Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2015_0598

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/NL/2011/100 from Monsanto under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/NL/2011/100 was submitted by Monsanto on 17 August 2011 for the marketing of genetically modified Soybean MON87705 x MON89788 for food and feed uses, import and processing within the framework of Regulation (EC) No. 1829/2003[1]. Soybean MON87705 x MON89788 is a stacked event obtained by conventional crossing of both parental strains MON87705 and MON89788, thereby expressing the CP4 EPSPS protein conferring tolerance to the herbicidal active substance glyphosate and containing a FAD2-1A/FATB1A suppression cassette resulting in an altered fatty acid profile.

The application was officially acknowledged by EFSA on 26 October 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). Nine 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 24 January 2013.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 25 June 2015 (EFSA Journal 2015;13(7):4178[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 22 July 2015 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/4178.htm

It should also be noted that the Belgian Biosafety Advisory Council assessed the single soybean events MON87705[3] and MON89788[4] and concluded that it would be unlikely that these will have any adverse effect on human and animal health or on the environment in the context of its proposed uses. In its advice[5] on additional exposure data including the possible use of MON87705 in commercial frying, the Biosafety Advisory Council also agreed with the opinion of the EFSA Scientific Panel[6] that the use of soybean MON87705 oil, even in (commercial) frying is very unlikely to have a negative impact on human health and nutrition. MON87705 and MON89788 are both authorised in the EU for food and feed uses with the exception of GMO cultivation[7].

Scientific evaluation

1. Environmental risk assessment

The Biosafety Advisory Council is of the opinion that it is unlikely that the accidental release of soybean MON87705 x MON89788 seeds (i.e. during transport and/or processing) into the European environment[8] 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 soybean MON87705 x MON89788, in comparison with its conventional counterpart, do not raise safety concerns.

The Biosafety Advisory Council also considers that, although not required by the OECD Document on compositional considerations for new varieties of soybean (OECD, 2001), it lacks the analysis on dietary fibre. The Biosafety Advisory Council recommends the analysis on dietary fibre since this concept is widely accepted in human food studies and recommends the adaptation of the OECD consensus document accordingly.

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] Advice of the Belgian Biosafety Advisory Council of 7 December 2012 on application EFSA/GMO/NL/2010/78 (ref WIV-ISP/BAC/2012_1009)
[4] Advice of the Belgian Biosafety Advisory Council of 26 September 2008 on application EFSA/GMO/NL/2006/36 (ref WIV-ISP/BAC/2008_0813)
[5] Advice of the Belgian Biosafety Advisory Council of 25 June 2014 on application EFSA/GMO/NL/2010/78 (ref WIV-ISP/BAC/2014_0366)
[6] See http://www.efsa.europa.eu/en/efsajournal/pub/3507.htm
[7] EU register of GM food and feed: http://ec.europa.eu/food/dyna/gm_register/gm_register_auth.cfm?pr_id=62 for MON87705 and http://ec.europa.eu/food/dyna/gm_register/gm_register_auth.cfm?pr_id=32 for MON89788
[8] As the application doesn't imply a cultivation of the GM crop in the EU, a full environmental assessment is not required according to EFSA procedure and was therefore not achieved.

3.3. Assessment of allergenicity

The potential allergenicity of the newly expressed CP4 EPSPS protein has been assessed in the context of this application but also in the context of several previous applications. There are no indications that MON 87705 X MON 89788 would differ from its conventional counterpart in terms of allergenicity.

3.4. Nutritional value

Based on the compositional data, the results of a feeding study in chickens and an exposure assessment, the Biosafety Advisory Council agrees with the EFSA GMO panel that food and feed derived from GM soybean MON87705 x MON89788 does not raise concerns from a nutritional point of view.

4. Monitoring

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

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, soybean MON87705 x MON89788 is unlikely to pose any risk to human and animal health.
Given the scope of the application of this herbicide tolerant soybean with altered fatty-acid profile (no cultivation in EU) and the fact that the establishment of volunteer plants would be unlikely (soybean cannot survive without human assistance and is not capable of surviving as a weed in Europe), the potential environmental release of MON87705 is unlikely to pose any threat to the environment.

