SCIENTIFIC OPINION

Guidance on selection of comparators for the risk assessment of genetically modified plants and derived food and feed[1]

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ABSTRACT

This opinion provides guidance in the area of comparators taking into account the requirements for the molecular characterisation, the food and feed and the environmental risk assessments. A key step in the risk assessment of genetically modified (GM) plants and derived food and feed is the identification of intended and unintended differences and equivalences between the GM plant and its comparator(s), taking into account the range of natural variation. In line with Regulation (EC) No 1829/2003 and Directive 2001/18/EC, the EFSA GMO Panel has, to date, required the use of non-GM lines with comparable genetic background as comparators. In the case of vegetatively propagated crops, these are the isogenic lines. In the case of sexually propagated crops these are non-GM lines as close as possible genetically to the GM plant under assessment. The identification and production of such comparators is becoming increasingly challenging due to the increasing complexity of GM plants, e.g. those developed by combining (stacking) events through conventional crosses, or those in which extensive compositional changes are targeted. Consequently, the EFSA GMO Panel has developed this guidance on the selection of comparators for the risk assessment of GM plants and derived food and feed. Whilst considering the requirements of Directive 2001/18/EC and Regulation (EC) No 1829/2003, the EFSA GMO Panel provides options which introduce flexibility in the selection of comparators based on sound scientific principles. This document addresses the selection of comparators for GM plants containing single or multiple events stacked by either conventional breeding, or by other approaches such as re-transformation, co-transformation and the use of multiple gene cassettes. The EFSA GMO Panel also considers situations where additional comparators may be required on a case-by-case basis and scenarios where appropriate comparators are not available (e.g. where extensive compositional changes are targeted). The EFSA GMO Panel recognises the different requirements for comparators for the molecular characterisation, food and feed and environmental components of the risk assessment.

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KEY WORDS

GMO, comparators, risk assessment, stacked events, stack, guidance, conventional counterpart, comparative approach, Regulation (EC) No 1829/2003, Directive 2001/18/EC, GM plant

SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Genetically Modified Organisms (GMO Panel) to develop further guidance in the area of comparators taking into account the requirements for the molecular characterisation, the food and feed and the environmental risk assessments. A key step in the risk assessment of GM plants and derived food and feed is the identification of intended and unintended differences and equivalences between the GM plant and its comparator(s), taking into account the range of natural variation. This information allows the assessment of the potential impact of the genetic modification with respect to human and animal health and the environment. Regulation (EC) No 1829/2003 on genetically modified food and feed defines the comparator (conventional counterpart) as "*similar food or feed produced without the help of genetic modification and for which there is a well-established history of safe use*". The EFSA GMO Panel has, to date, required as comparators either non-GM lines with a genetic background as close as possible to the GM plant under assessment in case of sexually propagated crops, or isogenic varieties in case of vegetatively propagated crops. The identification and production of such comparators is becoming increasingly challenging due to the increasing complexity of GM plants, e.g. those developed by combining (stacking) events through conventional breeding, or those in which significant compositional changes are targeted. The EFSA GMO Panel also considers situations where additional comparators may be required on a case-by-case basis and scenarios where appropriate comparators are not available (e.g. where extensive compositional changes are targeted). Whilst considering the requirements of Directive 2001/18/EC and Regulation (EC) No 1829/2003, the EFSA GMO Panel provides options which introduce flexibility in the selection of comparators based on sound scientific principles.

In summary the key conclusions and recommendations of this document are:

1. The EFSA GMO Panel supports the current concept that for GM plants containing a single event the choice of comparator must be the conventional counterpart which will be a non-GM genotype with a genetic background as close as possible to the GM plant. Applicants can also consider the use of additional comparator(s).

2. The same principle as outlined above applies to GM plants containing events stacked by conventional breeding or by other approaches, such as co-transformation, re-transformation and the use of multiple gene cassettes. In the case of GM plants containing stacked events, the risk assessment focuses on the potential interaction between the events present and their stability. However, where applicants can demonstrate that a conventional counterpart for the GM plant containing stacked events cannot be made available, applicants can use as comparators for the molecular characterisation (MC) and the food and feed (FF) risk assessment either:
   a. A negative segregant(s) - but only where segregants are derived from crosses between GM plants containing events which have been risk assessed previously and which are all stacked in the GM plant under assessment. This approach is only possible if either no unintended effects have been identified for the single events, or where the presence of such unintended effects in the GM plant containing the stacked events does not raise safety concerns.
   b. Any set of GM plants that have all been risk assessed on the basis of experimental data collected according to the principles of EFSA MC and FF risk assessment. This set of GM

plants must include, between them, all of the events stacked in the GM plant under assessment and no others.

For the environmental risk assessment (ERA), in case the conventional counterpart cannot be made available, different comparator(s) are appropriate depending upon the issue(s) under consideration.

3. In cases where appropriate comparators are not available (e.g. where significant compositional changes have been targeted) the EFSA GMO Panel considers to carry out a comprehensive safety/nutritional assessment on the GM plant *per se*.

