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Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants containing stacked transformation events

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The objective of this document is to set the scene with respect to the risk assessment, under Regulation (EC) No 1829/2003 and Directive 2001/18/EC, of Genetically Modified (GM) plants containing stacked transformation events. For the purpose of this document "stacked" events are defined as those combined by conventional breeding[1]. This document will be used by the EFSA Scientific Panel on Genetically Modified Organisms (GMO Panel) when reviewing and updating its current Guidance Document for risk assessment of genetically modified plants and derived food and feed.

1. Introduction

1.1 Background

Where GM events have been approved under Regulation (EC) No 1829/2003 or Directive 2001/18/EC, genotypes produced by crossing plants containing these events with non-GM plants are not required to undergo further risk assessment. However, where applications involve the crossing of plants to stack GM events, a risk assessment is required in the European Union. The stacking of approved events can arise from intentional crosses as well as unintentional crosses. However, the market approval procedure is designed for intended crosses only. The risk assessment for new events is required to take into account "the potential receiving environment" (Directive 2001/18/EC; Annex II, part C.1.) which would include other GM crop events incorporated into sexually compatible species.

The GMO Panel is aware that whilst current applications involve the stacking of two or three individual events, there is likely to be a move towards further increases in the numbers of events in GM stacks. As long as each event in the highest number of stacked events has been risk assessed, the risk assessment of the stacked events might also be applicable to GM stacks containing fewer of these events. Thus a single risk assessment of such a stack could cover all combinations with fewer of these events. However, applicants need to take into account the potential impact of any reduction in the number of events involved and should provide scientific argumentation for the absence of specific data on the stacked events with a lower combination of events.

[1] The stacking of events can also occur by the re-transformation of a plant containing an existing event. Constructs can also be designed which allow genes and traits to be stacked in a single transformation event. However, this type of stacking is not within the remit of this document.

Where an application for approval under Regulation (EC) No 1829/2003 (or Directive 2001/18/EC) for placing on the market in the European Union, involves plants produced by stacking of at least two events, the risk assessment should follow the requirements described in the present document.

1.2 General issues related to information required

Where events have been stacked in a plant, it is possible that neither of the individual events have been assessed previously, that one or more have, or that all of the events have undergone a risk assessment according to the EFSA Guidance Document. When assessing the safety of stacked events all of the requirements of the EFSA Guidance Document should be considered for each individual event that has not previously been assessed. To complete a risk assessment of stacked events all information on the potential risks of events already assessed must be made available, *e.g.* as a web link.

2. General approach to risk assessment of stacked events

The risk assessment of stacked events should follow the principles provided in the existing Guidance Document although, on a case-by-case basis, not all components of the Guidance Document may be relevant. Conversely, additional information may be required. Where all single events have been assessed, the risk assessment of stacked events should focus mainly on issues related to a) stability, b) expression of the events and c) potential interactions between the events.

Choice of comparators

In line with the EFSA Guidance Document, the most appropriate comparator(s) for the GMO plant containing the stacked events should include the GM parental materials as well as appropriate non-transgenic genotype(s). A genetic background comparable to the GMO containing the stacked events should be chosen. The applicant should provide detailed information justifying the choice of comparators.

3. Specific information required on stacked events

3.1 Molecular analysis

The molecular analysis should focus on a) the intactness and stability of events combined by crossing b) the expression of the traits and c) the potential interactions between the stacked events at the level of i) DNA, ii) protein and trait.

3.1.1 Assessment of the intactness and stability of the inserted events

The requirement is to establish that each of the events stacked in the plant has the same molecular properties and characteristics as in the individual events separately. This information will be important to

and trials involving animals. Intactness of events stacked in the plant is also a good indicator of trait stability. Comparisons between the insert structures in the original events and the GM stacks should be carried out on materials representative of those designed for commercial production, *i.e.* which will enter the environment and the food/feed chain.

To assess intactness, applicants should use appropriate molecular approaches, *e.g.* Southern and PCR analyses. The applicant should also ensure that probe-restriction enzyme combinations used are sufficient to prove intactness and stability of the insert and including the flanking regions.

3.1.2 Assessment of the expression of the traits and potential interactions between stacked events

Applicants are requested to carry out a risk assessment on the potential for any interactions between the stacked events which could impact on human or animal health and/or the environment. At the DNA level this would include, for example, assessing possibilities for gene silencing. Analyses of the levels of newly expressed proteins in GM stacks are also required. With regard to potential differences in the expression levels between GM stacks and the single events, the GMO Panel is aware that differences in expression of a range of proteins can be expected since they also occur in conventional crosses between non-GMOs as a consequence of differences in genetic backgrounds. Nevertheless, applicants are required to assess the impact of any difference in the expression of proteins and phenotype observed.