Prof. Maurice De Proft
President of the Belgian Biosafety Advisory Council

Annex I: Full comments of experts in charge of evaluating application EFSA/GMO/BE/2011/100 and *comments submitted on the EFSAnet (ref. BAC_2013_0050)*

Bioveiligheidsraad
Conseil de Biosécurité

24-January 2013

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC_2013_0050
Email: bac@wiv-isp.be

Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2011/100
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 12 November 2012
Coordinator: Dr. René Custers
Experts: Armand Christophe (UGent), Eddy Decuypere (KUL), Jacques Dommes (ULg), Leo Fiems (ILVO), Johan Grooten (UGent), André Huyghebaert (UGent), Peter Smet (Consultant), Frank Van Breusegem (VIB), Hadewijch Vanhooren (KUL)
Domains of expertise of experts involved: Molecular characterisation, genome analysis, genetic engineering, transgene expression, breeding techniques, plant biology, human and animal nutrition, biochemistry and analysis of food/feed, industrial processing of food/feed, toxicology in vivo & in vitro, immunology, alimentary allergology , plant allergens, soybean
SBB: Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman, Katia Pauwels

♦ INTRODUCTION

Dossier **EFSA/GMO/NL/2011/100** concerns an application submitted by the company **Monsanto** for authorisation to place on the market genetically modified **Soybean MON87705 x MON89788** 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 26 October 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 Scientific Opinion of the EFSA panel on GMO "Guidance for the risk assessment of food and feed from genetically modified plants" (EFSA Journal (2011), 9(5): 2150).
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

Comment 1

Both single events have already been evaluated. Only data related to the combination of both events are considered.

Comment 2

Plants with multiple insertion events are likely to have more transformation-induced mutations and thus carry a greater risk of exhibiting unintended consequences. Genetically modified soybean MON87705 x MON89788 is obtained by traditional breeding of 2 genetically modified maize lines. So, the chance for mutations is limited.
MON 89788 has already been approved for import, processing, and food and feed use in the EU (Anonymous, 2008), while EFSA (2012) stated that MON 87705 is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment. Furthermore, the modification of the fatty acid profile in foods can be beneficial for human health (Lichtenstein et al., 2006; Simopoulos, 2008).
In conclusion, the potential toxic effects of soybean MON87705 x MON89788 to humans and animals could be considered as negligible. However, some aspects of the quality of the end-products were lacking (see further).

A. HAZARD IDENTIFICATION AND CHARACTERISATION

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

Comments/Questions of the expert

Comment 1

P15: in the sentence about dietary supplements it is written that soybean is a rich source of phytochemicals as dietary supplements, including isoflavones and tocopherols. Furthermore, isoflavones have been shown to inhibit growth of cancer cells, lower cholesterol levels etc. while tocopherols have antioxidant activity, are anti-inflammatory, antineoplastic etc., suggesting that soybean is a very health-promoting substance, almost being more a medicament than a food.
Very often in the so-called health claims of food products, there is a large gap between the effective concentrations of these substances causing the claimed effects and the concentrations actually present in amounts of food products that are realistically consumed. The health claims on p15 of the dossier are therefore rather suggestive.

Comment 2

Information given is sufficient. No questions.

Comment 3

No comment.

Comment 4

No comment.

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

Comments/Questions of the expert

Comment 1

No comments

Comment 2

No comment.

Comment 3

No comment.

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

Comments/Questions of the expert

Comment 1

No questions

Comment 2

No comment.

Comment 3

No comment.

A.3. COMPARATIVE ASSESSMENT

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

Comments/Questions of the expert

Comment 1

No questions

Comment 2

1) Using the near-isogenic soybean variety A3525 as conventional counterpart is a logical choice.
2) In my opinion comparison of the results of MON 87705 x MON 89788 with those of parental lines grown **under the same conditions** is indicated as it would enable to determine whether there is an interaction between the stacked inserts or their gene products (Part II, Table 8, page 62-67, gives the results for MON 87705 and MON 89788 of crops grown in 2008 and 2005 respectively and for MON 87705 x MON 89788 of crops grown in 2009).

Comment 3

It is an important and positive step that the applicants included in their comparison also material from the MON 87705 × MON 89788 GMO treated before with the glyophosate herbicide.

