Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

# Advice of the Belgian Biosafety Advisory Council on application EFSA-GMO-BE-2016-138 (oilseed rape MS11) from Bayer CropScience under Regulation (EC) No. 1829/2003

1 July 2020
*Ref. SC/1510/BAC/2020_0616*

## Context

Application EFSA-GMO-BE-2016-138 was submitted by Bayer CropScience for the authorisation for the marketing of genetically modified (GM) oilseed rape MS11 for food and feed uses, import and processing (excluding cultivation) within the European Union, within the framework of Regulation (EC) No. 1829/2003[1].

Oilseed rape MS11 is intended to be used only to produce hybrid seed for breeding and thus is not meant to be commercialised as a stand-alone product for food and feed uses, import and processing.

Oilseed rape MS11 contains a single insert consisting of one copy of the *pat/bar*, barnase and barstar expression cassettes, conferring male sterility and tolerance to the herbicide glufosinate-ammonium.

The application was validated by EFSA on 8 March 2017 and a formal three-month consultation period of the Member States was started, lasting until 12 June 2017, in accordance with Articles 6.4 and 18.4 of Regulation (EC) No. 1829/2003 (consultation of national Competent Authorities within the meaning of Directive 2001/18/EC designated by each Member State in the case of genetically modified organisms being part of the products).

Within the framework of this consultation, the Belgian Biosafety Advisory Council (BAC), under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier, chosen from the common list of experts drawn up by the BAC and the Biosafety and Biotechnology Unit (SBB). Eight experts answered positively to this request, and formulated a number of comments to the dossier. See Annex I for an overview of all the comments and the comments sent to EFSA on 16 June 2017.

The opinion of the EFSA Scientific Panel on GMOs was published on 14 May 2020 (EFSA Journal 2020;18(5):6112[2]) together with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period. Those documents were forwarded to the experts on 18 May 2020, with an invitation to react if needed.

In delivering the present advice, the BAC considered in particular the comments formulated by the experts on application EFSA-GMO-BE-2016-138 and the opinion of EFSA.

[1] 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, p.1).
[2] See http://www.efsa.europa.eu/en/efsajournal/pub/6112

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## Scientific evaluation

### 1. Environmental risk assessment

The Biosafety Advisory Council is of the opinion that it is unlikely that the accidental release of oilseed rape MS11 (i.e. during transport and/or processing) into the European environment[3] will lead to environmental harm.

### 2. Molecular characterisation

With regard to the molecular characterisation, the Biosafety Advisory Council is of the opinion that the information provided is sufficient and does not raise safety concerns.

### 3. Assessment of food/feed safety and nutritional value

3.1. Assessment of compositional analysis

A comparative compositional assessment between the non-GM conventional counterpart and MS11 *B. napus* (both treated and not treated with trait-specific herbicide), was conducted. However, as MS11 is male sterile, one had to rely on donor pollen instead of self-pollination for reproduction. Plants not treated with trait-specific herbicide were cross-pollinated with an isogenic maintainer line; while plants treated with the trait-specific herbicide, were cross-pollinated with a herbicide-resistant restorer line (such as RF3). In case the herbicide was applied, this rendered the comparison between MS11 and its conventional counterpart difficult.

The Biosafety Advisory Council agrees with the GMO panel of EFSA that, considering the impossibility to collect an appropriate and complete data set, it is not possible to conclude on the compositional analysis without deviating from the requirements laid down in Regulation (EU) No 503/2013.
However, since oilseed rape MS11 is designed to be used only for the production of hybrid seed for breeding and thus is not expected to be commercialised as a stand-alone product for food/feed uses, the Biosafety Advisory Council is of the opinion that the information provided by the applicant is sufficient to conclude that the compositional analysis of this GM oilseed rape does not raise concerns in the context of its intended use.

3.2. Assessment of toxicity

The Biosafety Advisory Council agrees with the GMO panel of EFSA that the available data on the toxicity of the Barnase, Barstar and PAT/bar proteins, as expressed in oilseed rape MS11, does not raise safety concerns.
Since oilseed rape MS11 is designed to be used only for the production of hybrid seed for breeding and thus is not expected to be commercialised as a stand-alone product for food/feed uses, the Biosafety Advisory Council is of the opinion that the information provided is sufficient to conclude that the assessment of this GM oilseed rape does not raise toxicological concerns in the context of its intended use.