4. The risk assessment of GM plants, containing either single or stacked events, expressing specific traits such as herbicide tolerance, may require additional treatment comparisons.

The EFSA GMO Panel recognises that there may be different requirements for comparators for the molecular characterisation, the food and feed and the environmental components of the risk assessment and takes this into account in providing this guidance.

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BACKGROUND AS PROVIDED BY EFSA

The selection of appropriate comparators is central to the comparative approach in the risk assessment of genetically modified plants and derived food and feed. Regulation (EC) No 1829/2003 (EC, 2003) on genetically modified food and feed defines the comparator (conventional counterpart) as "*similar food or feed produced without the help of genetic modification and for which there is a well-established history of safe use*". Along the same lines, for molecular characterisation (MC) and food and feed (FF) risk assessment, Codex Alimentarius defines a conventional counterpart as a "*related organism/variety, its components and/or products for which there is experience of establishing safety based on common use as food"* recognising that "*for the foreseeable future, foods derived from modern biotechnology will not be used as conventional counterparts*" (Codex Alimentarius, 2009).

For environmental risk assessment (ERA), the European Commission Decision 2002/623/EC (EC, 2002) in support to Annex II of Directive 2001/18/EC (EC, 2001), state that "*identified characteristics of the GMO and its use which have the potential to cause adverse effects should be compared to those presented by the non-modified organisms from which it is derived and its use under corresponding situations*". The purpose of this comparison is to assist in identifying the particular potential adverse effects arising from the genetic modification. In addition the same EC Decision indicates that "*Information from releases of similar organisms and organisms with similar traits and their interaction with similar environments can assist the ERA*".

In line with the above, the EFSA GMO Panel has, to date, required as comparators either non-GM lines with a genetic background as close as possible to the GM plant under assessment in case of sexually propagated crops, or isogenic varieties in case of vegetatively propagated crops. The extent to which these non-GM comparators are genetically related to the GM plant under assessment varies depending upon the breeding scheme used for the production of both the GM plant and its comparator(s).

The identification and production of such comparators is becoming increasingly challenging due to the increasing complexity of breeding schemes and the GM plants themselves, e.g. those developed by combining (stacking) events through conventional breeding, or those in which significant compositional changes are targeted. Consequently the EFSA GMO Panel was requested to develop further guidance for the selection of comparators.

TERMS OF REFERENCE AS PROVIDED BY EFSA

The EFSA GMO Panel was requested by EFSA to develop a guidance document on the selection of comparators for the risk assessment of GM plants. Specific issues addressed in this guidance include:

- the selection of an appropriate comparator for the risk assessment of GM plants containing single or stacked events;
- the role of negative segregants in the risk assessment process;
- the selection of appropriate comparators in the case of GM plants containing stacked events obtained by techniques other than conventional breeding;
- the selection of comparators in cases where the current comparative approach may not be suitable for the risk assessment of the GM plants (e.g. where major compositional changes are targeted).

The EFSA GMO Panel was requested to draft a guidance to be released for public consultation. A draft guidance was published on the EFSA website from 15th November 2010 until 15th January 2011 for public consultation. At the deadline EFSA had received 139 submissions from 18 stakeholders. The table of all comments received, together with a summarised response to the most relevant ones, is published on the EFSA website http://www.efsa.europa.eu. A consultative stakeholder workshop was held after the public consultation (31st March 2011) to further discuss and clarify issues raised during the public consultation. Subsequently, the draft guidance was revised taking into account all of the scientific comments which enhanced both scientific quality and clarity.
The guidance was adopted on 14 April 2011.

1. Introduction

The current risk assessment strategy for GM plants and derived food and feed comprises a molecular characterisation of the genetic modification, a comparative analysis of the compositional, agronomic and phenotypic characteristics of the GM plant and its appropriate comparator(s), and an assessment of their potential impact on human and animal health and the environment. The starting point of the risk assessment is the identification of differences (intended and unintended) between the GM plant and derived food and feed, and its comparator(s) (EFSA, 2011a).

The MC component of the risk assessment is primarily focused on the analysis of the GM plant itself, but the inclusion of a non-GM comparator can provide valuable information on a case-by-case basis.

For GM plants containing stacked events the primary concern for the risk assessment is to establish that the combination of events is stable and that no interactions occur between the stacked events that may raise safety concerns compared to the single events. In addition, the ERA considers to what extent the combination of events in a GM plant results in changes in management systems which could lead to additional environmental impacts compared to the management of the GM plants containing these events independently.

Comparative studies are used as a major, but not unique, tool throughout the risk assessment and the selection of appropriate comparators for each of these comparative studies is crucial.

1.1. Comparative assessment: the difference and equivalence tests

The comparative analysis for FF risk assessment and ERA requires the simultaneous application of two complementary tests: the test of difference and the test of equivalence (EFSA, 2010a, 2011a).

The test of difference is used to verify whether the GM plant, apart from the introduced genetic modification(s), is different from its comparator and could have the potential to cause adverse effects.

