional herbicide regime);
- GMHT plant treated with the intended herbicide in addition to a subset of conventional herbicides used in the field management (partial replacement of the conventional herbicide regime).

In the case of GM plants that are tolerant to multiple herbicidal active substances, all or a subset of the intended herbicides can be applied either in sequence or in a tank mix, either alone or in combination with all or a subset of the abovementioned conventional herbicide treatments. Options for GM plants with tolerance to multiple herbicidal active ingredients include:

- GMHT plants treated with all the intended herbicides, either in sequence or in a tank mix, in addition to the full conventional herbicide regime (full additional treatment);
- GMHT plants treated with a subset of the intended herbicides, either in sequence or in a tank mix, in addition to the full conventional herbicide regime (partial additional treatment);
- GMHT plants treated with all the intended herbicides but with no conventional herbicide, either in sequence or in a tank mix (full replacement of the conventional herbicide regime);
- GMHT plants treated with a subset of the intended herbicides, either in sequence or in a tank mix, in addition to a subset of the herbicides used in conventional herbicide regimes (partial replacement of the conventional herbicide regime with partial treatment);
- GMHT plants treated with a subset of the intended herbicides but with no conventional herbicide, either in sequence or in a tank mix (full replacement of the conventional herbicide regime with partial treatment).

Applicants may consider adding additional weed control treatments to the three required by the Implementing Regulation, if deemed necessary to ensure representativeness and allow a proper assessment of GMHT plants.

3.3.1.2. Post-harvest conditions

Applicants should describe the conditions under which the harvested/sampled materials have been stored. If materials have been transported for analysis to facilities distant from the place of storage, then the conditions during transport should also be described (see EFSA GMO Panel, 2011a, for further details on sample storage).

3.3.2. Field trial design

A graphical representation of the design of the field trial covering the spatial distribution and size of the blocks and the plots within sites, the distances between them and the presence of buffer rows and outside border rows should be supplied.

3.3.2.1. Plot size, inter-plot distances and buffer/guard rows

Applicants should describe and justify the choice of plot size by accounting for the biological characteristics and common agricultural practices of the crop species. The minimum plot surface for

[18] Conventional herbicide regimes refer to the whole set of registered herbicides that can be applied on non-GM varieties of a given crop.

agronomic and phenotypic field trials should be between 20 and 30 m^2. Deviation from the proposed range should be explained and justified.

The selection of the plot size and shape should account for the inclusion of a representative number of plants so that reliable data may be gathered in the inner part of the plot without causing disturbance to test materials (EPPO, 2012). Plot size is highly dependent on the biology of the crop and on the genetic heterogeneity of the tested material (e.g. whether hybrid or line, etc.). In addition, for open-pollinated crops, the selection of plot size and distance between plots and the inclusion of buffer/guard rows should be made to minimise cross-fertilisation with adjacent plots in order to preserve the identity of the material that will be used for the compositional analysis. Manual pollination is not representative of common agricultural practice and should be avoided.

4. Agronomic and phenotypic endpoints

As specified in Implementing Regulation (EU) No 503/2013, "*the applicant shall provide a comparison between the genetically modified plant and its conventional counterpart. This comparison shall enable the applicant to identify unintended effects resulting from the genetic modification and shall address also plant biology and agronomic traits, including common breeding parameters (such as yield, plant morphology, flowering time, day degrees to maturity, duration of pollen viability, response to plant pathogens and insect pests, sensitivity to abiotic stress)*". To guide the selection of endpoints to optimise this comparison, it is essential that applicants follow closely the main objectives of characterisation and the potential contributions of the resulting dataset outlined in Section 1.1.

This section provides guidance on the selection of agronomic and phenotypic endpoints for GM plant applications for import/processing for food and feed uses and for cultivation.

A distinction is made between generic endpoints and case-specific endpoints. Generic endpoints contribute to several objectives of the agronomic and phenotypic studies and shall always be measured in accordance with the scope of the GM plant application (see Table 1 for import/processing for food and feed uses applications and Table 2 for cultivation applications). The case-specific endpoints are crop or trait related, and applicants can decide on a case-by-case basis whether or not these are to be considered further. Applicants shall provide a scientific rationale justifying the inclusion or exclusion of endpoints.

For all endpoints and performed measurements, applicants shall supply documentation of the sampling representativeness, and of the recording methods.

The use of internationally agreed units of measurement (Thompson and Taylor, 2008) and of growth scales (Meier, 2001) is advocated to ensure a more comprehensive interpretation of agronomic and phenotypic data and agricultural management practices.

4.1. Generic agronomic and phenotypic endpoints

Tables 1 and 2 summarise the minimum set of generic endpoints for GM plant applications for import/processing for food and feed uses and applications for cultivation. The tables include requirements for cotton, maize, oilseed rape and soybean, and they provide information on the entity to study, the methodology to follow, the units to use and the recommended growth stage(s) at which the necessary data should be gathered. The endpoints should be measured at all sites and on the whole set of test materials, including the selected non-GM reference varieties. The generic requirements listed can be applied to a wider range of plant species and can be adapted to other crops/species on a case-by-case basis.

Table 1: Overview of generic endpoints to consider in the case of GM plant applications for import/processing for food and feed uses when performing field trials for the agronomic and phenotypic characterisation of GM soybean, maize, cotton and oilseed rape

Phenological stage	Endpoint	Measure [a]	Unit	Growth stage [b]			
				Soybean	Maize	Cotton	Oilseed rape
Pre-sowing	Seed characteristics [c]	–	–	00–08	00–08	00–08	00–08
Vegetative	Early stand count	M	m^2	12–13	11–14	11–13	11–13
Reproductive	Days to flowering	V	Days	61–69	61–69	61–69	61–69
Maturity	Lodging [d]	V	No/%	89	89	89	80–89
	Final stand count	M	m^2	89	87–89	89	31–99
	Plant height	M	cm	89	69–89	69–89	71–89
	Days to maturity	V	Days	89	87	89	89
	Fruit count [e]	M	No	89	89	89	89
Harvest	Seed moisture	M	%	99	99	99	99
	Seed weight	M	g	99	99	99	99
	Yield	M	g/m^2	99	99	99	99
All phases after plant emergence	Biotic interactions	M/V	–	11–99	11–99	11–99	11–99
	Abiotic interaction	M/V	–	11–99	11–99	11–99	11–99

(a): M, measurement; V, visual estimation.
(b): Growth stage according to the BBCH scale of the crop. Indicates when the measurement/assessment of the endpoint should be conducted.
(c): Seed characterisation is conventionally performed in laboratory studies (see Section 3.1 for details).
(d): In the case of maize, stalk-lodged and root-lodged plants should be counted separately and a single value provided.
(e): For soybean and oilseed rape, pod count; for maize, ears per plant; for cotton, bolls per plant.