3.3. Assessment of allergenicity

The Biosafety Advisory Council agrees with the GMO panel of EFSA that the available data on the allergenicity of the Barnase, Barstar and PAT/bar proteins, as expressed in oilseed rape MS11, does not raise safety concerns.
Since oilseed rape MS11 is designed to be used only for the production of hybrid seed for breeding and thus is not expected to be commercialised as a stand-alone product for food/feed uses, the Biosafety Advisory Council is of the opinion that the information provided is sufficient to conclude that the

[3] As the application doesn't imply cultivation of the GM crop in the EU, a full environmental assessment is as in the case of a cultivation file is not warranted.

assessment of this GM oilseed rape does not raise concerns regarding the allergenicity in the context of its intended use.

3.4. Nutritional value

Since oilseed rape MS11 is designed to be used only for the production of hybrid seed for breeding and thus is not expected to be commercialised as a stand-alone product for food/feed uses, the Biosafety Advisory Council is of the opinion that the information provided is sufficient to conclude that the nutritional assessment of this GM oilseed rape does not raise safety concerns in the context of its intended use.

**4. Monitoring**

With regard to monitoring, the Biosafety Advisory Council is of the opinion that the information provided is sufficient.

## Conclusion

Based on the whole set of data on oilseed rape MS11 provided by the applicant, the scientific assessment of the dossier done by the Belgian experts, the opinion of EFSA, and the answers of the EFSA GMO panel to the questions raised by the Belgian experts, the Biosafety Advisory Council:

1) Agrees with the GMO panel of EFSA that the potential environmental release of oilseed rape MS11 is unlikely to pose any threat to the European environment;

2) Agrees with the GMO panel of EFSA that the characteristics of the introduced traits of oilseed rape MS11 challenge the comparative analysis to the extent that it is not possible to produce the materials and collect the data for the comparative analysis without deviating from the requirements laid down in Regulation (EU) No 503/2013;

3) Notes however that oilseed rape MS11 is intended to be used only to produce hybrid seed and thus not be commercialised as a stand-alone product for food and feed uses, import and processing. The BAC agrees with the GMO panel of EFSA that in the context of its proposed use, oilseed rape MS11 is unlikely to pose any risk to human and animal health;

4) Notes that this application for import and processing of MS11 was filed because authorisation of the single events included in a stacked is a prerequisite for a stacked event to be approved. The BAC is of the opinion that the scientific relevance of assessing such a 'hypothetical' product (MS11), not to be marketed, is highly questionable. The handling of this application is an example of a procedure leading to an unnecessary workload and a waste of time and money for the Biosafety Advisory Council.

Dr. ir. Geert Angenon
President of the Belgian Biosafety Advisory Council

*Annex I: Compilation of comments of experts in charge of evaluating the application EFSA-GMO-BE-2016-138 (ref. BAC_2017_0411)*

**Bioveiligheidsraad**
**Conseil de Biosécurité**

*16/06/2017*

**Secretariaat**
**Secrétariat**

O./ref.: WIV-ISP/41/BAC_2017_0411
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**Compilation of comments of experts in charge of evaluating the application EFSA/GMO/BE/2016/138**
**and**
**Comments submitted on the EFSAnet on mandate of the Biosafety Council**

**Mandate for the Group of Experts:** Mandate of the Biosafety Advisory Council (BAC) of 21 March 2017.
**Coordinator:** Prof. Maurice De Proft
**Experts:** Eddy Decuypere (KUL), Patrick du Jardin (ULg-Gembloux), Godelieve Gheysen (UGent), Johan Grooten (UGent), André Huyghebaert (UGent), Peter Smet (Consultant), Frank Van Breusegem (UGent), Jan Van Doorsselaere (KATHO).