The test of equivalence, in FF risk assessment, is used to verify whether the agronomic, phenotypic and compositional characteristics of the GM plant fall within the range of natural variation. The range of natural variation is estimated from a set of non-GM reference varieties with a history of safe use (EFSA, 2010a). Therefore these non-GM reference varieties fulfil the requirements of Reg. (EC) No 1829/2003, which states that the comparison of the GM plant should be made "*with a similar food or feed produced without the help of genetic modification and for which there is a well-established history of safe use*". The test of equivalence, in ERA, verifies whether the GM plant is equivalent or not to its comparator within bounds defined by so-called 'limits of concern', i.e. limits which if exceeded may potentially lead to environmental harm; these are estimated from literature data, modelling, existing knowledge and protection goals (Perry et al., 2009).

A description of the strategy recommended by the EFSA GMO Panel for the practical implementation of the comparative approach in the risk assessment of GM plants is available in the EFSA guidance document for the risk assessment of GM plants and derived food and feed (EFSA, 2011a). Such a strategy is also described in the EFSA GMO Panel opinion on the statistical considerations for the safety evaluation of GMOs (EFSA, 2010a) and is adopted in the EFSA guidance document on the ERA of GM plants (EFSA, 2010b).

The present document provides guidance on the criteria to follow for the selection of the most appropriate comparator(s) in the risk assessment of GM plants under different scenarios.

1.2. Comparator(s): current status

To date the EFSA GMO Panel has required the use of non-GM lines with comparable genetic background (i.e. near-isogenic lines in the case of sexually propagated crops and isogenic lines in the case of vegetatively propagated crops) as comparators in its evaluation of GM plant applications. The experience gained from the evaluation of GMO applications under Dir. 2001/18/EC and Reg. (EC) No 1829/2003 is that the extent to which such non-GM comparators are genetically related to the GM plant under assessment varies. Such variation may be related to the breeding scheme used for the production of both the GM plant and its non-GM comparator(s), and to the degree of complexity of the GM plant under assessment, as may be the case when several events are stacked. The potential variability in the degree of genetic similarity between the GM plant and its comparator(s) does not necessarily compromise the reliability of the safety assessment, provided that the comparator is genetically "as close as possible" to the GM plant with regard to its breeding pedigree. The comparator should preferably be derived from the breeding scheme used to derive the GM plant. For FF, the comparative approach in risk assessment requires the inclusion of non-GM reference lines in the equivalence test to verify whether any difference observed between the GM plant and its comparator(s) falls or not within the range of natural variation.

The EFSA guidance document for the risk assessment of GM plants and derived food and feed (EFSA, 2011a) states that:

"The EFSA GMO Panel recommends the use of the term "conventional counterpart" only when referring to: i) the non-GM isogenic variety, in the case of vegetatively propagated crops; ii) a genotype with a genetic background as close as possible to the GM plant, in the case of crops that are propagated sexually. [...] The risk assessment of GM plants containing single events should include the conventional counterpart, as defined above. Additional comparators, e.g. a negative segregant, may be included if deemed useful to support the risk assessment".

[...]

"In all cases, the applicant should provide information on the breeding scheme (pedigree) in relation to the GM plant, the conventional counterpart and/or other comparator(s) used in the risk assessment together with a clear justification for their selection".

The experience gained from the evaluation of GMO applications under Dir. 2001/18/EC and Reg. (EC) No 1829/2003 is that the extent to which such non-GM comparators are genetically related to the GM plant under assessment varies. Such variation may be related to the breeding scheme used for the production of both the GM plant and its non-GM comparator(s), and to the degree of complexity of the GM plant under assessment, as may be the case when several events are stacked. The potential variability in the degree of genetic similarity between the GM plant and its comparator(s) does not necessarily compromise the reliability of the safety assessment, provided that the comparator is genetically as close as possible to the GM plant with regard to its breeding pedigree. The comparator should preferably be derived from the breeding scheme used to derive the GM plant. For FF, the comparative approach in risk assessment requires the inclusion of non-GM reference lines in the equivalence test to verify whether any difference observed between the GM plant and its comparator falls or not within the range of natural variation.

The EFSA ERA guidance document (EFSA, 2010b) states that *"In an ERA, it is appropriate to draw on previous knowledge and experience and to use the conventional counterpart in order to highlight differences associated with the GM plant in the receiving environment(s).*"

1.3. Terminology

Comparator and Conventional Counterpart

Various terms have been used synonymously to describe non-GM comparators used in the risk assessment of GM plants. These include the terms control, non-GM comparator, conventional counterpart, non-GM reference lines and non-GM reference varieties.

For clarity the EFSA GMO Panel recommends the use of the term "conventional counterpart" only when referring to a non-GM comparator as described in the EFSA guidance document on the risk assessment of GM plants and derived food and feed (EFSA, 2011a) and in the EFSA ERA guidance document (EFSA, 2010b): i) in the case of vegetatively propagated crops, the conventional counterpart is the non-GM isogenic line; ii) in the case of crops that are propagated sexually, the conventional counterpart is a non-GM genotype with a genetic background as close as possible to the GM plant.