Table 2: Overview of generic endpoints to consider in the case of GM plant applications for cultivation when performing field trials for the agronomic and phenotypic characterisation of GM soybean, maize, cotton and oilseed rape

Phenological stage	Endpoint	Measure [a]	Unit	Growth stage [b]			
				Soybean	Maize	Cotton	Oilseed rape
Pre-sowing	Seed characteristics [c]	–	–	00–08	00–08	00–08	00–08
Vegetative	Early stand count	M	m^2	12–13	11–14	11–13	11–13
	Crop development	V	%	31–39	31–39	31–39	31–39
Reproductive	Days to flowering and duration	V	Days	61–69	61–69	61–69	61–69
Maturity	Lodging [d]	V	No/%	89	89	89	80–89
	Final stand count	M	m^2	89	87–89	89	31–99
	Plant height	M	cm	89	69–89	69–89	71–89
	Days to maturity	V	Days	89	87	89	89
	Seed loss [e]	M	No	89	89	89	89
	Fruit count [f]	M	No	89	89	89	89
Harvest	Seed moisture	M	%	99	99	99	99
	Seed weight	M	g	99	99	99	99
	Yield	M	g/m^2	99	99	99	99
All phases after plant emergence	Biotic interactions [g]	M/V		–	–	–	–
	Abiotic interaction [g]	M/V		–	–	–	–

(a): M, measurement; V, visual estimation.
(b): Growth stage according to the BBCH scale of the crop. Indicates when the measurement/assessment of the endpoint should be conducted.
(c): Seed characterisation is conventionally performed in laboratory studies (see Section 3.1 for details).
(d): In the case of maize, stalk-lodged and root-lodged plants should be counted separately and a single value provided.
(e): For soybean, shattering; for maize, dropped ear count; for oilseed rape, pod shattering.
(f): For soybean and oilseed rape, pod count; for maize, ears per plant; for cotton, bolls per plant.
(g): In the case of cultivation applications, biotic and abiotic interactions should be assessed following the approach outlined in EFSA (2010a).

4.1.1. Stand count

The early stand count provides information on the establishment of plants, while the late stand count indicates plant survival to maturity and capacity to complete the reproductive phase. Data generated for early stand count will deliver helpful information on emergence and early survival and subsequent death of plants before the final stand density is achieved, which might be indicative of differential development between the GM line(s), the conventional counterpart(s) and the non-GM reference varieties.

Method

The number of plants within a given area or distance (e.g. a given length of row or a quadrat of stated area) should be reported. If there are large numbers of plants per plot, then the quantification should be conducted by counting sub-sections of the entire plot (e.g. transects of 1 m length in two or more rows, 1-m^2 quadrats, etc.). Where possible, the same part of the plot should be used for measuring stand count at each different growth stage, in order to be in a position to make suitable comparisons.

Stand count is best measured at the seedling phase, when emergence and establishment is complete (after BBCH 10), and before any intervention in the field trial (e.g. first application of any post-emergence herbicide or thinning).

In the case of overwintering crops, an additional measurement should be conducted in spring to assess overwinter plant losses caused by factors such as pests or pathogens, chilling and waterlogging.

In high-density crops, it can be difficult to measure late stand count before harvest. In such cases, it may be preferable to assess it either during the stem extension phase or after harvest on the stubble.

Stand counts should be quantified as the total number of plants per square metre or per metre length of row converted to unit per area (m^2).

Recommendations

Unless thinning is the normal agronomic practice, it is strongly discouraged[19]. If carried out, thinning should be described in detail, explaining the time of execution, the method of removal of the plants and the target plant density. The decision to perform thinning must be based only on plant density and not on the phenotypic characteristics of the plants. Correct thinning is required to ensure a reliable comparative assessment of the agronomic and phenotypic endpoints to be measured later during the vegetative and reproductive phases of growth, including yield and yield components.

4.1.2. Plant development

The vegetative growth and development of crop plants should be measured, in order to assess the comparative vigour and rate of vegetative growth. This is related to the solar radiation intercepted by crops, which is a primary driver of productivity. When intercepted, radiation is accumulated over the growing season and it is strongly related to the dry matter produced by crops—the total cumulative intercepted radiation accounting for differences between crop types and years.

Method

The assessment can be conducted in different ways in accordance with the characteristics of the crop.

For most crops, percentage ground cover[20], estimated visually, is a useful and practical method (Steven et al., 1986). Generally, a 0.5 × 0.5 m quadrat is placed over the crop, and the amount of ground covered by the crop is estimated visually. For tall crops or crops with wide row spacing such as maize, measurement of plant height[21] will give a good assessment of vegetative growth. In general, other measurements that can provide information on crop development are considered appropriate, such as non-destructive quantification of leaf surface or leaf area index (e.g. Liu and Pattey, 2010). A detailed description of the applied methodology for the endpoint measurements should be provided.

The vegetative development should be measured during the elongation phase (BBCH 31–39). Vegetative development should be measured at least once during the season. Ideal timings to make the ground cover observations include during vegetative stem extension or prior to flowering. These phases can be identified between BBCH 16 and 39 but before BBCH 61.

4.1.3. Days to flowering

Differences in the periodicity of flowering can be used to discriminate between the reproductive phases of crop plants.

Method

Time to flowering can be visually estimated at the plot level between BBCH 61 and 69. In case of GM plant applications for import/processing for food and feed uses, time to flowering should be measured

[19] The EFSA GMO Panel considers that high heterogeneity in initial stand count can indicate heterogeneity in the quality of starting material. If such a difference was not captured in the germination tests, the identified difference should be attributed to a possible unintended effect and its consequences should be assessed over the entire life cycle rather than altered with thinning.

[20] Visual estimation of percentage crop ground cover (ranging from 0 % to 100 %).

[21] Plant height should be recorded during stem extension as distance (cm) from the base (soil level) of the plant to the uppermost terminal meristem (for further details on plant height, see Section 4.1.6).

at a specific growth stage (e.g. BBCH 65) and be provided as days from planting to when flowers occur on a specific percentage of the plants in the plot.

4.1.4. Flowering duration

Flowering duration provides information on differences in the duration of the flowering phase in test materials.

Method

Flowering is to be estimated visually at the plot level. The beginning and the end of the flowering period should be recorded as the dates when 10 % of the flowering is occurring and when 90 % of the plants have completed flowering, respectively. This will require visual observations to be carried from the onset to the completion of flowering (BBCH 61–69)[22].

4.1.5. Lodging

Lodging is an important agronomic characteristic that indicates the standing power of the crop. It also gives an indication of whether or not the plants grew and developed normally (e.g. whether they were unnaturally spindly) and the level of heterogeneity between test materials. Lodging can lead to uneven maturity, high seed/grain moisture content, loss of yield and grain quality and seed drop before harvest. Therefore, if lodging occurs, it may explain observed differences in other agronomic and phenotypic endpoints (such as maturation).

Method

Lodging should be measured at plant maturity, before harvest, by visually estimating the proportion of lodged plants per plot (to the nearest 10 %) inclined more than 45 ° from the vertical.