**Domains of expertise of experts involved:** Molecular characterisation, DNA/RNA/protein analysis, herbicide tolerance, animal and human nutrition, food/feed processing, toxicology, general biochemistry, statistics, immunology, alimentary allergology, plant allergens, agronomy, ecology, oilseed rape, breeding techniques, plant biology.

**SBB:** Didier Breyer, Fanny Coppens, Katia Pauwels.

## ♦ INTRODUCTION

Dossier **EFSA/GMO/BE/2016/138** concerns an application submitted by **Bayer CropScience** for authorisation to place on the market genetically modified **oilseed rape MS11** in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed.
The application has been officially acknowledged by EFSA on 10 March 2017.

The scope of the application is:
☒ GM plants for food use
☒ Food containing or consisting of GM plants
☒ Food produced from GM plants or containing ingredients produced from GM plants
☒ GM plants for feed use
☒ Feed produced from GM plants
☒ Import and processing (Part C of Directive 2001/18/EC)
☐ Seeds and plant propagating material for cultivation in European Union (Part C of Directive 2001/18/EC)

Depending on their expertise, the experts were asked to evaluate the genetically modified plant considered in the application on its 1) molecular, 2) environmental, 3) allergenicity, 4) toxicity and/or 5) food and feed aspects. It was expected that the expert should evaluate if the information provided in the application is sufficient in order to state that the marketing of the genetically modified plant for its intended uses, will not raise any problems for the environment or human or animal health. If information is lacking, the expert was asked to indicate which information should be provided and what the scientifically reasoning is behind this demand.

The comments are structured as in the "Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed" (EFSA Journal (2004), 99, 1-94). Items are left blank when no comments have been received either because the expert(s) focused on other related aspects, or because for this dossier the panel of experts who accepted to evaluate the dossier didn't have the needed expertise to review this part of the dossier.
It should be noted that all the comments received from the experts are considered in the evaluation of this dossier and in formulating the final advice of the Biosafety Advisory Council. Comments placed on the EFSAnet are indicated in grey.

**List of comments/questions received from the experts**

## GENERAL COMMENTS

*Comment 1*

No comments.

*Comment 2*

No comments.

*Comment 3*

The southern blot analyses have been done in great detail and the results are generally well described.

## A. HAZARD IDENTIFICATION AND CHARACTERISATION

### A.1. INFORMATION RELATED TO THE RECIPIENT OR (WHERE APPROPRIATE) THE PARENTAL PLANT

*Comment 1*

- Phenolic compounds/phytic acid in *Brassica napus* are anti-nutrients for proteins, complex carbohydrates and for important minerals (Ca, Mg, Fe…) but also for phosphor uptake: P is not mentioned here: why?
- Page 25: canola meal is considered a premium ingredient, due to its "high quality" of protein for milk production and growth: how is "high quality" defined here? Digestibility? Amino acid composition? This should be clearly and unequivocally defined.
- Page 31: It is stated that *Brassica* species show the highest nutritional demand for sulphur (linked to high levels of glucosinolates??), but is this also true for cultivars low in glucosinolates?

*Comment 2*

No comments.

*Comment 3*

No comments.

### A.2. MOLECULAR CHARACTERISATION

**A.2.1. INFORMATION RELATING TO THE GENETIC MODIFICATION** Including:

- Description of the methods used for the genetic modification
- Source and characterization of nucleic acid used for transformation
- Nature and source of vector(s) used

*Comment 1*

No questions: bar, barnase and barstar genes were introduced in a single gene construct via direct gene transfer. No unintended changes were found.

*Comment 2*

No comments.

*Comment 3*

No comments.

*Comment 4*

None.

**A.2.2. INFORMATION RELATING TO THE GM PLANT** Including:

- Description of the trait(s) and characteristics which have been introduced or modified
- Information on the sequences actually inserted or deleted
- Information on the expression of the insert
- Genetic stability of the inserted/modified sequence and phenotypic stability of the GM plant

*Comment 1*

No questions.

*Comment 2*

No comments.

*Comment 3*

- The quality of the Southern blot analysis of the transgenic locus (section 1.2.2.2.a. of the main dossier) is underlined: when some unexpected results were obtained (*e.g.* possible partial digestions, incomplete stripping of the membranes), the applicant spontaneously repeated the experiment to confirm the expected results.