The term "comparator" should be used in all other cases, i.e. cases in which the comparative assessment includes genotypes which do not fit with the definition of conventional counterpart as provided above.

Event

An event is the unique DNA recombination that takes place in one plant cell from which the entire GM plant is regenerated.

GM plant

Directive 2001/18 (EC, 2001) defines a genetically modified (GM) plant, as one in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination. Inclusions and exclusions from this definition are described in Annex 1a of the Directive.

Isogenic and near-isogenic lines

In the case of a GM plant, its isogenic line is the non-GM line from which the GM plant is derived. Thus, the only difference between the isogenic line and the derived GM plant is the presence of the recombinant DNA. Near-isogenic lines are lines genetically identical to the GM plant except for some loci.

Negative segregant (null-segregant)

Plants that are negative segregants lack the transgenic event and can be produced, for example, by self-fertilisation of hemizygous GM plants, or from crosses between hemizygous GM plants and non-GM plants.

Segregation

Segregation is the separation of hereditary genetic material into different cells during meiotic cell division. In meiosis, individual chromosomes of each chromosome pair are separated into daughter

cells. In the case of GM plants, segregation of stacked events can result in the production of GM plants (i.e. progeny) with a lower number of stacked events.

Stacked events

Events can be combined or "stacked" by conventional breeding or other approaches (e.g. re-transformation) to produce a GM plant containing stacked events.

2. The need for further elaboration on guidance for the selection of comparator(s)

Guidance on the criteria to be followed for the selection of suitable comparators(s) in GM plant risk assessment needs to be revised to accommodate advances in agricultural biotechnology research and development, particularly with respect to the increasing complexity of GM plants containing stacked events, and the traits likely to be modified in future GM plants. The main issues addressed by the EFSA GMO Panel in this document are listed below.

- Comparator(s) for GM plants containing single events

  In this document the EFSA GMO Panel confirms the current principles for the selection of comparator(s) for GM plants containing single events (EFSA, 2011a) and assesses the possible use of additional comparators.

- Comparator(s) for GM plants containing events stacked by conventional breeding

  When multiple events are combined into a new GM plant by conventional breeding between existing GM lines, the primary concern for both MC and FF risk assessment and ERA is to establish that this new combination of events is stable and does not result in interactions that may raise safety concerns, as compared to single events (EFSA, 2011a). The production of a conventional counterpart for GM plants with events stacked by conventional breeding is becoming increasingly difficult due to the complexity of the commercial breeding programs used, and the number of events combined in the GM plant.

- Comparator(s) for GM plants containing events stacked by methods other than conventional breeding

  To date guidance on the selection of comparators for GM plants containing stacked events has focused on stacking by conventional breeding. As other approaches can be used for the stacking of genes and events (e.g. multiple gene cassettes, co-transformation, and re-transformation) the EFSA GMO Panel has also considered in this document the selection of comparators in relation to the use of these approaches.

- Cases where appropriate comparators are not available and a comprehensive risk assessment is required

  The development of GM plants targeted towards major compositional changes is progressing rapidly. This includes, for example, the development of crops with modified metabolism and physiology to provide improved quality and enhanced nutritional profiles. In such cases plant composition may be modified to such an extent that for FF risk assessment an appropriate

comparator cannot be identified for the species in question. In such cases the risk assessment requires an alternative approach.

3. Guidance on the selection of comparator(s)

3.1. Comparator(s) for GM plants containing single events

For FF risk assessment (EFSA, 2011a) and ERA (EFSA, 2010b) the risk assessment of GM plants containing single events includes a conventional counterpart. In the case of crops vegetatively propagated the conventional counterpart is the non-GM isogenic line. In the case of crops propagated by sexual reproduction the conventional counterpart should have a genetic background as close as possible to the GM plant under assessment.

The ERA of GM plants involves generating, collecting and assessing information from a wide variety of sources (EFSA, 2010b) which include: data from ecological field trials, agronomic field trials, field surveys, semi-field trials, molecular characterisation data, compositional data, ecotoxicological testing, modelling, desk and literature studies. Among these, the majority of comparative studies will include the GM plant under assessment and its conventional counterpart, with both receiving appropriate treatments and management regimes according to the requirements of the field study. However, depending on the GM plant and on the problem formulation, additional treatments/management regimes may need to be considered. Furthermore, for some ERA field trials (e.g. to assess the effects of management systems), alternative non-GM comparators may be considered. These could include, for example varieties or plants with agronomic properties as similar as possible to the GM plant, depending on the hypothesis to be tested and the impacts to be assessed. The management techniques applied to the comparator should be compatible with the principles of good agricultural practice and Integrated Pest Management that are being introduced by Member States under Directive 2009/128/EC (EC, 2009) establishing a framework for Community action to achieve the sustainable use of pesticide (see http://ec.europa.eu/environment/ppps/home.htm).

The MC component of the risk assessment of GM plants containing single events is primarily focused on the analysis of the GM plant itself, the inserted DNA and the regions flanking the insert in the GM plant. Information is also required on the expression of the insert. Data on the conventional counterpart may be required on a case-by-case basis, e.g. when the expression of an endogenous gene has been targeted for modification (EFSA, 2011a).

