Bioveiligheidsraad
Conseil de Biosécurité

02-09-2014

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2014_0592

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/BE/2011/101 from Monsanto under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/BE/2011/101 was submitted by Monsanto on 8 September 2011 for the marketing of genetically modified oilseed rape MON88302 for food and feed uses, import and processing within the framework of Regulation (EC) No. 1829/2003[1]. Oilseed rape MON88302 contains a single insert expressing the CP4 EPSPS protein conferring tolerance to the herbicidal active substance glyphosate.

The application was officially acknowledged by EFSA on 02 April 2012. On the same date EFSA started the formal three-month consultation period of the Member States, 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, which were edited by the coordinator. See Annex I for an overview of all the comments and for the list of comments actually placed on the EFSAnet on 9 July 2012.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 21 May 2014 (EFSA Journal 2014; 12(6):3701 [2], and published together with the responses from the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period.

On 20 June 2014 the opinion of EFSA was forwarded to the Belgian experts. They were invited to give comments and to react if needed to the answers given by the EFSA GMO Panel, in particular in case the comments formulated in their initial assessment of the dossier were not taken into account in the opinion of EFSA. The comments formulated by the experts together with the opinion of EFSA including the answers of the EFSA GMO Panel form the basis of the advice of the Biosafety Advisory Council given below.

[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/3701.htm

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 MON 88302 seeds (i.e. during transport and/or processing) into the European environment[3] will lead to any unwanted effects.

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

The Biosafety Advisory Council agrees with the GMO panel of EFSA that the compositional data of GM oilseed rape MON 88302, in comparison with its conventional counterpart and the set of non-GM oilseed rape varieties, do not raise safety concerns.

3.2. Assessment of toxicity

The Biosafety Advisory Council is of the opinion that the information provided is sufficient and does not raise safety concerns.

3.3. Assessment of allergenicity

The Biosafety Advisory Council agrees with the EFSA GMO Panel that there are no indications that the newly expressed CP4 EPSPS protein in GM oilseed rape may be allergenic in the intended use. Oilseed rape is not considered to be a common allergenic food and there are no indications that the genetic modification might change this.

3.4. Nutritional value

Based on compositional data the Biosafety Advisory Council agrees with the EFSA GMO panel that the nutritional value of food and feed derived from oilseed rape MON 88302 is not expected to differ from that of food and feed derived from non-GM oilseed rape varieties.

4. Monitoring

The Biosafety Advisory Council is of the opinion that the environmental monitoring plan proposed by the notifier in case of accidental spillage of reproducible material remains very general. The plan does provide information on which organizations are involved in the monitoring for unanticipated adverse effects and the fact that they will submit yearly reports to either the applicant or EuropaBio, but does not provide more detailed information on how the monitoring is actually going to be performed.

[3] As the application doesn't imply a cultivation of the GM crop in the EU, a full environmental assessment is not required in EFSA procedure and was not achieved.

Conclusion

Based on the scientific assessment of the dossier done by the Belgian experts, taking into account the opinion of EFSA, the answers of the EFSA GMO Panel to the questions raised by the Belgian experts, the answers of the applicant to the EFSA GMO Panel questions and considering the data presently available, the Biosafety Advisory Council is of the opinion that in the context of its proposed uses, MON 88302 oilseed rape is unlikely to pose any risk to human and animal health.

Other considerations

The Biosafety Advisory Council wants to point out that the environmental monitoring plan, proposed by the applicant is not fully satisfactory and could be improved. In particular, it should be more precise at the level of the identity, training and expertise of the people involved in the monitoring, at the level of the monitoring methods (including types of unanticipated effects to be looked at and sites to be monitored) and time-frame planning, and at the level of identification methods, and we suggest to include into the monitoring farmers organizations as well.