- When using Southern blots to confirm the absence of backbone sequences (section 1.2.2.2.b. of the main dossier), considering the presence of the barnase gene on both the T-DNA and the vector backbone, the applicant decided not to use a barnase probe. In consequence, there is no full coverage of the vector backbone by the probes, as requested by the EFSA guidelines, but the applicant performed a PCR test specific for this barnase backbone sequence to compensate for this. This approach is sound and gives the expected result.

- The study of the expression of the Barnase/Barstar/PAT protein in plant tissues concludes (p.88 of the main dossier): “the expression of the Barnase, Barstar and PAT/bar proteins in different tissues of MS11 *B. napus* collected at different developmental stages were shown to be comparable to the expression in tissues of the respective non-GM conventional counterpart”. This is surprising since no expression at all is obviously expected in the non-GM counterpart, as the three genes are of bacterial origin and no expression of plant homologs is expected in the non-GM counterpart. When looking to the report M-549123-01-1 presenting the details of the study, no such conclusion can be found, instead (page 18 of this report): “While the expression levels of PAT/bar and Barstar were quantifiable in most of the matrices, the measured expression levels of Barnase in MS11 were all below LLOQ. The expression levels of PAT/bar, Barstar, and Barnase in all matrices were similar between MS11 *B. napus* treated with trait-specific herbicide and untreated MS11 *B. napus*.”
Does the applicant in the main dossier mean that he considers that values below LLOQ are considered as “no expression”, hence that the GM and non-GM are considered as equivalent to each other? By non-GM-counterpart, does the applicant mean the negative segregant that was included in this study? I guess so, but this wording is misleading. I do not see a safety issue here, but a problem in the formulation of the conclusions.

- In the study on protein expression levels (M-549123-01-1), there is no indication that the extraction efficiency of the tissue proteins was quantified. As it is not described in the detailed methods of the study M-549123-01-1, it may be concluded that this has actually not been done. Extraction efficiencies may vary significantly from one plant tissue to another and the applicant should justify why he did not

quantify and include this parameter in the calculations of the protein levels. This seems especially relevant when values < LLOQ are concluded.

- For the analysis of "phenotypic stability", the applicant only measures the PAT activity by a test called "lateral flow strip analysis" and no information on the Barnase/Barstar associated trait is given. The test is thus considered as a proxy for the phenotypic assessment of all traits of the MS11 event, but this is implicit and the applicant should have discussed this. However, there is no safety issue associated with a hypothetic phenotypic instability, in my view, and I consider the data as sufficient for the safety assessment.

*Comment 4*

- P46-48 and p54: page 48 describes in detail the results and interpretation of the Southern blot on page 47. However, several fragments are not described, nor commented on. Upon comparison with the figure on page 46, it is clear that these additional fragments result from a small overlap with the probe and are therefore not unexpected. It would be better if these fragments are explained in the text on page 47. They do appear on the table of page 54 as expected and observed fragments due to small probe overlap.
- P56: it is not clear to me why the 11280 bp fragment is visible with the Pta29 probe. It is not a problem for backbone presence as this probe does not detect vector backbone. Legend: "framgents" should be "fragments".
- P74-75: the results of probe 28-1,-2,-3 and -10 are difficult to understand as the figures are not shown. While the text on page 72 states that the positive controls, also the 0.1 equimolar amount, showed the expected fragments, the tables are in contradiction with this as the 0.1 equimolar amount does not in all cases show the expected fragment (tables on p74-75 Exp Yes Obt. No).
- P85: the T-DNA is inserted near an endogenous gene, but deduced from the fact that the coding region is not interrupted, it is concluded that it is unlikely that the insertion interrupts the transcriptional or translational activity of that gene. Interruption of transcriptional activity cannot be excluded from this, has any analysis of RNA levels of the endogenous gene been performed?
- P91: how was the zygosity of the individual plants confirmed? By segregation analysis of the progeny?
- P92: please rename the figures in the table to fit the description in the text, Figure 2 in table = 2.26 in text, etc.