Additional comparators, e.g. a negative segregant, may be included if deemed useful to support the risk assessment.

In all cases, information on the breeding scheme (pedigree) in relation to both the GM plant and the conventional counterpart, together with a clear justification for the use of the selected conventional counterpart and, if appropriate, alternative or additional comparators shall be provided.

Field trials design

For compositional, phenotypic and agronomic comparative analyses , field trials will include: the GM plant under assessment, its conventional counterpart and non-GM reference-varieties, representative of those that would be normally grown in the areas where the field trials are performed (EFSA, 2010a, 2011a).

For ERA, field trials for comparative assessment will include the GM plant under assessment and its conventional counterpart, with both receiving appropriate treatments and management regimes according to the requirements of the field study. However, depending on the GM plant and on the problem formulation, additional treatments and management regimes or alternative comparators (e.g. varieties with agronomic properties as similar as possible to the GM plant) may need to be considered (EFSA, 2010b).

3.2. Comparator(s) for GM plants containing events stacked by conventional breeding

The EFSA guidance document on the risk assessment of GM plants and derived food and feed (EFSA, 2011a) and EFSA ERA guidance document (EFSA, 2010b) indicate that the risk assessment of GM plants containing stacked events requires the previous risk assessment of the GM plants containing these events independently (i.e. GM plants containing single events).

For GM plants containing stacked events, the primary concern for MC and FF risk assessment and ERA is to establish that the combination of events is stable and does not result in interactions that may raise safety concerns, as compared to single events. The risk assessment of GM plants containing stacked events shall then mainly focus on issues related to the stability of the inserts, and the potential synergistic or antagonistic effects resulting from the combination of the events.

In addition, the ERA considers to what extent the combination of events in a GM plant results in changes in management systems which could lead to additional environmental impacts compared to the management of the GM plants containing these events independently.

For FF risk assessment of GM plants containing events combined by conventional breeding the first choice of comparator is the conventional counterpart as defined in this document. Where applicants can demonstrate that a conventional counterpart is not available then applicants could use:

- Negative segregant(s), but only where the segregants are derived from crosses between GM plants containing events which have been risk assessed and which are all stacked in the GM plant under assessment. The breeding scheme used to produce the negative segregant(s) should be clearly illustrated and the negative segregant should be genetically as close as possible to the GM plant under assessment. This approach is only possible if either no unintended effects have been identified for the GM plants containing the single events or where the implications for the presence of such unintended effects in the GM plant containing the stacked events have been evaluated.

and/or

- Any set of GM plants that have all been risk assessed on the basis of experimental data collected according to the principles of EFSA MC and FF risk assessment (EFSA, 2011a). This set of GM plants must include between them all of the events stacked in the GM plant under assessment, and no others. This allows the analysis of potential interactions which may impact on safety. This set of GM plants may include either parental GM lines, if previously risk assessed, or GM plants containing the single events in case the parental GM line(s) has not been risk assessed. Additional comparators, e.g. negative segregants, can be included if deemed useful to support the risk assessment.

For example, if a GM plant containing five events has been produced by crossing a parent containing three events with a parent containing two events and no conventional counterpart is available, there are different possible scenarios:

- both GM parental plants have been risk assessed previously. These can be used as the comparators;

- the GM parental plant containing three events has been risk assessed, but not the one containing two events. The GM parental plant containing three events can be used as one comparator alongside the two already risk assessed GM plants containing the single events present in the other GM parental line;

- neither of the parental plants was risk assessed before. The comparators should be the five already risk assessed GM plants containing the single events stacked in the GM plant under assessment.

Similarly to what has been described in Section 3.1, the ERA of GM plants containing stacked events also encompasses a wide variety of different studies and the majority of comparative studies include the GM plant under assessment and its conventional counterpart, when this is available. However, depending on the GM plant under assessment and on the problem formulation, additional treatments and management regimes and/or alternative non-GM comparators may need to be considered, particularly for field trials. In addition to stability, expression and potential synergistic effects of the events, the ERA should consider to what extent the combination of events results in changes in management systems, which could lead to additional environmental impacts compared to the management of the GM plants containing these events independently.

As indicated in Section 1.1, the MC component of the risk assessment is primarily focused on the analysis of the GM plant itself, but some analyses on a non-GM comparator can provide valuable information. This may include, for example, data on the levels of specific proteins present in the non-GM plant which are the targets for gene silencing. For the MC assessment of interactions between events that could impact on the levels of the specific proteins (or in some cases specific RNAs or metabolites) under assessment, any set of GM plants that have all been risk-assessed and which include between them all of the events stacked in the GM plant under assessment but no others can be used as comparators.

In all cases information on the breeding scheme in relation to both the GM plant containing stacked events and the selected comparator(s), together with clear justification for the use of the comparator(s), shall be provided.

Field trials design

For compositional analysis in FF risk assessment, field trials will include: the GM plant containing stacked events under assessment, its conventional counterpart and non-GM reference-varieties, representative of those that would be normally grown in the areas where the field trials are performed (EFSA, 2010a, 2011a). In case a conventional counterpart is not available, it may be replaced by appropriate negative segregant(s) and/or the set of GM plants as defined above.