If brackling occurs, then this can be measured following the same method described for lodging and measured along with lodged plants.

4.1.6. Plant height

Information on plant height will give general information on the development and the vegetative growth of the test materials and their heterogeneity. Plant height also serves as a general indicator of vegetative vigour and plant biomass.

Method

Measurements should be performed at completion of stem elongation, which is usually during or at the end of flowering (BBCH 69). A representative number of plants per plot should be measured for length (cm) from the base (soil level) of the plant to the uppermost terminal meristem, or in maize to the base of the tassel.

Recommendations

The total number of sampled plants per plot is to be justified and reported, as well as the individual values measured.

4.1.7. Maturity

Maturation is a general characteristic of the crop, which delivers information on its life cycle and whether it developed normally.

[22] Flowering time should be provided as duration of flowering in days and as days from planting to the date when flowers occur on 10 % and 90 % of the plants in the plot.

Method

Visual estimation of fruit/kernel/seed maturity is to be measured when the plants reach full physiological maturity (BBCH 89) by recording days from planting to the date when mature fruits/kernels/seeds occur on 90 % of the plants.

Recommendations

If it is not possible to assess the physiological maturity of the crop (for instance, owing to the use of desiccants, swathing), then applicants should characterise the level of maturity (BBCH growth stage) at the time of the intervention.

4.1.8. Seed loss[23]

Either way, seed loss results in an underestimate of plant productivity and associated errors in fruit number, seed number, seed per fruit and mean seed weight. In addition, it provides an indication of the potential of the plant to introduce seed into the soil, and this may indicate its potential to create a seed bank and may be correlated with relative survival rates and the production of volunteer or feral plants. All propagules should be considered: for example, loss by tubers and true seed should be considered for potatoes.

Method

Record the number of dropped ears/cobs/seeds per plot or shattered pods per specific area of plot. In some instances, "seed rain" trays may be positioned beneath the plots to collect falling or shattered seed (e.g. oilseed rape). Observations should ideally be made at plant maturity before harvest. Total number of mature dropped cobs or seeds per plot, or total number of shattered pods per specific length or area of the plot, should be recorded. Measurements should be converted to numbers per unit area (m^2).

For potatoes, standard areas of plots should be examined after harvest and the numbers of small tubers not harvested and fruit should be counted.

Recommendations

For some crops, such as oilseed rape, the measurement of seed loss can be challenging owing to the natural seed loss during inspection. This may lead to an overestimation of losses and, in those cases, alternative approaches can be proposed by applicants. For example, in the case of oilseed rape, seed loss can be measured by sampling pods at different intervals up the main stem and then assessing those for losses (so no plants are removed) or, alternatively, placing trays on the ground prior to pod ripening to collect the dropped seeds and recording just before harvest.

4.1.9. Fruit or ear count[24]

The fruit or ear count per plant is an important yield component, providing an indication of plant fertility.

Method

Record the number of fruits per plant from a representative sample of plants in the plot at maturity (BBCH 89).

[23] The term varies between plant species. It refers to shattering in the case of soybean and oilseed rape, dropped ears in the case of maize, and tuber and true seed loss in the case of potato.

[24] Number of pods per plant in the case of soybean and oilseed rape; number of ears per plant in the case of maize; and number of bolls per plant in the case of cotton. (In the case of potato, this character is the number of harvestable tubers per plant but it can also be reported as the number of true fruit produced per plant.)

Recommendations

The total number of sampled plants per plot shall be justified and reported, as well as the individual values measured. Number of fruits can also be recorded as number of fruits per square metre or per metre length of row by factoring the plant stand and the number of fruits per plant.

4.1.10. Seed moisture at harvest

The moisture content of harvested products varies with crop maturity, variety, soil type and weather. In order to measure the dry matter yields of crops, the moisture content is required and a correction is then applied to the fresh yield recorded at harvest.

Method

Seed moisture content analysis should be based on representative samples taken from the entire harvest. Samples should be tested immediately after harvest (BBCH 99) and before any processing (e.g. natural or artificial air drying, storage). "Weight loss on drying", moisture meters or other suitable devices can be used to determine moisture content. Specification of the methodology applied should be always provided.

Moisture content should be recorded as percentage water of the fresh harvested weight. The remainder is the dry matter.

4.1.11. Seed weight

Seed weight is an important yield component, often strongly related to fruit and seed number. If a crop is source limited (i.e. there are more seeds than the available plant assimilate can fill), then there is commonly a trade-off between seed number and seed weight—generally, the fewer seeds per plant, the larger the seeds. However, if a crop is sink limited (i.e. there are fewer seed sites than can be filled by the available assimilate), then seed weight is more stable. The relation between seed weight and number is an important attribute which is often more discriminating between varieties than is seed weight.

Method

A representative sample of harvested (BBCH 99) seeds per plot is weighed and counted in order to determine the mass (g) of 100 or 1 000 seeds. The value obtained is then corrected to a standard moisture content using the moisture content assessment (see Section 4.1.10).

4.1.12. Yield

Yield is a measure of the utilisable fraction of the crop at harvest and provides an assessment of the agronomic performance of the test materials. Yield is related to several of the agronomic and phenotypic endpoints described above, and so differences in yield may be attributed to differences in other endpoints.

Method

Harvest a representative area or the whole plot and weigh the harvested plant material. Yield can be expressed as mass (g or kg) of the harvested material per sampled plot area (m^2) corrected for moisture content. Alternatively, yield can also be converted to tonnes or kilograms per hectare.

Recommendations

Harvested plant material should be as free as possible of non-harvest fractions (e.g. straw, stems, leaves) and contaminants (e.g. soil for potato). Secondary cleaning can be done to remove these materials. In addition, it should be clearly stated whether the yield refers to grain, grain and cob, or grain plus attached husk. In the case of crops harvested before physiological maturity (e.g. sweetcorn or forage maize), the maturity and/or growth stage of the crop at harvest should be described.

4.1.13. Plant responses to biotic stressors

Agronomic and phenotypic field studies can provide information on whether plants differ in susceptibility to the activity of specific arthropods and/or plant diseases. Moreover, an early phytosanitary assessment may help to confirm the health of the test materials (seed/tubers), and may detect the presence of pathogens and pests in the soil and their pattern of distribution throughout the testing field.

The following characterisation is requested only for GM plant applications for import/processing for food and feed uses. In the case of a GM plant application for cultivation, the requirements for assessing plant response to biotic stressors are those indicated in the EFSA GMO Panel guidelines on the environmental risk assessment of GM plants (EFSA GMO Panel, 2010a).

Methods

Arthropods: The necessary surveys or monitoring programmes in field trials will depend on the biology of the herbivorous arthropods and plant phenology, and therefore they will have to be determined on a case-by-case basis.

