Biosafety Advisory Council

Secretariat

Scientific Institute of Public Health

Division of Biosafety and Biotechnology

O./ref.: WIV-ISP/BAC/2007_PT_535
Email: bac@sbb.ihe.be

Guidelines for Genetically Modified Stacked Events to be Placed on the Market

revised version[1]

Adopted by the Belgian Biosafety Advisory Council on 11 June 2007

1. Introduction

This document is for use of notifiers who intend to apply for the release of genetically modified (GM) stacked events under existing Community legislation and for risk assessors. GM stacked events are defined as plants obtained from crosses of GM (inbred) lines, each transformed with different events. The guidelines seek to provide guidance and assistance in preparation and evaluation of genetically modified organism (GMO) applications intended for commercial release.

The scope of this document is limited to the evaluation of GM stacked events obtained from inbred parental lines proven to be safe for human health and the environment for the same uses as the GM stacked event[2]. These guidelines are set up to address all market situations (import, processing, cultivation and use as food/feed) and should be consulted in the light of the requested use of a GMO.

The guidance proposes criteria for the risk assessment of GM stacked events combining positively assessed GM parental lines. For this type of GM stacked events, the safety

[1] replaces document BAC_2007_PT_496 of 23 April 2007

[2] An event is considered to be safe in case an authorisation for marketing has been granted in the EU under Directive 2001/18/EC or Regulation (EC) 1829/2003 or if EFSA has published a favourable opinion.

assessments of the parental GM events form a good basis for the evaluation of the GM stacked event. However, additional information proving the validity of the studies carried out on the GM parental lines for the GM stacked event will be needed to complete the risk assessment together with data proving the biosafety of the GM stacked events. Minimum data requirements are highlighted in bold. The guidance is not intended to be prescriptive in terms of what methods to use. However, it is emphasized that the quality of the experimental data provided should be sufficient to verify clearly any statements made by the applicant. The guidelines may develop further in function of the biotechnological developments and regulatory demands, and on the basis of further discussion with (inter)national fora that have put the risk assessment of GM stacked events on their agenda (e.g. EFSA).

The different sections apply to the different risk assessment criteria needed for evaluation of GMOs as set out in Community legislation and the guidance notes of EFSA[3], including molecular characterisation (section 2), comparative analysis (section 3), environmental aspects (section 4), toxicology testing (section 5), allergenicity testing (section 6) and food/feed nutrition evaluation (section 7). Each section gives a concise overview of the data required for risk assessment of the aforedescribed GM stacked events and explains briefly the scientific bases for data requirements. More information on the rationales underlying data requirements can be found in the following publication: De Schrijver *et al.* (2007) Risk assessment of GM stacked events obtained from crosses between GM events. Trends in Food Science and Technology, 18, 101-109.

2. Molecular characterisation

In order to prove the transfer of the transgenes through conventional breeding to the GM stacked event, a Southern Blot should be provided to demonstrate the **presence** and to determine the **copy number** of the parental introduced/modified DNA sequences. The presence of the total introduced/modified DNA sequence (including the flanking regions) needs to be demonstrated. Demonstrating the maintenance of the regions flanking the insert is particularly relevant since this will allow extrapolation of the bio-informatic analysis carried out on the junction regions of the single GM events.

The **expression** of the introduced/modified trait (at protein level) in the stacked GM event should be analysed in order to demonstrate that gene expression in relevant tissues falls within the range of the ones determined in the GM parental lines.

The **stability** of the parental introduced DNA sequences should be determined at the molecular level in case the progeny of the GM stacked events (>F_1) will be used for cultivation. Given the prolonged environmental exposure in case of farm-saved seed, data on the stability of the insert over several generations are considered relevant.

[3] EFSA (2006). Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. The EFSA Journal, 374, 1-115.

3. Comparative analysis

Agronomic, morphological and compositional studies on the GM stacked event should be carried out. Comparative analysis might identify unintended effects resulting from the interbreeding of GM varieties (e.g. synergistic or antagonistic interactions of the transgenic proteins) compared to the traditionally grown crop or the parental GM events.

4. Environmental aspects

Although the GM stacked event is considered as a new GMO, the environmental risk assessment data on the recipient, the genetic modification and the potential receiving environment of the single GM events will remain valid for the GM stacked event. Aspects linked to the GM crop that might alter its interactions with the environment will be relevant to take into consideration during risk assessment.

The overall impact on the environment of the GM stacked event needs to be assessed on a case-by-case basis since, compared to single events, potential effects and/or interactions of the regulatory sequences, gene products or metabolites might affect differently the agro-ecosystem.

Additional environmental studies and/or post-market monitoring will be relevant if there is an increased risk of potential adverse agro-ecological effects as a result of the combined presence of several traits.

5. Toxicology testing

When the expression level of an introduced/modified trait in the GM stacked event falls outside the range of the one determined in the GM parental line (see 2), the possible need for whole GM food/feed toxicology studies of the GM stacked event should be considered. An increase in the amount of newly expressed protein could lead to a toxic effect if the protein is potentially toxic.

The overall toxicity testing of the GM stacked event needs to be assessed since potential interactions of newly introduced genes, regulatory sequences and proteins (or its metabolites) with the host genome of the GM stacked event, might lead to a change in overall toxicity of the plant.

If compounds with a synergistic toxic potential for animals and/or humans are combined in the GM stacked event, additional toxicity testing should determine whole GM food/feed safety. Toxins individually considered safe, might lead to unacceptable health effects when exposure is to a combination.

6. Allergenicity testing

The overall allergenicity of the GM stacked event needs to be assessed since potential interactions of newly introduced genes, regulatory sequences and proteins (or its metabolites)

with the host genome of the GM stacked event, might lead to a change in overall allergenicity of the plant.

7. Food/Feed nutrition evaluation

Compositional analyses carried out on parental GM events combined in a stack cannot be extrapolated *per se* to the stacked event. Through hybrid breeding the composition of a crop might change. Therefore, compositional analysis of the GM stacked event is relevant for risk assessment to identify if any possible adverse effects might result from a change in composition.

Compositional analysis should be done on the raw agricultural commodities to identify if any change in composition has occurred compared to the comparator. On a case-by-case basis, the processed fractions should be assessed for key nutrients as well as naturally occurring anti-nutrients, toxicants and other secondary plant metabolites (EFSA, 2006).

Further nutritional analysis, including animal performance, feed and digestion studies should be considered on a case-by-case basis.