For ERA, field trials for comparative assessment should include the GM plant containing stacked events under assessment and its conventional counterpart. In case a conventional counterpart is not available, different comparator(s) may be appropriate depending upon the issue(s) under consideration. In particular:

- where studies utilise data arising from the field trials for compositional analysis mentioned above (often used to assess agronomic and phenotypic characteristics), the comparators will be identical to those listed above for FF risk assessment;

- to evaluate the impact on persistence and invasiveness, target organisms, non-target organisms, effects of management, cultivation and harvest, and biogeochemical processes the conventional counterpart can be substituted, on a case-by-case basis, by another non-GM line derived from the same breeding scheme used to develop the GM plant. Such a line could be genetically more distant from the GM plant than the conventional counterpart, but can still serve as an appropriate comparator. Alternatively, a non-GM line with agronomic properties as similar as possible to the GM plant containing stacked events can be used as an appropriate comparator. Applicants must justify the choice explicitly in such cases. The assessment of the effects of persistence and invasiveness requires information from specific experiments which tend to be of a case-specific, research-driven nature. The selection of the appropriate comparator should therefore be made on a case-by-case basis according to the effect studied.

For cultivation, it should be stressed that consideration of management is essential since interactions between the events on biota may occur even if the products of the genetic modification themselves do not interact directly. Applicants should consider whether the use of additional comparators, such as the parental lines, or negative segregants, may be appropriate.

3.3. Comparator(s) for GM plants containing events stacked by methods other than conventional breeding

To date the EFSA approach on the selection of comparators for GM plants containing stacked events has focused on stacking by conventional breeding. However, other approaches can be used for the stacking of genes and traits (e.g. co-transformation, re-transformation, and multiple gene cassettes). Here the EFSA GMO Panel considers the selection of comparators in relation to the use of these approaches.

3.3.1. Re-transformation

If an existing GM line (containing either single or multiple events) is re-transformed, the same principles apply as for Sections 3.1 and 3.2 above. This requires that the new event is segregated and compared with a conventional counterpart. However, in the unlikely situation that the new-event integrates at the same locus as the existing event(s), then applicants should provide evidence that independent segregation of the events is not possible.

Where applicants can demonstrate that a conventional counterpart does not exist then the comparator for a GM plant containing stacked events produced by re-transformation can be:

- For FF either the negative segregant or the recipient GM plant which must have been risk assessed previously (see Section 3.2).

- For ERA either another non-GM line used to develop the GM plant, or a non-GM line with agronomic properties as similar as possible to the GM plant under assessment.

For the MC assessment of interactions between events that could impact on the levels of specific proteins (or in some cases specific RNAs or metabolites) under assessment, any set of GM plants that have all been risk-assessed and which include between them all of the events stacked in the GM plant (and no others) used for re-transformation, should be included as comparators.

Field trials design

For compositional analysis in FF risk assessment, field trials will include: the GM plant under assessment, its conventional counterpart and non-GM reference-varieties, representative of those that would be normally grown in the areas where the field trials are performed (EFSA, 2010a, 2011a). In case a conventional counterpart is not available, it may be replaced by appropriate negative segregant(s) and/or the set of GM plants as defined above.

For ERA, field trials for comparative assessment will include the GM plant under assessment and the conventional counterpart, or if this is not available, either another non-GM line used to develop the GM plant, or a non-GM line with agronomic properties as similar as possible to the GM plant under assessment. The inclusion of the GM parental line is recommended as an additional comparator.

3.3.2. Co-transformation

Multiple genes or sequences that modify gene expression can be co-transformed into plants using two or more individual DNA molecules, each harbouring different transformation cassettes. If the receiving plant is non-GM, the comparator should be the conventional counterpart as in the case of GM plants containing single events (see Section 3.1). In co-transformation the transformation cassettes may or may not integrate at the same locus within the genome. If they do not then independent segregation of inserts derived from each cassette in subsequent progenies is likely. The applicant should either provide evidence that segregation of the functional inserts and traits does not occur or, where segregation is possible, provide a risk assessment of the GM plants containing the segregating single events, including all their possible sub-combinations. In this case the comparator should be the conventional counterpart. If co-transformation is used to re-transform an existing GM plant the applicant should follow the guidance for FF and ERA provided in section 3.3.1.

3.3.3. Transformation cassette containing multiple genes

If a GM plant has been produced by inserting, in a non-GM line, a single cassette with multiple genes or sequences which will modify gene expression, it is expected that the insert will occur at a single locus. Therefore, independent segregation of the elements of this cassette is not likely. However, the potential effects of a loss of function of genetic elements within the event need to be considered (EFSA, 2011a). With regard to the choice of comparator this case should be treated as a GM plant containing a single event (see Section 3.1). Where the cassette is introduced into an existing GM line, the comparators should be selected using same the principles set out in Section 3.3.1. Re-transformation of existing GM plants should use guidance provided in section 3.3.1.