Prof. Maurice De Proft

President of the Belgian Biosafety Advisory Council

Annex I: Minority declaration

Annex II: Full comments of experts in charge of evaluating application EFSA/GMO/BE/2011/101 and *comments submitted on the EFSAnet (ref. BAC_2012_0664)*

Minority Declaration of P. Baret, M. Lateur and D. Perreaux

Uncontrolled spread of GM oilseed rape in the environment already occurred in Europe and can realistically not be avoided. Risk assessment as reported does not sufficiently take into consideration long term effects of feral GM plants, as the probable introgression of the genetic modification into wild populations of related species (stability of the transgene for example). Should environmental problems be detected in the future, it will likely be impossible to retrieve GM plants from the environment. Moreover, risks related to the marketing of conventional grain or seed produced in EU (outside of the scope of coexistence regulation) are not taken into consideration. Beside those considerations, GM plants developed to withstand “higher rate” of herbicide are likely to cause environmental problems, wherever they are grown and those problems cannot be ignored. The Biosafety Advisory Council should then give a negative advice regarding the environmental safety of this event.

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N./réf. : WIV-ISP/41/BAC_2012_0664
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09-07-2012

Compilation of comments of experts in charge of evaluating the application EFSA/GMO/BE/2011/101 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 17 April 2012
Coordinator: Dr. René Custers
Experts: Armand Christophe (UGent), Johan Grooten (UGent), Jean-Luc Hofs (CIRAD), Birgit Mertens (WIV-ISP), Peter Smet (Consultant), Jan Van Doorsselaere (KATHO Roeselaere), Bart Van Droogenbroeck (ILVO), Michel Van Koninckxloo (HEP Hainaut-Condorcet)
Domains of expertise of experts involved: molecular characterization, human and animal nutritioni, biochemistry of food/feed, toxicology, risk analysis, immunology, alimentary allergology , plant allergens, agronomy, ecology, plant biology, herbicide tolerance, biosafety, oilseed rape
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman, Katia Pauwels

INTRODUCTION

Dossier **EFSA/GMO/BE/2011/101** concerns an application of the company **Monsanto** for the marketing authorisation of the genetically modified **oilseed rape MON88302** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 03 April 2012.
The scope of the application is:
(a) GM food
☒ Food containing or consisting of GM plants
☒ Food produced from GM plants or containing ingredients produced from GM plants
(b) GM feed
☒ Feed containing or consisting of GM plants
☒ Feed produced from GM plants
(c) GM plants for food or feed use
☒ Products other than food and feed containing of consisting of GM plants with the exception of cultivation
☐ Seeds and plant propagating material for cultivation in the EU

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 received from the experts

GENERAL COMMENTS

Comments/Questions of the expert(s)

Comment 1

1) Some of the statements in the application are not correct or at least debatable from a scientific point of view (e.g. Part II, page 131, lines 6-8: "Rapeseed oil contains…a high level of…oleic acid *which has been shown to reduce serum cholesterol levels*" (arguable); Part II, page 106, lines 3-4: "Humans consume the oil *extracted* from seeds" (In Europe edible oil obtained from rapeseed by cold pressing is available; Spielmeyer et al., 2009). Of course these statements have no bearing on safety aspects.

2) Reviewing would be easier if the applicant would adhere to the same numbering as in the review document.

Clarification from the SBB
The applicant has for the content of this dossier followed the new EFSA guidance on submission of applications[1] while the commenting form given to the experts and the commenting form on EFSAnet still follows de guidance of 2004[2].

Comment 2

I'm convinced that the relevance and usefulness of the trait introduced, i.e. tolerance to glyphosate during the sensitive reproductive stages of growth, and possible application of glyphosate at higher rates up to first flower, will be questioned by many people (opponents). The possibility to use higher glyphosate rates, during longer growth periods automatically triggers the question: what about the possibility to create glyphosate resistant weeds? It would be relevant to include information about relevant strategies to avoid the development of such glyphosate resistant weeds, wherever relevant.
Comment from the coordinator:
This comment is relevant for cultivation, and not for import and processing, which is the scope of this notification.

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

Detailed general information is provided. This information is sufficient.

[1] EFSA guidance on the submission of applications for authorisation of genetically modified food and feed and genetically modified plants for food or feed uses under Regulation (EC) No 1829/2003 - EFSA Journal 2011; 9 (7):2311
2 EFSA 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

Comment 2

The information provided in the application is sufficient.