Stability of protein expression and phenotype should be assessed on materials representative of those designed for commercial production, *i.e.* which will enter the environment and the food/feed chain.

In cases where altered expression of the gene products and/or phenotype is viewed as a potential safety issue, further assessment will be required on a case-by-case basis, *e.g.* additional field trials, appropriate animal feeding studies and environmental studies.

3.2 Comparative analysis

3.2.1 Compositional assessment

For the stacked events at least one year of field trial data is required, with trials performed together with appropriate controls in geographical localities representative of the climatic conditions under which such crops will be cultivated. Based on the outcomes of this assessment, additional analysis of compositional characteristics of plants containing the stacked events may be required, *e.g.* from additional field trials.

The selection of the nutrients, anti-nutrients, allergens and natural toxins to be analysed and considered in the comparative assessment should be carried out according to OECD consensus documents on the key components. Where appropriate, on a case-by-case basis additional compounds could be selected for analysis depending upon the introduced traits.

3.2.2 Agronomic traits

In addition to possible compositional modifications of stacked events, there may be changes to agronomic and phenotypic characteristics. These may be indicative of unintended effects such as modified susceptibility to biotic and abiotic stresses. Possible differences in phenotypic characteristics and agronomic properties of stacks must be assessed in field trials over at least one season, as indicated above. Again, on a case-by-case basis, additional information on agronomic traits of the stacked events may be required from additional field trials.

3.3 Food and Feed assessment

3.3.1 Toxicology, allergenicity and nutritional assessment of GM food/feed

An assessment of any potential for increased toxicity and/or allergenicity to humans and animals or for modified nutritional value due to the stacked events should be provided. These potential effects may arise from additive, synergistic or antagonistic effects of the gene products or by these produced metabolites and may be particularly relevant where the combined expression of the newly introduced genes has unexpected effects on biochemical pathways. This assessment will clearly require a case-by-case approach.

3.4 Environmental Risk Assessment

The environmental risk assessment should take into account the evaluation of the individual events and additional data from molecular characterisation and comparative compositional analysis of the stacked events when determining potential interactions between genes or between gene products. The environmental risk assessment should evaluate any interactions between the stacked events which could result in modified environmental effects of the GM plant.

In particular the combination of transgenes may result in changes in expression levels which may lead to a significant biological impact that may need to be assessed. However, it should be noted that expression levels may vary significantly also in the individual events.

The guidelines below set out certain minimum requirements for the provision of information. If possible adverse effects have been identified through experimentation or if there are scientific reasons to believe they might exist then further data should be provided or information given.

3.4.1 Invasiveness and selective advantage or disadvantage

Comparison between plants containing the stacked events and the most appropriate comparators during one representative growing season and multiple geographical locations representative of the various environments in which the GM plants will be cultivated are necessary. Additional field data may be required if changes are observed in *i.e.* behaviour, fitness, reproduction, survivability or dissemination.

3.4.2 Interactions between the stacked events and target organisms

In order to evaluate/identify possible altered efficacy of biocidal gene products to target organisms in the stacked events as compared to the individual events, the potential impact on target organisms should be assessed in one year field trials initially. If biologically relevant changes are observed, additional studies might be required.

3.4.3 Interactions between the stacked events with non-target organisms

Stacked biocidal events may have different effects on non-target organisms when compared with the individual events. Therefore there is a need to focus on changes in sensitivity of non target organisms and/or specificity of biocidal gene products. To test the hypothesis that such combined events do not interact, a minimum of one year field trials are required. Where appropriate, further laboratory tests on a range of relevant non-target organisms representing ecological functions, using plant material containing the combined events may be required.

3.4.4 Impacts of the specific cultivation, management and harvesting techniques

Differences in the specific cultivation, management and harvesting techniques between plants containing the stacked events and the parental lines, and any environmental impacts of such differences, should be evaluated and, where appropriate, supported by relevant data.

3.5 Environmental Monitoring Plan

The general principles of the Post-Market Environmental Monitoring (PMEM) as described in the Guidance Document of the GMO Panel are retained for applications concerning stacked events. Case-specific monitoring should take into account the results of the environmental risk assessment, plus any monitoring already proposed or established for individual events previously approved. Consideration should be given to any additional environmental exposure or other effect due to the combination of events identified in the environmental risk assessment. General surveillance should proceed as for any other GM crop and take account of any general surveillance plans already proposed or established for individual events previously approved.