As it will be impossible to consider all potentially exposed arthropods, common pest species to be surveyed should be identified at an early stage of the field experiments considering the expected/actual presence of plant pests locally (see Section 3.2.4). Problem formulation will therefore serve as a relevant tool to select suitable arthropod species.

The following criteria should be considered when selecting relevant arthropod species to be surveyed in field trials:

Feeding mode. The first requirement for the selection of a relevant species is that it feeds on the plant (such as a sap sucker, foliage chewer, leaf miner/stem borer, gall former, root feeder, flower feeder, seed/grain feeder). Species using the plants as shelter and reproduction sites are not necessarily directly feeding on the plant, and therefore they are not optimal to assess plant–arthropod stressor interactions. Applicants are asked to consider the mode of feeding/feeding guild of the arthropods when selecting relevant species. Ideally, the different abovementioned categories of feeding mode should be covered, including one pest species for each feeding habit, ranging from foliage chewers to sap suckers to root feeders, where relevant for the crop and the area of the study.

Biological and economic relevance. The biological and economic relevance of the pest species is to be considered too, as potential changes in abundance and frequency may lead to populations exceeding economic injury levels. Measuring the biological features of these species will enable anticipation of unintended changes with potentially relevant economic consequences.

Plant diseases: Disease incidence or severity per plot is to be measured or estimated visually. All relevant diseases should be considered during the survey.

The appearance of symptoms in the field depends on the interaction between the host, the pathogen and the environment, and may vary from year to year. It is therefore necessary to prioritise the observations and to make an appropriate selection of plant diseases to be surveyed.

The following criteria should be considered when selecting relevant plant diseases to be surveyed in field trials:

Threat to crops in field trials. The species that will be surveyed in a given testing location should cover diverse categories of etiological agents (fungi, viruses, bacteria, etc.) and/or mechanisms of pathogenesis (biotrophic, necrotrophic) depending on the local phytosanitary situation. Moreover, for non-systemic diseases, the different organs (leaves, stem/stalk/trunk, roots, flowers, fruit) or

plant parts (e.g. aerial parts vs. subterranean parts) that can be affected by the disease should be considered.

Biological and economic relevance. Diseases known to have a severe biological/economic impact should be prioritised in the selection process.

The applicant must provide a scientific rationale justifying the selection of diseases to be surveyed in field trials. Expert knowledge and supporting scientific literature on the various crop diseases, such as the information available in the CABI Crop Protection Compendium (CABI, 2014), should be accounted for when selecting relevant species. Likewise, applicants are asked to provide a specification for the survey methodology used.

Timing

Arthropods: Overall, more than one sampling occasion is necessary to infer patterns of herbivore infestation. The most relevant information necessary to determine the timing of surveys is a knowledge of the biological cycle of the species and the pest–plant interactions for each insect instar. Voltinism is another very relevant feature that should determine (on the basis of the local agroenvironmental conditions) the optimal timing for surveys. In addition, plants may be more/less susceptible to herbivory during plant development (e.g. some maize varieties are naturally resistant to corn borers in their early growth phases). In addition, repeated samplings will also enable, when relevant, estimation of the presence/incidence of natural enemies of the chosen herbivore species.

Plant diseases: The timing for scoring disease level(s) and the appropriate number of observations to be conducted depends on each plant–pathogen interaction and should be defined on a case-by-case basis, accounting for the disease progression at each testing location. Disease assessment should be conducted according to plant phenology and pathogen epidemiology to ensure the generation of appropriate data on plant response to infection. Disease assessment scales appropriate for different crops should be used.

For plants whose harvested product is an organ growing partly or totally under the ground (e.g. beet roots, potato tubers), an additional assessment of disease must be done on a representative, cleaned sample of the subterranean organs immediately after harvest.

Applicants shall justify their sampling/observation strategy and report the plant growth stages at which disease scorings were performed.

Measurement unit

Arthropods: The presence and incidence of arthropod pests can be estimated in different ways. Standards proposed by international organisations (e.g. the European and Mediterranean Plant Protection Organization (EPPO)[25]) are available and can be readily adopted. Although a specific discussion for each species is beyond the scope of the present document, as a general guide to applicants, it is advisable to use one of the two following approaches:

- A measure of the abundance of herbivores, when and where relevant, on plants via visual observations (directly or after removing plant parts or the organisms living on them, e.g. via sucking devices or knock-down systems).

- In the case of high infestation levels, counts can be done by pooling instars (e.g. aphid colonies, egg masses, cohorts of young larvae, etc.) or by estimating actual densities (e.g. more than 20 individuals per plant). In many cases, damage classes, percentage of damaged

[25] For the complete list of standards for plant protection product efficacy (PP 1), see the EPPO website: http://archives.eppo.int/EPPOStandards/efficacy.htm

leaves, fruits, etc., or infestation level over the plots (percentage of damaged plants) can be used as an estimate of plant response to biotic stressors. It is recommended that these endpoints are used where the relevant scientific literature and expertise already exists.

Plant diseases: Appropriate scales should be adopted for measuring each crop–pathogen interaction. EPPO protocols and other international standards are available for many interactions and can be adopted for estimating disease incidence/severity.

Recommendations

Biotic interactions in the field trials shall be regularly monitored during the entire crop cycle, in order to ensure that appropriate crop protection measures are taken timeously, so as to avoid a build-up in populations that could overexploit the crop. These measures should effectively reduce the pathogen/pest pressure to comparable levels at the different selected sites for the field trial, thereby reducing the incidence of targeted harmful organisms and the potential plant damage caused by them.

In addition to the quantitative visual scores, any anomaly observed in the response of plants towards biotic stressors (e.g. presence/absence of halos around leaf spots that may suggest differences in the response of the GM plant to a stressor) must be reported, and crop protection measures applied shall be described. In the case of observed differences, applicants should provide their interpretation of such results.

4.1.14. Plant responses to abiotic stress

If GM plants have traits specifically intended to reduce the susceptibility of the plant to a defined stressor, then substantial field trials would be required as part of a much broader risk assessment (see Section 4.2). These trials might be carried out across sites over a natural gradient of the stressor or through local manipulation of the environment.

For GM plants that are not intended to have traits making them less susceptible to stressors, measurements should be made as part of standard agronomic and phenotypic assessment, in order to look for any unintended, differential response to the abiotic environment between the GM line(s), the conventional counterpart(s) and the non-GM reference varieties. Applicants should record damage due to abiotic stress routinely, and typically at the same time as other phenotypic assessments.

Given the wide range and type of damage, the method of assessment and descriptors or units of damage are not prescribed but should be justified by the applicant. Damage shall be assessed at plot, plant and organ levels and the likely causes defined. The aim is to assess whether the GM plant differs from the other test materials in response to abiotic stress experienced during the trial[26].

Methods

In addition to the generic endpoints, applicants should record visually any damage to leaves, stems and reproductive structures related to abiotic stressors. The range and type of possible visible damage are very wide and varied, but might include leaf scorch and discolouring, leaf mottling, patterning due to nutrient deficiency or the effects of herbicide, weakness or imbalance of the stems causing lodging or a fractured canopy, failure of flowers to open fully and damage to reproductive organs (stamens and stigmas) preventing effective pollination.