Comment 3

No major remarks.
Typo's/Missing references:
- Part II – Scientific information - Point 1.2., pg. 13 – one but last paragraph - B. napus should be italic
- Part II – Scientific information - Point 1.2., pg. 13 – Reference, "(USDA-FAS, 20103)" is missing in the reference list.

B. INFORMATION RELATING TO THE RECIPIENT OR (WHERE APPROPRIATE) PARENTAL PLANTS

Comments/Questions of the expert(s)

Comment 1

The information given is sufficient.

Comment 2

The information provided in the application is sufficient.

Comment 3

No major remarks.
Typo's:
Part II – Scientific information - Point 2.1., pg. 19– first sentence, and pg 21 first paragraph, - *Agrobacterium* should be italic.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

No comments.

Comment 2

No major remarks.

D. INFORMATION RELATING TO THE GM PLANT

D.1 DESCRIPTION OF THE TRAITS AND CHARACTERISTICS WHICH HAVE BEEN INTRODUCED OR MODIFIED

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

Comment 2

No comments.

Comment 3

Cfr. General remark formulated above.

D.2. INFORMATION ON THE SEQUENCES ACTUALLY INSERTED OR DELETED

Comments/Questions of the expert(s)

Comment 1

No comments.

Comment 2

From the thorough molecular analysis, it is clear that the selected event contains the T-DNA at single detectable locus of integration in MON 88302 and contains no detectable vector backbone sequence.

To verify the organization and sequence of the insert and adjacent genomic DNA in MON 88302, PCR amplification and sequencing of the obtained amplicons was performed (pg 46 and following pages). To do this, primers based on the 5' and 3' flanking regions were used. For reasons of completeness and clarity of the text it seems relevant to describe how these primers were developed in the dossier as well.

Additional comment from SBB:
Taking into consideration pg 46- 48 and following pages, we do not see which information not currently present in the dossier, relative to development of the primers would be relevant to clarify the text.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

No comments.

Comment 2

The data included in the dossier only reports about the expression level of the insert in seeds (Table 5 & 6, pgs. However, as MON 88302 utilizes a FMV/Tsf1 chimeric promoter sequence to drive CP4 EPSPS expression in different plant tissues, it would be relevant to show data of all the relevant tissues an not only the seeds. By virtue of CP4 EPSPS expression in pollen, MON 88302 provides tolerance to glyphosate during the sensitive reproductive stages of growth, and enables the application of glyphosate at higher rates up to first flower with no detectable impact to male fertility. So, especially expression data of the CP4 EPSPS protein in pollen is relevant at least.

Additional comment from coordinator:
Because one cannot fully exclude that some MON 88302 plants would grow in the EU following accidental seed spillage of imported MON 88302, and one cannot fully exclude that some MON 88302 pollen would be produced as a result of that, it seems relevant to also have data on the expression of the CP4 EPSPS protein in pollen.

Additional comment from SBB:
This comment is also related to comment 2 under D.7.9. saying that a significant level of pollen-derived CP4 EPSPS protein is likely to be present in honey for human consumption. It is therefore appropriate that the applicants determine CP4 EPSPS expression level in pollen.

D.4. INFORMATION ON HOW THE GM PLANT DIFFERS FROM THE RECIPIENT PLANT IN: REPRODUCTION, DISSEMINATION, SURVIVABILITY

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

Comment 2

This part is not included in the dossier.

Comment 3

No major remarks.

D5. GENETIC STABILITY OF THE INSERT AND PHENOTYPIC STABILITY OF THE GM PLANT

Comments/Questions of the expert(s)

Comment 1

No comments.

Comment 2

No major remarks. From the SB analysis it is clear that the insert is stably integrated into the host plant genome.

D.6. ANY CHANGE TO THE ABILITY OF THE GM PLANT TO TRANSFERR GENETIC MATERIAL TO OTHER ORGANISMS

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

Comment 2

This part is not included in the dossier.