3.4. Additional comparisons required on a case-by-case basis

Risk assessment of GM plants and derived food and feed should be carried out in an integrative manner and, on a case-by-case basis, depending on the type of genetic modification, should take into

consideration environmental factors including cultivation practice that may influence food and feed safety.

GM plants carrying specific traits, e.g. herbicide tolerance, require appropriate treatment comparisons to evaluate FF, MC and environmental safety. Such GM plants may include cases in which the traits are stacked to provide tolerance to multiple herbicides.

As indicated in Section 1.1 and 3.2, the MC component of the risk assessment is primarily focused on the analysis of the GM plant itself. In the MC risk assessment of the herbicide-tolerant GM plant containing single events, the experimental design should always include the following test materials: the GM plant exposed to the intended herbicide, and the GM plant treated with the conventional herbicide management regimes. For GM plants containing stacked events, comparison of conventional and specific treatments linked to the trait(s) (e.g. use of herbicides) are only necessary if data obtained from the respective GM plants containing the single events indicate a potential safety concern.

In the FF risk assessment of herbicide-tolerant GM plants, containing single or multiple events, the experimental design should include the following test materials: the GM plant exposed to the intended herbicide(s), the comparator treated with conventional herbicide management regimes and the GM plant treated with the same conventional herbicide management regimes.

The same three test materials are recommended for the ERA of GM plants containing single events (EFSA, 2010b). For GM plants containing stacked events that include herbicide-tolerant traits, only two test materials are mandatory: the GM plant exposed to the intended herbicide(s) and the comparator treated with the appropriate conventional herbicide management regime. However, on a case-by-case basis, and particularly when assessing the effects of changes in management, it may also be necessary to include the GM plant treated with the same conventional herbicide management regimes. In the case of GM plants containing stacked events that are tolerant to multiple herbicides, there are several possible options for the management of the GM plants. An appropriate choice must be made on a case-by-case basis (EFSA, 2010b) and clear justification shall be provided by the applicant.

In addition to cases of herbicide tolerance, there are other situations where the inclusion of comparators, other than those described in this document, may provide useful information for the risk assessment. For example, for the assessment of insect-resistant plants, comparisons may involve a range of pest control practices.

4. Challenges and limitations to the selection of comparators

The majority of GM plants applications concern modifications to agronomic traits such as herbicide tolerance and/or insect resistance. Currently, GM plants are being developed with quality traits modified by major modifications in metabolic pathways, possibly leading to extensive compositional alterations. Examples include nutritionally enhanced foods with qualitative and quantitative changes in proteins, amino acids, carbohydrates, oils/lipids, vitamins and minerals. Other GM plants will have new traits which facilitate adaptation to environmental stress conditions such as drought or high salinity. These crops may be cultivated in areas where they have never been grown before.

The selection of appropriate comparators for the risk assessment of these GM plants with complex modifications may be difficult. When no appropriate comparator is available, the risk assessment

should be based primarily on the evaluation of the characteristics of the GM plant and derived products themselves.

Such a scenario is addressed in the guidance on the risk assessment of GM plants and derived food and feed (EFSA, 2011a) where it is stated that: "*Where no comparator can be identified, a comparative risk assessment cannot be made and a comprehensive safety and nutritional assessment of the GM plant and derived food and feed itself should be carried out. This would, for instance, be the case where the food and/or feed derived from a GM plant is not closely related to a food and/or a feed with a history of safe use, or where a specific trait or specific traits are introduced with the intention of changing significantly the composition of the plant".* In this guidance data requirements for the safety assessment of the GM plant and derived food and feed for which no appropriate comparator is available are listed and discussed in details.

The risk assessment of such GM plants should be focused on specific characteristics of the genetic modification, on food/feed constituents and on the whole food/feed. Data are required on:

a) characteristics of the donor organisms and recipient plant;

b) genetic modification and its functional consequences;

c) compositional characteristics of food and feed derived from the GM plant;

d) potential toxicity and allergenicity of gene products (proteins, metabolites) and the whole GM plant and its derived products;

e) dietary intake and potential for nutritional impact;

f) influence of processing and storage on the characteristics of the derived products.

A description of the compositional analysis and specific toxicological/nutritional analyses requirements, selected according to the compositional properties of the GM plant and the derived food and feed, is provided elsewhere (EC, 1997; EFSA, 2011a).

Depending on the available data, animal feeding trials with *whole food or feed* using laboratory animal species (rodents) and/or target animals should be considered, on a case-by-case basis. Approaches and test protocols for animal feeding trials with GM plants which have been extensively modified in composition, are described in the Report of the EFSA GMO Panel on the role of animal feeding trials (EFSA, 2008), and the opinion of the EFSA Scientific Committee on 90-day feeding trial protocol (EFSA, 2011b).