[26] Applicants are encouraged to supply the data gathered from preliminary tests, if available, conducted with the appropriate materials and under controlled conditions mimicking the occurrence of abiotic stressors (e.g. salinity, heat, cold, drought, etc.). Those data may add to the weight-of-evidence approach followed for the identification of unintended hazards in GM plants.

Measurements on three scales are useful, each by rapid visual assessment. They are:

- the average proportion of the field plot (i.e. of all plants) that is affected;
- the proportion of organs on damaged plants that is affected;
- the proportion of the damaged organ that is affected.

Measurement units will vary with the type of damage. Typical examples of the three scales are percentage of plot affected; percentage of plant organs affected on damaged plants; and percentage of single organs affected (e.g. 10 % of a plot is affected by leaf scorch; on damaged plants, 20 % of leaves are affected; on damaged leaves, 50 % of the surface is browned owing to drought). Alternatively, damage can be defined semi-quantitatively by, for example, “high”, “medium” and “low”, but these categories should be defined.

Assessments should be made throughout the growth period, typically at the same time as morphological measurements—e.g. at the vegetative stage before flowering, during full flower and when the crops are maturing before harvest. For crops that overwinter, a second vegetative score should be made after winter to note wind chill or ice damage.

4.1.14.1. Pesticide, especially herbicide, injury

Pesticide application may cause plant stress (phytotoxicity). Herbicide and other pesticide injury data are required to assess whether there are differences in sensitivity to the applied pesticides between the test materials. In the case of GMHT plants, sensitivity to the intended herbicide treatment(s) is also considered through this endpoint. Herbicide injury can result in differences observed in other agronomic and phenotypic endpoints (such as initial stand count) and can alter quality and yield characters.

Method

Visually estimate leaf chlorosis, necrosis or rolling, or any other symptoms (e.g. flower deformation) that are indicative of pesticide injury.

Record and assess pesticide injury after treatments have been applied and a sufficient interval has passed to allow full expression of the damage.

Record percentage of leaf area injured or other plant damage averaged over the entire plot, with a short description of the symptoms. For a description of observed symptoms in the different crops, the EPPO standard (EPPO, 2014) should be followed.

Recommendations

Where pesticide injury is recorded, it should be noted whether other factors may have contributed to the effect, such as extremes in weather at or immediately after applications, or tank mixes/combinations of pesticides.

4.2. Case-specific agronomic and phenotypic endpoints

In addition to the generic agronomic and phenotypic endpoints outlined in Section 4.1, applicants should consider the relevance of including other additional relevant endpoints on a case-by-case basis. Whenever relevant, the following three categories of case-specific endpoints should be considered:

- trait-specific endpoints;
- endpoints related to potential unintended effects;
- endpoints related to persistence and invasiveness.

4.2.1. Trait-specific endpoints

Trait-specific endpoints go beyond the spectrum of generic agronomic and phenotypic endpoints proposed in Section 4.1. Depending on the trait intentionally introduced into the GM plant, applicants may decide to measure additional plant characteristics (e.g. pollen characteristics, flower (corolla) colour, number of flowers per plant, or other fruit characteristics) to further characterise the plant's biology and performance. For example, it can be important to assess the efficacy of male sterility and restorer genes (e.g. barstar/barnase) and to assess whether pollen characteristics have been altered in the case of sterile GM plants. Likewise, GM plants with traits specifically intended to reduce the susceptibility of the plant to a defined (a)biotic stressor may necessitate the consideration of additional endpoints (Sections 4.1.13 and 4.1.14).

If the objective of the genetic modification is to alter the composition of the plant, then it is recommended that additional agronomic and phenotypic endpoints associated with this intended compositional change be measured in field trials. For example, pest susceptibility could be a relevant endpoint for a GM plant exhibiting an altered level of an anti-nutrient or toxicant (reviewed by Hopkins et al., 2009; Bjorkman et al., 2011) or dormancy and survival characteristics, in the case of a GM plant with modified seed/tuber composition.

In specific cases, the genetic modification is designed to target specific pollen characteristics. Depending on the plant species, trait and the intended uses, altered pollen characteristics may affect pollen viability and thus exposure. Therefore, further characterisation of pollen characteristics may be required for those GM plants for which increased pollen viability is hypothesised.

4.2.2. Endpoints related to potential unintended effects

The selection of additional case-specific endpoints can also be driven by the identification of a potential unintended effect of the genetic modification, which could require further comparative analyses. Indications of potential unintended effects are therefore usually obtained after the agronomic and phenotypic comparison. However, the molecular characterisation might have already identified possible unintended effects, which may further structure the comparative analyses through the selection of endpoints related to the identified possible effects.

As comprehensive assessment of the entire agronomic and phenotypic dataset may be feasible only after completion of the field trials, it is recommended that representative samples are retained enabling supplementary analyses on a case-by-case basis.

4.2.3. Endpoints related to persistence and invasiveness

One source of potential environmental harm arising from the cultivation or import of GM plants is that they will persist in agricultural land or become invasive in non-agricultural land. Detailed guidelines on the assessment of the persistence and invasiveness potential of GM plants are available (EFSA GMO Panel, 2010a), which specifies the necessary information to test risk hypotheses concerning the persistence and invasiveness of GM plants.

Agronomic and phenotypic data may provide information relevant to the assessment of the persistence and invasiveness of some GM plants, as some intended and unintended differences may be associated with changes in the plant's biology and/or life cycle characteristics. Therefore, where considered relevant, agronomic and phenotypic data can be part of the weight of evidence that is used in the environmental risk assessment to evaluate whether the GM plant is likely to have significantly altered characteristics indicative of a change in persistence and invasiveness.

On an optional basis (e.g. when it could avoid the need for additional specific environmental risk assessment-oriented field trials), applicants can decide to expand the agronomic and phenotypic characterisation of the GM plant with additional measurements that are relevant to the persistence and

invasiveness assessment of GM plants, depending upon the plant species, the intended traits and the scope[27].

Many general predictors of persistence and invasiveness have been proposed in the literature, but only a few hold up under quantitative analysis. The most important are the ability to make a long-lived soil seed bank, small seeds, a short vegetative period before seeds are produced and a long flowering period, very high seed output and seed shattering (reviewed by Warwick et al., 2005; Kos et al., 2011). Focus on these characteristics will help to estimate the potential persistence and invasiveness of the GM plant, as changes in these endpoints may alter weediness or invasiveness and thus the plant's potential to cause environmental harm.