D.7. INFORMATION ON ANY TOXIC, ALLERGENIC OR OTHER HARMFUL EFFECTS ON HUMAN OR ANIMAL HEALTH ARISING FROM THE GM FOOD/FEED

D.7.1 Comparative assessment

Comments/Questions of the expert(s)

Comment 1

Minor remark: although suitable for difference and equivalence testing, the methodology used for the determination of carbohydrates (by difference) and for fibre gives values which are not very relevant from a nutritional point of view. It is suggested that EFSA considers including the methodology to be used for these proximates in guidance documents when they are reviewed in the future.

Clarification from the SBB:
The applicant follows the recommendations from the OECD 2001 document whereas in the draft OECD 2010 consensus document, it is suggested to determine acid detergent fibre and Neutral detergent fibre in seed/meal for feed use.

Comment 2

Information concerning erucic acid seems to be missing.

Comment from the SBB:
While no measurement data relative to erucic acid were given in the dossier, the applicant did provide some information .The applicant states that ***'seed samples were analysed for key nutrients (protein, amino acids, total fat, fatty acids (C8-C24, including erucic acid),*** *ash, moisture, carbohydrates by calculation, acid detergent fiber (ADF), neutral detergent fiber (NDF), total dietary fiber (TDF), vitamin E (α-tocopherol), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), and vitamin E) and key antinutrients (glucosinolates (alkyl, indolyl and total), phytic acid, sinapine or sinapic acid (by location), and total tannins). Moreover in (Lundry et al., 2012(confidential appendices) it is stated that 'Of the evaluated components across both studies, 14 fatty acids (**including 22:1 erucic acid)** and sodium had more than 50% of the observations below the assay limit of quantitation (LOQ), and as a result, were excluded from the statistical analyses.'*

There seems to be no significant differences between the event and its control concerning the toxin (*Glucosinolates*) and antinutrient (*Phytic Acid, Sinapine/Sinapic Acid and Tannins*) content.

Additional information from SBB:
On p91 of the dossier the applicant concludes that *'All observed differences in this statistical analysis conducted under EFSA guidance were characterized by a lack of compositional relevance. Magnitudes of difference between MON 88302 and the control were small and of no relevance from a food and feed perspective particularly in the context of the naturally occurring wide range of values for the conventional counterpart. Values for MON 88302 and the counterpart components overlapped extensively highlighting their compositional parity.'*
*The applicant refers to the EFSA guidance for RA of food and feed from GM plants (*EFSA Journal 2011; 9(5):2150) and states that levels of alkyl, indolyl and total glucosinolates + phytic acid + tannins were determined to be equivalent between MON 88302 and the set of conventional reference varieties (equivalence category I, Figure 1 in EFSA guidance 25) at the 95% confidence level and there were no differences at the 10% significance level between MON 88302 and the control for glucosinolates (Outcome Type 1).
However for tannin, outcome type II was observed (p78), but here again the applicant concludes that the range of sinapine values for MON 88302; 0.82 to 1.00% dwt, was entirely within the range of control values (p77).

Comment 3

The information provided in the application is sufficient.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

The information provided in the application is sufficient.

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

In addition to the anti-nutritional components which were analysed, FAO (http://www.fao.org/ag/Aga/AGAP/FRG/afris/Data/724.htm) considers rape mucilage as an "anti-quality" factor. As rape foliage is seldom consumed by domesticated animals (but sometimes by wild animals; http://www.oilseedrape.org.uk/html/toxicity.htlm) and not expected to be imported in Europe, determination of this component in imported rape is of minor importance.

Comment 2

The information provided in the application is sufficient.

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

In the point "how the plant is typically cultivated", weed control, diseases control and mineral fertilization are not treated.

Comment 2

(1) Phenologic traits (3.4.2 Agronomic and phenotypic results, page 94) of non-treated MON 88302 don't show increasing weediness and invasiveness potential under American (North and Southern) conditions. MON 88302 would be rather a late and medium-yielding cultivar in these conditions, which doesn't correspond to the profile of an invasive organism.

- Are these environmental conditions comparable to those of the European "receiving environment", in the case of accidental release (spillage)?