*Comment 5*

Page 88: the following conclusion is drawn:
The expression of the Barnase, Barstar and PAT/bar proteins in different tissues of MS11 *B. napus* collected at different developmental stages were shown to be comparable to the expression in tissues of the respective non-GM conventional counterpart.
Since the non-GM conventional counterpart does not contain Barnase, Barstar or bar, it is not possible that these genes are expressed in the non-GM counterpart. This conclusion should be clarified.

Page 100: the segregation ratios for the T3, T4 and T5 are "un-expected" (?) since for a hemizygous condition one would expect a 3/1 ratio and for a homozygous (MS11 positive) line one would expect 1/0.
So therefore it should be mentioned if the plants are a selection of different (homozygous and non-GMO) lines.

### A.3. COMPARATIVE ASSESSMENT

#### A.3.1. CRITERIA FOR THE SELECTION OF COMPARATOR(S)

*Comment 1*

No questions.

*Comment 2*

Oilseed rape MS11 is compared with its conventional counterpart and six non-GM reference varieties.
No remarks.

*Comment 3*

No comments.

### A.3.2. FIELD TRIALS: EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

*Comment 1*

Field trials (at 10 locations) consisted of six entries: the non-GM counterpart with conventional herbicide treatment, MS11 *Brassica napus* with conventional and with intended herbicide and 3 of the 6 reference varieties.

*Comment 2*

No remarks.

*Comment 3*

No comments.

### A.3.3. COMPOSITIONAL ANALYSIS

*Comment 1*

Since MS11 *B. napus* is not a stand-alone product but includes 3 lines (as explained on p 109), differences between MS11 and the conventional counterpart were more pronounced when MS11 was treated with the intended herbicide, because with such treatment the fertile segregants are removed and MS11 *B. napus* will cross-pollinate with pollen from neighbouring plots, instead of the usually occurring 70% self-pollination of the fertile segregants (not tolerant to glufosinate ammonium).
Cross-pollination with the B-line which is an isogenic line to the sterile A line, results in fertile segregants which are not removed when there is no spraying of glufosinate ammonium, and therefore the MS11 *B. napus* (CHM, conventional herbicide management) is less different to the conventional counterpart than MS11 *B. napus* (Test TIH).

*Comment 2*

The OECD (2011) revised Consensus Documents was followed for the selections of compounds for analysis.
To my knowledge it is the first time the term toxicants in addition to anti-nutrients is used. Food safety is indeed an essential element in the assessment of food quality.

- Proximates:
Carbohydrates are given as a group without any differentiation; fiber is assessed as neutral and detergent fiber; the major use of oilseed rape after extraction of the oil is indeed in animal feed; the information is appropriate.
Amino acids are covered in detail, including the essential amino acids.
Fatty acids are also described in detail, including the long chain fatty acids with 20 to 24 carbon atoms; these fatty acids are of great importance for oilseed rape, among others erucic acid, known to have adverse health effects; the erucic acid content is very low.
- Minerals: no further comments

- Vitamins: according to the actual knowledge of the vitamin content of oilseed rape, particularly the oil, information is given about the important tocopherols and vitamin K.
- Anti-nutrients: the data contain important information about the different glucosinolates, phytic acid and tannins.

Data have been statistically evaluated. In most cases no significance differences have been observed. In case values are significantly different they are always within the range of literature data for the particular constituent.

I agree with this conclusion.

*Comment 3*

Secondary metabolites and antinutrients: Either no significant difference from the control or equivalent to the reference lines.

### A.3.4. AGRONOMIC AND PHENOTYPIC CHARACTERISTICS

*Comment 1*

No questions (see also comments above under A.3.3).

*Comment 2*

This chapter contains important information on disease stress rating and insect stress rating. This is important information in relation to food safety aspects.
The applicant concludes that identified differences in agronomic and phenotypic endpoints are not considered biologically relevant, taking into account natural variation.
I agree with this conclusion.

*Comment 3*

No comments.

### A.3.5. EFFECTS OF PROCESSING

*Comment 1*

No questions.

*Comment 2*

Potential effects on processing characteristics have been thoroughly evaluated. The analysis covers not only the composition of the fractions obtained in terms of nutrients but also in anti-nutrients.
No biologically relevant effects have been observed.
I agree with this conclusion.