For ERA, the main focus should be on the environmental impacts and the management of the GM plant compared to what is currently grown and/or against environmental protection goals (EFSA, 2010b). Comparators should be chosen on a case-by-case basis. Dependent on the issue(s) under consideration, choices might include: a non-GM line derived from the breeding scheme used to develop the GM plant; a non-GM plant with agronomic properties as similar as possible to the GM plant under assessment; and/or a non-GM line having other characteristics as close as possible to those of the GM plant, except for the intended modification. Additional comparators could be considered on a case-by-case basis, including plants of other species appropriate to the environmental conditions. Applicants should justify their choice in all cases. Further guidance on this topic may be derived from the ERA Guidance (EFSA, 2010b).

5. Conclusions

A key step in the safety assessment of GM plants and derived food and feed is the identification of differences (intended and unintended) and equivalences between the GM plant and its comparator(s), taking into account natural variation. This information will assist the identification of potential adverse effects arising from the genetic modification. Within this risk assessment framework, the EFSA GMO Panel has, to date, required the use of non-GM lines with comparable genetic background as comparators. In the case of vegetatively propagated crops, these are the isogenic lines. In the case of sexually propagated crops these are non-GM lines as close as possible genetically to the GM plant under assessment. The identification and production of such comparators is becoming increasingly challenging due to the increasing complexity of breeding approaches and of the GM plants themselves, e.g. those developed by combining (stacking) events through conventional breeding, or those in which significant compositional changes are targeted. Consequently the EFSA GMO Panel has developed further guidance in this area.

For the FF risk assessment (EFSA, 2011a) and the ERA (EFSA, 2010b) of GM plants containing single events the EFSA GMO Panel confirms that the risk assessment must include a conventional counterpart. The EFSA GMO Panel also indicates the possible use of additional comparators, such as negative segregants, if deemed useful to support the risk assessment. In addition, for some ERA field trials and specific agronomic traits, depending upon the objective of the study (EFSA, 2010b) and only if there is explicit justification, the applicant may use a non-GM variety, with agronomic properties as similar to the GM plant as possible, as appropriate comparator. In all cases, information on the breeding scheme in relation to both the GM plant and the conventional counterpart, together with a clear justification for the use of the selected conventional counterpart and, if appropriate, alternative or additional comparators should be provided.

For the FF risk assessment of GM plants with traits combined by conventional breeding the first choice of comparator is the conventional counterpart. Where applicants can demonstrate that a conventional counterpart is not available, then applicants have two options: 1) the use of an appropriate negative segregant(s) where the segregants are derived from crosses between GM plants containing events which have been risk assessed and which are all stacked in the GM plant under assessment. This approach is only possible if either no unintended effects have been identified for the single events, or where the presence of such unintended effects in the GM plant containing the stacked events does not raise safety concerns. The breeding scheme used to produce the segregant(s) should be clearly illustrated; and/or 2) the use of any set of GM plants that have all been risk assessed on the basis of experimental data collected according to the principles of EFSA MC and FF risk assessment (EFSA, 2011a). This set of GM plants must include between them all of the events stacked in the GM plant under assessment, and no others. Additional comparators may be included if deemed useful to support the risk assessment.

For the ERA of GM plants with traits combined by conventional breeding the comparator is normally the conventional counterpart. In cases where a conventional counterpart is not available, different comparator(s) might be considered, depending upon the issue(s) under consideration. Where studies utilise data arising from the field trials for compositional analysis, to assess agronomic and phenotypic characteristics, the comparators will be identical to those for the FF risk assessment. For other ERA field studies, the conventional counterpart can be substituted, on a case-by-case basis, by either another non-GM line derived from the same breeding scheme used to develop the GM plant. Such a line will be genetically more distant from the GM plant than the conventional counterpart, but can still serve as an appropriate comparator. Alternatively a non-GM line with agronomic properties as similar as possible to the GM plant under assessment can serve as an appropriate comparator.

The MC component of the risk assessment of GM plants containing single or stacked events is primarily focused on the analysis of the GM plant itself. In case of GM plant containing single events

data on the conventional counterpart may be required on a case-by-case basis. In case of GM plants containing stacked events, the MC assessment of interactions between events that could impact on protein expression levels (or in some cases specific RNAs or metabolites), requires as comparators any set of GM plants that have all been risk assessed. This set of GM plants must include between them all of the events stacked in the GM plant under assessment, and no others.

In cases the stacking of events is performed applying stacking methods other than conventional breeding (such as co-transformation, re-transformation and multiple gene cassettes) similar principles as described for stacking by conventional breeding apply.

In cases where appropriate comparators are not available a comprehensive safety and nutritional assessment on the GM plant and derived food and feed itself is required as for other novel foods (ref to guidance on novel foods). Further development of a comprehensive safety and nutritional assessment strategy is needed.

For ERA, the main focus should be on the environmental impacts and the management of the GM plant compared to what is currently grown and/or against environmental protection goals. Thus, the comparator should be chosen on a case-by-case basis according to the issue(s) under consideration.

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612 **ABBREVIATIONS**

613	DNA	Deoxyribonucleic Acid
614	EC	European Commission
615	EFSA	European Food Safety Authority
616	ERA	Environmental Risk Assessment
617	FF	Food and Feed
618	GM	Genetically Modified
619	GMO	Genetically Modified Organism
620	MC	Molecular Characterisation

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