Additional information from SBB :
p153 part II, the applicant states that, *'there is no evidence to suggest that MON 88302 plants would be any different from conventional oilseed rape regarding their ability to persist in agricultural fields (in-field) or invade non-agricultural habitats (offfield) as evidenced by the extensive phenotypic/agronomic characterization conducted with MON 88302 (Sections E.2.2.3 and A.3.3.4 and Appendices D and G, thereby referring to the results from the field trials in 2009 in the US and Canada and 2009 2010 in Chile)'*

(2) Environmental interaction (page 98) was studied in Canada and Chile. In McPherson 2012a (CI), tables of results (page 73 and following) show a majority of absence (0) of damage to abiotic and biotic stresses in both test and control.

- Does it mean that there was no pressure from most of the biotic and abiotic stressors under the field conditions? In this case comparison wouldn't be relevant and no conclusion should be drawn.
-

Additional information from SBB:
As recognized by applicant on p 154 part II: *'The expression of CP4 EPSPS in a MON 88302 volunteer plant or any progeny resulting from hybridization and introgression with sexually compatible species would only confer a selective advantage over conventional oilseed rape when plants would be treated with glyphosate herbicide (Squire et al., 2011; Warwick et al., 2008). The ability to survive after herbicide treatment (a specific selective advantage) does not change the plants' innate biological or ecological properties related to persistence or invasiveness. Furthermore, the use of herbicides to control plants is a condition which is predictable, is spatially and temporally limited, short in duration, and has negligible consequences to natural environments'.*

- Is there information about the current status of MON 88302 and its counterpart for tolerance/susceptibility to the mentioned diseases and others (not mentioned in the study) that exist in Europe such as Ring Spot (*Mycosphaerella brassicicola*), (Inman et al., 1991) and Light Leaf Spot (*Cylindrosporium concentricum* and *Pseudocercosporella capsellae*),? Tolerance to disease could contribute to a better fitness of ferals resulting from seed spillage.

Comment from the coordinator:
There are no reasons to suspect that a tolerance to glyphosate would alter the susceptibility to diseases and in this way influence the fitness of ferals resulting from seed spillage.

(3) Evaluation of pollen viability (page 104). Alexander's stain is a preliminary test to assess pollen grain maturity **before** testing viability (Dafni, 1992). Further testing must be carried out to assess pollen viability or germination capacity: Tetrazolium test (Barrow, 1983) and in vitro germination test (Taylor, 1972). Thus it is possible that information about viability is not accurate.

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

It is claimed that the oil is the sole product of oilseed rape with direct human consumption (Technical dossier pp 15).
Questions:
1) Can intake by humans of the rape itself or of other rape products than the oil be excluded? The composition of rapes appears in food tables (e.g. NUBEL 2009; 5th edition, page 48) suggesting that rapes are consumed in Europe as such. Furthermore, I am aware of one study where the biological value of oilseed rape protein has been determined in humans (Bos et al. 2007).
2) It is stated that MON 88302 will be imported as oil and seed (Part II, page 169) but the scope of the application is for all uses as any other oilseed rape, excluding cultivation. Can import of rapes be excluded?

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

No data on rape consumption in Europe by humans are provided (vide D.7.5). It is stated that oilseed rapes can be included in animal diets (Part II, page136).

Comment 2

The information provided in the application is sufficient.

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

Note that carcinogens are formed when refined rapeseed oil is heated during cooking in excess of 200°C. In this respect, rapeseed oil appears to be more hazardous to health than most other cooking oils (http://www.oilseedrape.org.uk/html/toxicity.htlm). No comparison was made between the thermal/oxidative stability of the refined oil of the GM rape and its conventional comparator. Factors affecting the oxidative stability of rapeseed oil have been published (e.g. Tynek et al., 2012). The linolenic acid content was found to be a major factor reducing the oxidative stability (Tynek et al., 2012). (Note in this respect that the mean value and upper range of linolenic acid in the GM-oil is somewhat higher than in its conventional counterpart and in other reference oils; Part II, Table 13, page 87).

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

Based on the data presented, including the mouse acute toxicity study, the newly expressed CP4 EPSPS protein seems to be safe.
Question. Has the possibility been excluded that other proteins are formed? (introduction of new open reading frames by the genetic modification?).