Comment 4

Soybean MON 87705 x 89788 is compared with a near isogenic line A3525, a conventional counterpart.
No comments.

Comment 5

No comment.

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

Comments/Questions of the expert

Comment 1

In paragraph 3.2.3 it is stated that difference testing was performed at the 10% level of significance, and equivalence testing was performed at the 5% level. Why is this difference?
Is equivalence testing one-sided? Why an equivalence testing? Normally the zero-hypothesis is difference (and should be at the 5% level), and the alternative hypothesis is equivalence of means (http://v8doc.sas.com/sashtml/analyst/chap12/sect10.htm).

Comment from the SBB:
From the Scientific Opinion of the EFSA panel on GMO "Guidance for the risk assessment of food and feed from genetically modified plants" (EFSA Journal (2011), 9(5): 2150) we can read on page 16 : "Both the difference test and the equivalence test are implemented using the correspondence between hypothesis testing and the construction of confidence limits. In the case of equivalence testing the approach used follows the two one-sided tests (TOST) methodology (e.g. Schuirmann, 1987) by rejecting the null hypothesis of non-equivalence when both confidence limits fall between the equivalence limits. The choice of 90% confidence limits corresponds to the customary 95% level for statistical testing of equivalence."
Furthermore, on page 45 of Part II, the applicant states that it has followed the Scientific Opinion of the EFSA panel on GMO Statistical considerations for the safety evaluation of GMOs (EFSA Journal 2010; 8(1):1250)

Comment 2

No questions

Comment 3

Both varieties were grown at nine field sites, in the soybean growing areas of the US.
No particular remarks.

A.3.3. COMPOSITIONAL ANALYSIS

Comments/Questions of the expert

Comment 1

P60: I accept the argument that the small magnitude of differences between MON87705xMON89788 and the control, are not relevant for a food or feed safety perspective, particularly in the context of naturally occurring wide range of values for the conventional control.
But why no attempt for a scientific and/or statistical explanation of these differences?
Is the conclusion then not a bit overthrown?

There is compositional similarity to the conventional soybean at least if under conventional soybean is understood the range of values of 18 commercial varieties and not the control, non-GMO isogenic parental line; this could be understood as conventional (non-GMO), but it is not clear and left to the interpretation of the reader

In table 8, p64: why are the levels of palmitic, stearic, oleic, linoleic, linolenic acid so different for the control group US2008 (2nd column) and the control Arg2004-20054 (8th column) as well as the control US20055 (11th column) and MON89788 (US20055 or Arg 2004-20054)?

The specific manipulation in MON87705 or MON87705xMON89788 results in intentionally changed and decreased levels of palmitic and stearic acid and increased levels of oleic acid and associated decrease in linolenic acid.
But there should be no differences between the different controls or with MON89788.
All other tables give no differences between the different controls for whatever component, except for the fatty acids on p64: no explanation is given??

Comment 2

1) The data given for fatty acid composition for MON 89788 and its controls in part II, Table 8, page 63, are impossible and should be corrected.
2) In part II, page 36, it is mentioned that MON 87705 x MON 89788 and its 2 parental lines grown in the US in 2009 were compared for CP4 EPSPS protein levels.

Questions:

1) One would expect that the level of CP4 EPSPS in the stacked crop would be significantly higher of that in its parental lines, both expressing this protein. Is this the case? (Part II, Table 4, page 39).
2) Comparing the results of crops grown under different conditions is tricky. Why was this done in table 8, page 62-67? (different years).
3) Is there an explanation why the ranges in fatty acids (expressed as %, Table 8, page 64) are considerably larger in MON 87705 x MON 89788 than in MON 87705?

Minor comments:

1) Note that the symbol % is used in two different ways. To describe unintended differences between MON 87705 x MON 89788 and its control it is used in the sense of % points (page 46 ssq) whereas in the other case it is used in the sense of % (e.g. change in fatty acid composition). This way of presentation results in seemingly smaller differences for unintended effects and larger ones for intended effects.

The headings in Table 8 are confusing, e.g. (range) means different things. This makes comparison of the results difficult.