## A.4. TOXICOLOGICAL ASSESSMENT

### A.4.1. METHODOLOGY USED FOR TOXICITY TESTS

*Comment 1*

The mode of action of Barnase, Barstar and Barnase/Barstar complex proteins is well explained. Protein characterization is well described ad repeated dose toxicity studies in mice by gavage did not induce any treatment-related changes.

### A.4.2. ASSESSMENT OF NEWLY EXPRESSED PROTEINS including:

- Molecular and biochemical characterisation of the newly expressed proteins
- Up-to-date bioinformatic search for homology
- Information on the stability of the protein under the relevant processing and storage conditions for the food and feed derived from the GM plant
- Data concerning the resistance of the newly expressed protein to proteolytic enzymes
- Repeated dose toxicity studies using laboratory animals

*Comment 1*

No questions.

*Comment 2*

Degradation in SGF/SIF

The Barnase protein was degraded very rapidly (within 30 seconds) in simulated gastric fluid, but was still visible at 60 minutes when incubated in the presence of pancreatin, indicating a slow degradation rate in simulated intestinal fluid.
The Barstar protein was degraded very rapidly (within 30 seconds) in simulated gastric fluid, and (>90% of the Barstar protein) was degraded within 10 minutes in simulated intestinal fluid.
The Barnase/Barstar protein complex was degraded very rapidly (within 30 seconds) when incubated with SGF. Small molecular weight residual fragments (2.5 to 3.5 kDa) were fully degraded within 5 minutes. The Barnase/Barstar protein complex was not degraded within 60 minutes when incubated with SIF; the Barnase protein band showed a partial digestion in that there was a decrease in intensity of the bands with time, while the Barstar protein bands showed no decrease in intensity.
The PAT/bar protein was degraded very rapidly (within 30 seconds) in simulated gastric fluid, as well as in simulated intestinal fluid.
=> These data indicate a rapid degradation of the proteins in the gastrointestinal tract.

28 Day Repeat Dose Toxicity Study by Oral Gavage in Rats

The Barnase protein administered by gavage to CD1 mice, for at least 28 days, at the measured dose of up to 9.5 mg/kg/day did not induce any treatment-related changes.
The Barstar protein administered by gavage to CD1 mice, for at least 28 days, at the measured dose of up to 10 mg Barstar protein/kg body weight/day did not induce any treatment-related changes.
The Barnase/Barstar complex protein administered by gavage to CD1 mice, for at least 28 days, at the measured dose of up to 20 mg Barnase/Barstar complex protein/kg body /weight/day did not induce any treatment-related changes.
The EFSA GMO Panel has previously evaluated the safety of the PAT/bar protein in the context of several applications for the placing on the EU market of GM crops expressing this protein, and no safety concerns were identified.

Sequence homology with known toxins

In conclusion, there were no toxicological *in silico* findings associated with the Barnase, Barstar and PAT/bar proteins.

*Comment 3*

- When assessing the equivalence between the plant-expressed Barnase and Barstar proteins on one hand with the *E. coli*-expressed proteins on the other hand, the applicant is challenged by the very low expression in plant tissues, making it practically impossible to extract sufficient amounts of proteins to perform the expected comparisons with the *E. coli* proteins. For the Barnase protein, the expression of which is driven by the NOS promoter and which can be found in root tissues in reasonable amounts, experimental data on the immunoreactivity and molecular mass were obtained by western blot analysis. In figure 2 of the M-548891-01-1 report, the MS11 *B. napus* protein extract is run on a SDS-PAGE gel in parallel with a non-GM protein sample spiked with the *E. coli* purified protein. The applicant concludes that the MS11 immunoreactive protein and the spiked protein show 'comparable' apparent Mw. In fact, and refering also to the Coomassie-stained gel shown in figure 1 where the migration of the extracted samples in the different lanes can be analysed (no apparent retardation of the proteins in lane 2 as compared with 3-to-6), there is a slight, but significant, difference in the mobility between the two immunoreactive proteins, which questions the conclusion of the applicant. One possible explanation of the lower apparent mobility of the MS11 protein could be glycosylation, but the report M-471279-04-1 seems to rule out this possibility based on the bioinformatic analysis of potential glycosylation sites. Could the applicant comment on this issue?