Additional information from SBB:
According to the applicant p52, Part II and based on bioinformatic analysis (theoretical), *'there is no reason to suspect or evidence to indicate the presence of transcripts spanning the flank junctions. No empirical evidence exists to suggest that any of the 11 sequences are produced or found in planta. Likewise, other than translation of CP4 EPSPS, no evidence exists to indicate that any other sequence from the T-DNA is translated. Rather, the results of the flank junction and TDNA bioinformatic analyses indicate that in the unlikely occurrence that any of the 11 peptides analyzed herein is found in planta, or translation of sequence other than CP4 EPSPS was to occur, none would share significant similarity or identity to known allergens and toxins, or other biologically active proteins that could affect human or animal health'.*

Comment 2

a) Degradation of the protein in simulated gastric fluid (based on earlier studies)).

It was demonstrated earlier that this protein rapidly degrades in SGF.

b) Degradation of the protein in simulated intestinal fluid (based on earlier studies)).

It was demonstrated earlier that this protein rapidly degrades in SIF.

c): Acute Oral Toxicity Study in Mice (based on earlier studies)).

From earlier studies, it was demonstrated that the No Observable Adverse Effect Level (NOAEL) for CP4 EPSPS was **572 mg/kg bw**, the highest dose tested.

d): Repeated dose oral toxicity (28-day feeding) study in mice ()).

Not performed. No further action is needed at the moment.

e): Assessment of Amino Acid Sequence Homology with Known Toxins (Tu and Silvanovich (2011)).

The results of these data indicate that no biologically relevant sequence similarities were observed between the CP4 EPSPS protein and toxins, or other biologically active proteins. These results and conclusion are consistent with those previously reported by Tu and Silvanovich (2010), which concluded that CP4 EPSPS demonstrated nostructurally relevant sequence similarity to toxins or biologically active proteinsthat could be harmful to human or animal health.

Comment 3

Protein used for safety assessment

The inserted sequence in MON 88302 oilseed rape encodes for the 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein. Given the low expression level of CP4 EPSPS in MON 88302, the applicant used a microbial analogue of the CP4 EPSPS protein, produced in *E. Coli,* for safety testing. Physicochemical and functional identity of the CP4 EPSPS protein produced in *E. Coli* and in MON 88302 was demonstrated by analyzing the amino acid sequence of the protein, the enzymatic activity of the protein and the homology of the active site residues and by studying the protein molecular weight, glycosylation of the protein and its immunoreactive properties.

Toxicological assessment of the expressed novel protein in MON 88302

History of safe use of MON 88302

The CP4 EPSPS protein is already expressed in commercial Roundup Ready® crop products. Since their introduction, food and feeds containing these biotechnology-derived plants have been found to pose negligible risk to human and animal health. In addition, homologous EPSPS enzymes present in food crops and common microorganisms have not been reported to cause toxic effects.

Bioinformatic searches

Bioinformatic analyses demonstrated that CP4 EPSPS protein is not structurally or functionally related to toxic or allergenic proteins that adversely affect human or animal health.

Acute oral toxicity

An acute oral (gavage) toxicity study with CP4 EPSPS protein in male and female CD-1 mice with various doses of the CP4 EPSPS protein showed no adverse effects up to the highest dose administered (572 mg/kg body weight). These data were already presented and evaluated by Monsanto in other applications of CP4 EPSPS containing crops.

Comment 4

The information provided in the application is sufficient.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

Based on the results of the comparative assessment, it does not seem likely that new constituents are formed. As non-GM rape can not be treated with glyphosate whereas MON 88302 rapes can, glyphosate and its major metabolites may be present in MON 88302.

Comment 2

Since no new constituents other than CP4 EPSPS were expressed in MON 88302, a toxicological assessment is not applicable.

Comment 3

The information provided in the application is sufficient.

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

The whole GM food has not been tested. It is suggested that this will be done for the rape as such because rapes are consumed by humans (vide supra) and no comparative assessment has been made for this food.

Comment from SBB:
For the moment and according to the EFSA guidance on RA of food and feed of GM plants, a whole GM food and feed test is not mandatory when the compositional analysis shows substantial equivalence between the GM plant and non-GM comparator. The possibility that humans consume oilseed rape will not change that.