This is mostly relevant for species that are persistent in agricultural fields (e.g. potato, oilseed rape under cultivation conditions) and/or species that can establish temporary or persistent feral populations (e.g. oilseed rape under cultivation and import/processing conditions). For these species, it is helpful to make measurements of the ability of the plant to form a persistent seed bank and how this is affected by the genetic modification (e.g. if seeds have different oil contents). In such cases, measurements of the soil seed bank and population demographics will be required for the persistence and invasiveness assessment (as outlined in EFSA GMO Panel, 2010a). For species that do not persist in agricultural fields and/or establish temporary or persistent feral populations, such as maize and soybean in the EU, these additional measurements are not necessary.

- Seed dormancy potential tested under controlled conditions[28]:
  - viability testing of dormant seeds (to examine the fraction of seeds that remain viable after having acquired secondary dormancy (dark dormancy)) (Schatzki et al., 2013);
  - temperature-dependent germination testing in Petri dishes (see de Jong et al., 2013)[29]
- Seed survival tested under field conditions:
  - viability testing of seeds buried in the field at 10 to 20 cm depth for one year;
  - survey of volunteers in subsequent years in the field trials.

[27] Exposure and potential impact are expected to be the highest under cultivation conditions (Devos et al., 2012). However, under import/processing conditions, the context usually differs, as only a few GM plants are expected to be present in the environment. Therefore, low-exposure scenarios can be expected to reduce the likelihood of one or more steps in a risk scenario, compared with a cultivation scenario (Roberts et al., 2014). It is therefore important to account for the intended uses of the GM plant when determining the necessary level of agronomic and phenotypic characterisation of GM plants (e.g. high-exposure scenarios for GM plant applications for cultivation and low-exposure scenarios for GM plant applications for import).

[28] Seed germination testing in Petri dishes establishes how many seeds have primary dormancy. Many weeds in agricultural fields (e.g. *Papaver* spp.) have strong primary dormancy. In crop plants, oilseed rape included, there is typically no primary dormancy and all viable seeds germinate readily under optimal conditions. Nevertheless, such species can develop a persistent seed bank in the soil through secondary dormancy. For instance, oilseed rape seeds germinate in light or dark, but when they are kept in the dark for a period of a few weeks and their germination is prevented, for instance because there is no water available, they will develop secondary dormancy (dark dormancy). When remaining in the dark and conditions become suitable (e.g. by adding water), the seeds no longer germinate. To estimate how this behaviour affects the ability to establish a persistent seed bank in the soil, additional methods are needed (other than ISTA guidelines) that are tailored to specific cases. For oilseed rape, specific methods for testing dark dormancy in the laboratory have been used (see Schatzki et al., 2013, and references therein) that are valid in the field (Gruber et al., 2004).

[29] If seeds do not germinate at low temperatures, then there is a greater chance that they will not germinate for a while and will develop secondary dormancy during that period.

5. Data analysis

In this section, recommendations are given for the analysis of agronomic and phenotypic data. Moreover, clarifications on specific aspects of the data analysis requirements set in previous existing guidance documents are offered (EFSA GMO Panel, 2010b, 2011a).

5.1. Data submission

Implementing Regulation (EU) No 503/2013 states that "*raw data should be provided in all cases and be in a suitable electronic format*".

Depending on the nature of the agronomic and phenotypic endpoints, data should be measured either as one value per plot (e.g. stand count or lodging) or as multiple values per plot (e.g. plant height or seed count per plant). For both type of endpoints, the raw data must be provided in a workable format, possibly suitable for direct analysis with the EFSA GMO statistical software[30,31]. If different statistical software is used, then the complete code, as well as all the specific files required for the correct execution of the analysis, should be provided.

In the case of endpoints producing multiple values per plot, applicants are requested to include the mean and standard error for each plot.

In designing the field trials, applicants should consider the likelihood that some sites and/or replicates may not produce data of an appropriate quality for a range of reasons outside the control of the applicant.

In line with the requirements outlined in Implementing Regulation (EU) No 503/2013 and EFSA guidelines (EFSA GMO Panel, 2010a, b), the field trial design should ensure that a representative portion of the environmental variability is captured (see Section 2). Therefore, applicants shall plan a sufficient number of sites and replicates to ensure representativeness and to account for possible unexpected damage on sites and/or plots. In any case, the number of sites and replicates shall never be less than eight sites replicated four times.

Whenever some sites and/or replicates must be (completely or partially) disregarded for reasons outside the control of the applicants, these shall be explicitly described.

If sites and/or replicates are discarded during the growing season (e.g. in the event of a hailstorm leading to poor data on yield), then the datasets already gathered and fulfilling quality standards must be included in the statistical analysis. In addition, the disregarded datasets must be submitted, even if they are of poor quality. These data may be informative for the assessment of the agronomic and phenotypic characteristics of the GM plant and the risk assessment in general.

5.2. Statistical analysis

Specific requirements for the analysis of field trial data, including agronomic and phenotypic data, are provided in the EFSA guidelines on the statistical considerations for the safety evaluation of GMOs (EFSA GMO Panel, 2010b) and the guidance document on risk assessment of food and feed from GM plants (EFSA GMO Panel, 2011a).

As discussed in van der Voet et al. (2011), there may be situations in which the test of equivalence cannot be carried out because of zero or very small variance between the non-GM reference varieties. The practical experience gained during the assessment of recent GM plant applications identified several of these situations. This may occur because of the nature of the endpoint, by chance or because

[30] http://www.efsa.europa.eu/en/gmo/gmoanalysissoftware.htm.
[31] The annex within the supporting documentation of the EFSA GMO statistical software provides a detailed description of the requested information.

of some unexpected event, such as specific climatic conditions minimising variance between the non-GM reference varieties. This makes it impossible to categorise the outcome of the analysis into any of the seven types of outcomes described in the guidance document on risk assessment of food and feed from GM plants (EFSA GMO Panel, 2011a). If this occurs for specific endpoints, owing to their nature (e.g. lodging) the applicant can categorise the outcome only as (1) no-difference or (2) difference from its conventional counterpart. However, if this occurs for several uncorrelated endpoints, it may indicate that the non-GM reference varieties and/or REs did not sufficiently capture the range of natural variation. Should this occur, applicants may be requested to submit additional data to ensure sufficient representativeness (see Section 2.1). Practical experience gained during the assessment of recent GM plant applications also indicates the sporadic occurrence of an outcome type not anticipated in the guidance documents on the risk assessment of food and feed from GM plants (EFSA GMO Panel, 2010b, 2011a). This corresponds to the case of no difference between the GM line and its conventional counterpart, and of no equivalence with the non-GM reference varieties. This might occur by chance for a single or a few endpoint(s) if a very large number of endpoints are tested. However, if several uncorrelated endpoints have this outcome, then it may indicate that the GM plant and its conventional counterpart are derived from varieties with characteristics not present in the non-GM reference varieties and, consequently, the test material may not have been chosen appropriately.

Finally, for the endpoints for which the statistical approach described in the abovementioned EFSA guidance documents (EFSA GMO Panel, 2010b; 2011a) cannot be implemented, a proper statistical analysis should be submitted (e.g. through non-parametric methods) and its implications for the risk assessment discussed.