- Furthermore, for the comparison of the 'immunoreactivity' of the MS11-expressed and *E. coli*-expressed proteins, the applicant says (page 12 of report M-548891-01-1) that "the immuno-reactivity of the Barstar protein in MS11 *B. napus* total protein extract was considered comparable to the recombinant 1340_Barstar (spiked into the non-GM counterpart) when the signals were observed at comparable migration distances. No Barstar protein specific signal was expected for the non-GM counterpart." Comparing the migration distance does not provide information on 'immunoreactivity' *sensu stricto*, but a quantification of the band intensities should have been performed for addressing this issue, based on the amounts of the spiked *E. coli* proteins in the western blot analysis and on the measured concentration of the Barstar protein in the MS11 protein extracts. The presented data do not support the conclusion.

In conclusion of the assessment of the protein equivalence between the plant-expressed and *E. coli*-expressed proteins, the applicant seems to have done his best to provide data complying with the requirements of the regulation and guidelines, but the technical obstacles prevented him to do so. Still, there are problems of interpretation of the available data. Overall and from a safety perspective, taking into account the nature of the imported materials in the EU and the very low expression of the Barnase/Barstar proteins in these materials, any risk associated to the newly expressed proteins may be considered as negligible.

### A.4.3. ASSESSMENT OF NEW CONSTITUENTS OTHER THAN PROTEINS

*Comment 1*

Not applicable.

### A.4.4. ASSESSMENT OF ALTERED LEVELS OF FOOD AND FEED CONSTITUENTS

*Comment 1*

No questions.

### A.4.5. ASSESSMENT OF THE WHOLE FOOD AND/OR FEED DERIVED FROM GM PLANTS

*Comment 1*

The 90-day feeding study in Sprague-Dawley rats showed no biologically or toxicologically effects and therefor the conclusion of the GM-MS11 *B. napus* to be as safe a as consumption of products from conventional oilseed rape seems warranted.

*Comment 2*

90-Day rat feeding study
Based on the results of this study, dietary administration of MS11 *B. napus* meal for at least 90 consecutive days at a concentration of 15% in the diet had no adverse effects on the growth or health of Sprague Dawley rats.
Why was a concentration of 15% chosen? Why wasn't a higher dose included?

## A.5. ALLERGENICITY ASSESSMENT

**A.5.1. ASSESSMENT OF ALLERGENICITY OF THE NEWLY EXPRESSED PROTEIN** including:

- Amino acid sequence homology comparison between the newly expressed protein and known allergens using a comprehensive database
- Specific serum screening
- Pepsin resistance and in vitro digestibility tests
- Additional tests

*Comment 1*

No questions.

*Comment 2*

No comments on the bioinformatic search.

*Comment 3*

The weight of evidence approach followed by the applicant did not indicate a risk for allergenicity of the newly expressed Barnase, Barstar, and PAT proteins. In addition, the PAT protein has been evaluated before by EFSA, concluding that it is unlikely that the PAT protein is an allergen. On this basis I comply with the applicant's conclusion that the newly expressed proteins are unlikely to be allergenic.
I have no further comments.

### A.5.2. ASSESSMENT OF ALLERGENICITY OF THE WHOLE GM PLANT

*Comment 1*

No questions.

*Comment 2*

Low to near absent protein levels in rapeseed oil for human consumption along with the absence of biologically relevant differences in the comparative analysis support the conclusion that no increased allergenicity is to be anticipated for the MS11 *B. napus* GM plant.
Is there any indication of the levels the barnase protein, selectively expressed in pollen (?!), may reach in honey collected from MS11 *B. napus* fields? In my opinion such honey constitutes a second food product derived from the GM plant besides the rapeseed oil.
I have no further comments.

### A.5.3. ADJUVANTICITY

*Comment 1*

No questions.

*Comment 2*

No comments.