Comment 2

a) 42-day feeding study in broiler chickens ()
Not performed. No further action is needed at the moment.

b) 90-Day rat feeding study ()).

Not performed. No further action is needed at the moment.

Comment 3

No study with the whole food was performed. However, comparative analysis did not detect biologically relevant differences in the compositional, phenotypic and agronomic characteristics of MON 88302 and its conventional counterpart. Furthermore, its composition fell within the range of non-GM oilseed rape varieties and there were no indications of unintended effects of genetic modification. Together with the safety data of other CP4 EPSPS containing crops, these data do not require animal safety studies with whole food/feed.

Comment 4

The information provided in the application is sufficient.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

It is claimed that there are no reports of allergic reactions to oilseed rape **oil** (Part II, pages 131 and 168). Note that an association has been described between consumption of rapeseed oil and risk for allergy and respiratory health (Takaoka M et al., 2008). Of course an association does not prove that there is a cause-effect relationship.

Comment 2

Assessment of allergenicity of (i) the whole GM plant, (ii) the newly expressed CP4 EPSPS protein, and (iii) the newly expressed CP4 EPSPS protein in the seed and oil was performed in line with EFSA guidelines and guidelines from the Codex Alimentarius. This assessment supports the conclusion that the CP4 EPSPS protein in the GM plant is not likely to be allergenic and that the food/feed derived from MON 88302 is not likely to be more allergenic than the comparator.

Yet I have one remark and question to the applicant: MON 88302 differs from other commercial Roundup Ready crops that similarly express the CP4 EPSPS protein by the expression of CP4 EPSPS protein also in pollen. As pollination by insects, especially bees, is crucial for seed formation, a significant level of pollen-derived CP4 EPSPS protein is likely to be present in honey for human consumption produced by local bee colonies. While clearly honey production from the GM plant is not the application foreseen by the applicant, it nevertheless is a real outcome and a potential source of allergenicity. It is therefore appropriate that the applicants determine CP4 EPSPS protein levels in honey from MON 88302 fields in order to assess the potential for allergenicity through this route of human consumption.

Additional comment from the SBB:
Here we would also like to refer to comment 2 under section D3. saying that it would be appropriated to determine the expression level of CP4 EPSPS.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

The **seed** composition of MON 88302 is not different from that of a set of reference varieties. No data are given on the composition of the rape itself. As rapes are not a major food, either comparing anti-nutritional factors or a 90 day rodent study may suffice for their safety evaluation.

Additional comment from the SBB (cfr D.7.8.4):
For the moment and according to the EFSA guidance on RA of food and feed of GM plants, a whole GM food and feed test is not mandatory when the compositional analysis shows substantial equivalence between the GM plant and the non-GM comparator. The possibility that humans consume oilseed rape will not change that.

Comment 2

The information provided in the application is sufficient.

D.7.11 Post-market monitoring of GM food/feed

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

D.8. MECHANISM OF INTERACTION BETWEEN THE GM PLANT AND TARGET ORGANISMS (IF APPLICABLE)

Comments/Questions of the expert(s)

Comment 1

Not applicable.

D.9. POTENTIAL CHANGES IN THE INTERACTIONS BETWEEN THE GM PLANT WITH THE BIOTIC ENVIRONMENT RESULTING FROM THE GENETIC MODIFICATION

D.9.1. Persistence and invasiveness

Comments/Questions of the expert(s)

Comment 1

Persistence of seeds of oilseed rape outside cultivated fields has been shown (Pessel et al., 2001).

Recently data have been published suggesting intraspecific transgene flow between escaped B. napus populations and introgression of an herbicide-resistance gene into related species (Aono et al., 2011).
A report on the potential for dispersal of herbicide tolerance genes from genetically-modified, herbicide-tolerant oilseed rape crops to wild relatives has been published (Daniels et al., 2005).

Additional information from SBB :
The applicant refers to Devos et al 2011. Not to the publication of Aono 2011.

Comment 2

Question: (p.29 Main Text) "MON 88302 provides tolerance to glyphosate during the sensitive reproductive stages of growth...with no detectable impact to male fertility", in this case, why (p.104) "pollen was collected from glyphosate untreated MON 88302" and not from treated MON 88302?