5.3. Analysis of genotype × environment interactions

Implementing Regulation (EU) No 503/2013 states that "*in the case of significant difference and/or lack of equivalence for any particular endpoint, further statistical analysis shall be carried out to assess whether there are interactions between any of the test materials and site*".

In addition, the statistical guidance document (EFSA GMO Panel, 2010b) states that "*to aid the identification of unintended effects that might otherwise be missed in an overall analysis*", applicants "*should allow for the possibility of checking for possible site-specific effects, i.e. genotype by site interactions*".

The analysis of G × E interactions is mandatory in the case of significant differences and/or lack of equivalence of agronomic and phenotypic characteristics. In the case of significant interactions, applicants are requested to perform a per-site analysis to verify whether the observed change(s) occur under specific environmental and agricultural conditions (e.g. whether differences between the GM plant and its comparator occur only in a specific soil type across sites) and to discuss the implications in terms of risk assessment.

In addition, in order to ensure that the outcomes of the agronomic and phenotypic characterisation carried out in the application provide enough confidence to draw conclusions on the GM event, whatever the RE in which it may be used, the EFSA GMO Panel recommends that applicants assess, in all cases, to what extent the environmental and agricultural conditions under which the GM plant may be grown affect any differences observed between the GM plant and the test materials.

If significant G × E interactions are identified, then a per-site analysis should be carried out to provide input for further assessment of the experimental data. Applicants are requested to interpret any major variability in observed differences from site to site within the comparative analysis and to assess whether site differences are related to specific characteristics of REs (e.g. whether differences between the GM plant and its comparator occur only in a specific soil type across sites), and to analyse their implications in terms of the risk assessment. Such an analysis usually requires a more detailed description of sites in terms of the meteorological, agronomic and biotic factors, as outlined in Section 3.2.

5.4. Correlated endpoints

The endpoints used to support the agronomic and phenotypic characterisation of GM plants cover plant development along the whole growing season, from seeding to harvesting, characterising the development of the GM plant at different stages. Many of the selected endpoints are correlated (e.g. initial plant stand and final plant stand, number of fruit and seed weight) and thus are not independent. Therefore, differences observed in specific endpoints may be due to differences in other endpoints and not to the genetic transformation. In order to decipher the source of differences and to help draw conclusions on the unintended effects, in the case of differences and/or lack of equivalences, applicants are requested to discuss the relationship between yield components (yield components analysis) such as the number of pods vs. final plant density, number of seeds per area unit vs. plant density, and seed size vs. number of seeds per unit area.

DOCUMENTATION PROVIDED TO EFSA

1. Proposal for a self-task mandate of the EFSA GMO Panel to establish a new Working Group to supplement the guidelines on the agronomic and phenotypic characterisation of GM plants. May 2013. Submitted by the Chair of the EFSA GMO Panel.

2. Acceptance of the self-task mandate of the EFSA GMO Panel to establish a new Working Group to supplement the guidelines on the agronomic and phenotypic characterisation of GM plants. July 2013. Submitted by the Executive Director of EFSA.

3. Request of deadline extension of the self-task mandate of the EFSA GMO Panel to publish the guidelines on the agronomic and phenotypic characterisation of GM plants. March 2015. Submitted by the Chair of the EFSA GMO Panel.

REFERENCES

Bjorkman M, Klingen I, Birch ANE, Bones AM, Bruce T, Johansen T, Meadow R, Molmann J, Selijasen R, Smart L and Stewart D, 2011. Phytochemicals of Brassicaceae in plant protection and human health—influences of climate, environment and agronomic practice. Phytochemistry, 72, 538–556.

CABI, 2014. Crop Protection Compendium. Available online: http://www.cabi.org/cpc

Codex Alimentarius, 2009. Food derived from modern biotechnology, 2nd edition. WHO/FAO, Rome. Available online: ftp://ftp.fao.org/codex/publications/Booklets/Biotech/Biotech_2009e.pdf

de Jong T, Isanta MT and Hesse E, 2013. Comparison of the crop species *Brassica napus* and wild *B. rapa*: characteristics relevant for building up a persistent seed bank in the soil. Seed Science Research, 23, 169–179.

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

Devos Y, Aguilera J, Diveki Z, Gomes A, Liu Y, Paoletti C, du Jardin P, Herman L, Perry JN and Waigmann E, 2014. EFSA's scientific activities and achievements on the risk assessment of genetically modified organisms (GMOs) during its first decade of existence: looking back and ahead. Transgenic Research, 23, 1–25.

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010a. Guidance on the environmental risk assessment of GM plants. EFSA Journal 2010;8(11):1879, 111 pp. doi:10.2903/j.efsa.2010.1879

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2010b. Statistical considerations for the safety evaluation of GMOs. EFSA Journal 2010;8(1):1250, 59 pp. doi:10.2903/j.efsa.2010.1250

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011a. Guidance for risk assessment of food and feed from GM plants. EFSA Journal 2011;9(5):2150, 37 pp. doi:10.2903/j.efsa.2010.2150

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), 2011b. Guidance document on selection of comparators for the risk assessment of GM plants. EFSA Journal 2011;9(5):2149, 20 pp. doi:10.2903/j.efsa.2010.2149

EFSA (European Food Safety Authority), 2014. Indicative timelines for submitting additional or supplementary information to EFSA during the risk assessment process of regulated products. EFSA Journal 2014;12(1):3553, 37 pp. doi:10.2903/j.efsa.2010.3553

EPPO (European and Mediterranean Plant Protection Organization), 2012. Design and analysis of efficacy evaluation trials. Standard PP 1/152(4). OEPP/EPPO Bulletin, 42, 367–381. Available online: pp1.eppo.int/downpdf.php5?id=569f48de0f4516cef66fae830dbf5898le

EPPO (European and Mediterranean Plant Protection Organization), 2014. Efficacy evaluation of plant protection products. Standard PP 1/135 (4). OEPP/EPPO Bulletin, 44, 265–273. Available online: http://pp1.eppo.int/downpdf.php5?id=3001dc9e238cd0ccf3d75f66acb9df62le

EU (European Union), 2008. Genetically modified organisms (GMOs). Council conclusions. Available online: http://register.consilium.europa.eu/doc/srv?l=EN&f=ST%2016882%202008%20INIT

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization), 2000. Safety aspects of genetically modified foods of plant origin. Report of a Joint FAO/WHO Expert Consultation on Foods Derived from Biotechnology, Geneva, Switzerland, 29 May to 2 June. WHO, Geneva. Available online: http://www.fao.org/fileadmin/templates/agns/pdf/topics/ec_june2000_en.pdf

Garcia-Alonso M, 2010. Current challenges in environmental risk assessment: the assessment of unintended effects of GM crops on non-target organisms. IOBC/WPRS Bulletin, 52, 57–63.