### A.6. NUTRITIONAL ASSESSMENT

### A.6.1. NUTRITIONAL ASSESSMENT OF FOOD DERIVED FROM GM PLANTS

*Comment 1*

No questions.

### A.6.2. NUTRITIONAL ASSESSMENT OF FEED DERIVED FROM GM PLANTS

*Comment 1*

No questions.

## B. EXPOSURE ASSESSMENT - ANTICIPATED INTAKE/EXTENT OF USE

*Comment 1*

No questions.

## C. RISK CHARACTERISATION

*Comment 1*

No questions.

## D. POST MARKET MONITORING (PMM) OF FOOD AND FEED DERIVED FROM GM PLANTS

*Comment 1*

No comments.

## E. ENVIRONMENTAL RISK ASSESSMENT

### E.1. INTRODUCTION

*Comment 1*

No comments.

*Comment 2*

No comments.

### E.2. General approach of the ERA

*Comment 1*

No comments.

*Comment 2*

No comments.

### E.3. Specific areas of risk

As stated in the EFSA guidance on the environmental risk assessment of genetically modified plants (EFSA Journal 2010, 8(11):1879) the objective of the ERA is on a case-by-case basis to identify and evaluate potential adverse effects of the GM plant, direct and indirect, immediate or delayed (including cumulative long-term effects) on the receiving environment(s) where the GM plant will be released. For each specific risk the ERA consists of the six steps described in Directive 2001/18/EC:
1. Problem formulation including hazard identification,
2. Hazard characterisation,
3. Exposure characterisation,
4. Risk characterisation,
5. Risk management strategies,
6. Overall risk evaluation and conclusions.

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

*Comment 1*

No questions.

#### E.3.2. Plant to micro-organisms gene transfer

*Comment 1*

No questions.

#### E.3.3. Interaction between the GM plant and target organisms

*Comment 1*

Not relevant.

#### E.3.4. Interaction between the GM plant and non-target organisms (NTOs)

*Comment 1*

Negligible
In the conclusions of the interactions of the GM plant with non-target organisms, and given the scope of this application and considering the low levels of exposure and absence of identified unintended

differences, it is stated that the uncertainty associated with this risk characterization can be considered low. Why??
If the risk estimation which is a combination of consequence assessment and likelihood assessment, is estimated as negligible, why then another uncertainty evaluation linked on this risk characterization or estimation???

#### E.3.5. IMPACTS OF SPECIFIC CULTIVATION AND MANAGEMENT AND HARVESTING TECHNIQUES

*Comment 1*

Not applicable.

*Comment 2*

No comments.

#### E.3.6. EFFECTS ON BIOGEOCHEMICAL PROCESSES

*Comment 1*

Not relevant.

*Comment 2*

No comments.

#### E.3.7. EFFECTS ON HUMAN AND ANIMAL HEALTH

*Comment 1*

No questions.

#### E.3.8. OVERALL RISK EVALUATION AND CONCLUSIONS

*Comment 1*

No questions.

*Comment 2*

No comments.

### E.4. POST MARKET ENVIRONMENTAL MONITORING PLAN

#### E.4.1. INTERPLAY BETWEEN ENVIRONMENTAL RISK ASSESSMENT AND MONITORING

*Comment 1*

No questions.

*Comment 2*

No comments.

### E.4.2. CASE-SPECIFIC GM PLANT MONITORING

*Comment 1*

No questions.

*Comment 2*

No comments.

### E.4.3. GENERAL SURVEILLANCE FOR UNANTICIPATED ADVERSE EFFECTS

*Comment 1*

No questions.

*Comment 2*

In the section dedicated to the general surveillance with the PMEM plan, the web link indicated as informing operators about the currently approved GM plant products subject to general surveillance (www.europabio.org/information-operators, see page 214 of Main dossier) does not work anymore and should be updated.

### E.4.4. REPORTING THE RESULTS OF MONITORING

*Comment 1*

No questions.

*Comment 2*

In the section dedicated to the general surveillance with the PMEM plan, the web link indicated as informing operators about the currently approved GM plant products subject to general surveillance (www.europabio.org/information-operators, see page 214 of Main dossier) does not work anymore and should be updated.