Comment 3

For GM Canola, it is generally said that there is no evidence of more invasive or more persistent behaviour than non GM counterparts, in undisturbed or disturbed habitats (Crawley et al 1993, 2001). However these early studies are complemented by more recent studies and conclusions are not exactly the same. For example, Elling et al. (2009) show that in NW Germany, feral sites were inhabited by plants for at least 2 years. The proportion of feral population setting seeds was variable but high. Within-population genetic diversity of feral oilseed rape populations was high. Repeated escapes of different varieties and hybridisation between these varieties were identified as the most important sources of genetic variation in feral populations. Among it, three hybrids between different varieties were detected indicating that feral oilseed rape populations may persist via self-recruitment. Elling et al. (2009) conclude by "these results highlight the evolutionary potential of feral oilseed rape populations".

In addition, comments made in section D 7.4., about susceptibility to pathogens, would help in preventing unintended growth of feral populations.

Additional information from SBB:
The applicant refers to a more recent review of Devos et al 2011 in which the conclusions of the paper of Elling et al., 2009 were taken into account. The applicant also refers to another recent study Warwick et al., 2008 that reported a decrease of hybrids resulting from crosses between B. napus and B rapa decreased over 3-year period from 85 surviving plants out of ~200 to five surviving plants out of ~200 under normal agro-environment field conditions. Devos et al 2011 concludes that feral GM herbicide tolerant (HT) oilseed rape has the potential to introduce HT traits to volunteer weeds in agricultural fields but that it would only be amplified if the selection (herbicide to which HT volunteers are tolerant) were used routinely in the field.
It should also be noted that introgression of alleles (the stable incorportaion of genes from one species into another species) does not only involves the gene exchange between species: it first involves the viable hybrid formation upon gene exchange and it also needs the pick-up of the newly introduced alleles by selection. A recent publication on the modeling of hybrid populations resulting from crosses and back crosses of B. napus (AACC) with B. rapa (AA) (de Jong et al 2012) also studied the influx of unpaired C-chomosomes into wild B.rapa population, which is thought to be an indicator of introgression. By using a model of unbiased transmission the authors conclude that the continued presence of plants with extra C-chromosomes depends on seletion in the adult stage. A transgene present on a C-chromosome could be selected for when the selective advantage is greater than the disadvantage of being aneuploid. The authors also conclude that insertion of transgenes on C-chromosome results in much lower chance of introgression compared to transgenes on A-

chromosomes. ***Therefore it could be relevant to consider whether the transgene in MON 88302 has been inserted on the A or the C-chromosome.***

Comment from coordinator:
Introgression of a EPSPS gene per se cannot be categorized as environmental damage.

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

Comment 1

Not relevant.

D.9.5 Interactions of the GM plant with non-target organism

Comments/Questions of the expert(s)

Comment 1

Not relevant.

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1

No negative effects of the **oil** derived from MON 88302 rapes are expected. No data are given to judge the safety of the rape itself.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

No negative effects of feeds derived from MON 88302 rapes are expected.

Comment 2

The information provided in the application is sufficient.

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

D.9.9 Impacts of the specific cultivation, management and harvesting techniques

Comments/Questions of the expert(s)

Comment 1

Not relevant.

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

Comment 2

Tolerance or susceptibility to non mentioned diseases or pathogens currently found in Europe should be investigated (*Mycosphaerella brassicicola*, *Cylindrosporium concentricum* and *Pseudocercosporella capsellae*) and other.

Comment of SBB:
There are no indications given that expresion of CP4 EPSPS would confer tolerance or susceptibility to diseases. This question is out of the scope of this application which does not cover cultivation in Europe.

D.11.4 General surveillance of the impact of the GM plant

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

Comment 2

New data related to feral populations settings (as mentioned in D 9.1) should be taken into account in the general surveillance plan.

Additional information from the SBB:
The applicant refers to the conclusions of Devos et al 2011. Devos et al 2011 takes into account the results of Elling et al 2009.

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

Comment 1

The information provided in the application is sufficient.

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