Garcia-Alonso M, 2013. Safety assessment of food and feed derived from GM crops: using problem formulation to ensure "fit for purpose" risk assessments. Collection of biosafety reviews. Available online: http://www.icgeb.org/~bsafesrv/pdffiles/GarciaAlonso.pdf

Gower JC, 1967. Multivariate analysis and multidimensional geometry. The Statistician, 17,13–28.

Gray A, 2012. Problem formulation in environmental risk assessment for genetically modified crops: a practitioner's approach. Collection of Biosafety Reviews. Available online: http://www.icgeb.org/~bsafesrv/pdffiles/Col6_Gray.pdf

Gruber S, Pekrun C and Claupein W, 2004. Seed persistence of oilseed rape (*Brassica napus*): variation in transgenic and conventionally bred cultivars. Journal of Agricultural Science, 142, 29–40.

He H, de Souza Vidigal D, Snoek LB, Schnabel S, Nijveen H, Hilhorst H and Bentsink L, 2014. Interaction between parental environment and genotype affects plant and seed performance in *Arabidopsis*. Journal of Experimental Botany, 65, 6603–6615.

Hopkins RJ, van Dam NM and van Loon JJA, 2009. Role of glucosinolates in insect–plant relationships and multitrophic interactions. Annual Review of Entomology, 54, 57–83.

Horak MJ, Rosenbaum EW, Woodrum CL, Martens AB, Mery RF, Cothren JT, Burns JA, Nickson TE, Pester TA, Jiang C, Hart JL and Sammons B, 2007. Characterization of Roundup Ready Flex Cotton, "MON 88913", for use in ecological risk assessment: evaluation of seed germination, vegetative and reproductive growth, and ecological interactions. Crop Science, 47, 268–277.

Inselberg A, 2009. *Parallel coordinates: visual multidimensional geometry and its applications*. Springer-Verlag New York, Inc., Secaucus, NJ, USA.

ISRIC (World Soil Information), 2002. Available online: http://www.isric.org/isric/webdocs/docs/ISRIC_TechPap09_2002.pdf

ISTA (International Seed Testing Association), 2015. International Rules for Seed Testing. Seed health testing. ISTA, Bassersdorf, Switzerland. Available online: http://www.ingentaconnect.com/content/ista/rules/2015/00002015/00000001

Kok EJ and Kuiper HA, 2003. Comparative safety assessment for biotech crops. Trends in Biotechnology, 21, 439–444.

Kos SP, de Jong TJ and Tamis WLM, 2012. Can transgenic crops go wild? A literary study on using plant traits for weediness pre-screening. COGEM Research Report: CGM 2012-01. Available online: http://www.cogem.net/showdownload.cfm?objectId=0C4BCF62-1517-64D9-CC425A5BA49399BA&objectType=mark.hive.contentobjects.download.pdf

Liu J and Pattey E, 2010. Retrieval of leaf area index from top-of-canopy digital photography over agricultural crops. Agricultural and Forest Meteorology, 150, 1485–1490.

Macdonald P, 2012. Developing workable regulatory frameworks for the environmental release of transgenic plants. Collection of Biosafety Reviews. Available online: http://www.icgeb.org/~bsafesrv/pdffiles/Col6_Macdonald.pdf

Meier U (ed.), 2001. Growth stages of mono-and dicotyledonous plants, 2nd edition. BBCH Monograph. Federal Biological Research Centre for Agriculture and Forestry, Bonn, Germany.

MoE, (Ministry of the Environment) 2004. Concerning the application for approval of type 1 use regulations with regard to the genetically modified plants, the production or circulation of which falls within the jurisdiction of the Minister of Agriculture, Forestry and Fisheries, Tokyo. English translation available online: http://www.bch.biodic.go.jp/english/law.html

Nickson TE, 2008. Planning environmental risk assessment for genetically modified crops: problem formulation for stress-tolerant crops. Plant Physiology, 147, 494–502.

OECD (Organisation for Economic Cooperation and Development), 1993. Safety evaluation of foods derived by modern biotechnology. Concepts and principles. OECD, Paris. Available online: http://www.oecd.org/env/ehs/biotrack/41036698.pdf

OECD (Organisation for Economic Cooperation and Development), 1999. The application of the GLP principles to field studies. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, No 6. OECD Publishing, Paris.

OGTR (Office of the Gene Technology Regulator), 2009. Application for licence for dealings with a GMO involving intentional release of the GMO into the environment (DIR). OGTR, Canberra. Available online: http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/content/dirform-1/$FILE/dirform5.pdf

Raybould A, Tuttle A, Shore S and Stone T, 2010. Environmental risk assessments for transgenic crops producing output trait enzymes. Transgenic Research, 19, 595–609.

Raybould A, Higgins LS, Horak MJ, Layton RJ, Storer NP, De La Fuente JM and Herman RA, 2012. Assessing the ecological risks from the persistence and spread of feral populations of insect-resistant transgenic maize. Transgenic Research, 21, 655–664.

Roberts A, Devos Y, Raybould A, Bigelow P and Gray A, 2014. Environmental risk assessment of GE plants under low-exposure conditions. Transgenic Research, 23, 971–983.

Schnell J, Steele M, Bean J, Neuspiel M, Girard C, Dormann N, Pearson C, Savoie A, Bourbonniere L and Macdonald P, 2015. A comparative analysis of insertional effects in genetically engineered plants: considerations for pre-market assessments. Transgenic Research, 24, 1–17.

Schatzki J, Allam M, Klöppel C, Nagel M, Börner A and Möllers C, 2013. Genetic variation for secondary seed dormancy and seed longevity in a set of blackseeded European winter oilseed rape cultivars. Plant Breeding, 132, 174–179.

Steven MD, Biscoe PV, Jaggard KW and Paruntu J, 1986. Foliage cover and radiation interception. Field Crops Research, 13, 75–87.

Tabery J, 2008. R.A. Fisher, Lancelot Hogben, and the origin(s) of genotype–environment interaction. Journal of the History of Biology, 41, 717–761.

Thompson A and Taylor BN, 2008. The International System of Units (SI). Special publication 330. National Institute of Standards and Technology, Gaithersburg, MD, USA.

Tufte ER, 1990. *Envisioning information*. Graphics Press, Cheshire, CT, USA.

USDA (United States Department of Agriculture), 1996. Guide for preparing and submitting a petition for genetically engineered plants. USDA Animal and Plant Health Inspection Service, Washington, DC, USA. Available online: http://www.aphis.usda.gov/brs/pdf/usergen8.pdf

USDA-NCRS (United States Department of Agriculture– Natural Resources Conservation Service), 1999. Soil taxonomy. A basic system of soil classification for making and interpreting soil surveys. Available online: http://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_051232.pdf

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

Warwick SI and Stewart CN Jr, 2005. Crops come from wild plants—how domestication, transgenes, and linkage together shape ferality. In: *Crop ferality and volunteerism*. Ed. Gressel J. CRC Press, Boca Raton, FL, USA, 9–30.