Additional comments from the coordinator

In part II, table 8, page 64 of the dossier the data are given for the fatty acid composition for the single events and the stack in comparison with different reference varieties. According to the table this fatty acid composition is given as the percentage of the total fatty acid (TFA). The data given for MON 89788 in this table, however, cannot be given as the percentage of the TFA, as the numbers do not add up to 100%. The numbers given for MON 89788 are in percentage of the dry weight, which makes it impossible to compare the numbers for MON 89788 with the numbers for the stacked event. The applicant is requested to correct this table and present correct data using the same unit for all events.

With a double herbicide tolerance one would expect the amount of CP4 EPSPS protein to be higher than in the single events. Table 4 indeed shows that this is the case, but there is a difference between forage and seeds. In forage the amount of CP4 EPSPS protein is doubled in seeds it is less than doubled. Is there an explanation for this, even though this does not raise any safety concerns?

Comment 3

A traditional approach was followed: a comparison of the nutritional composition of Soybean MON 87705 x 89788 and the isogenic line. The OECD document was followed for the selection of nutrients and anti-nutrients. It was accepted that any modification in the nutrient and anti-nutrient composition will be revealed by this in depth analysis.
In addition to the analytical comparative analysis, published data were also used in the evaluation.
Constituents studied for soybeans include:

- proximates: ash, fat , moisture, protein, carbohydrates by difference, acid detergent and neutral detergent fibre,
- 18 amino acids, fatty acids C8 – C24,
- vitamine E (no other vitamins) ,
- isoflavones: daidzein, glycitein and genistein,
- flatulence factors: raffinose and stachyose (not verbascose),
- phytic acid, lectin ans trypsin inhibitors.

Forage was analysed for proximates.
A statistical analysis according to the guidelines of EFSA, allows to conclude that Soybean MON 87705 x 89788, treated and non treated with glyphosate, is compositionally equivalent to conventional soybean except for the intended changes in fatty acids.
Results of the statistical analysis are presented in detail for beans and forage of Soybean MON 87705 x 89788 treated and not treated with glyphosate.
Although the conclusion is evident from the data presented, I have some comments:

- protein, amino acids, fat and fatty acids are well studied,
- anti-nutrients are also well covered,
- within the range of vitamins only vit E or α-tocopherol is studied; the applicant emphasizes that this constituent is particularly important in relation to the oxidation stability; it is well known that other tocols are present in soybean such as γ-tocopherol with the highest anti-oxidative capacity of all tocopherols; some information about the tocotrienols could also be given,
- no other vitamins and no minerals are included,
- the information about carbohydrates, with the exception of the flatulence factors, is not according to “the state of the art” of nutritional composition; carbohydrates by calculation (or by difference) is an obsolete concept; this also applies for the fibre constituents studied.

In previous applications the analysis was much more elaborated. As the application refers only to a combination of two events that have been evaluated before I agree with this limited and partial approach and with the conclusion of compositional equivalence.

Additional remark from SBB:
We note that fibers have been analysed according to the recommendations of OECD.
For consistency with previous dossiers, and more particularly for conistency with the evaluation of dossier EFSA/GMO/NL/2010/78 (MON 87705), we inform that several similar comments (as regards the saponins, the requirement to determine dietary fibre, the analysis of other vitamins than only vit E) were made and rephrased into the following comment (sent to EFSA) :

"Although the OECD consensus document on "Compositional considerations for new varieties of soybean: key food and feed nutrients and anti-nutrients" does not prescribe the analysis of saponins, desmethyltocopherols, phytosterols and phytosterol glycosides, one expert has suggested to include these components in the compositional analysis. It is also recommended that the same OECD consensus document is adjusted on the point of the analyses of fibres,and that for human food uses, the requirement will become to determine dietary fibre instead of ADF and NDF. Moreover, the document should consider more recent soybean foods such as soybean drinks and derived foods. These types of foods may make it more relevant to look at other vitamins, instead of only vitamin E.
In its previous advices the Biosafety Advisory Council underlined that in the revised version of the OECD Consensus Document on Compositional Considerations for New Varieties of soybean (still under discussion at OECD level), Vitamin K is also listed as suggested constituent to be analysed related to food use. The Biosafety Advisory Council is of the opinion that data provided by the applicants should comply with the latest scientific standards."

As regards the assessment of the carbohydrates, the following comment has been transmitted to EFSA within the context of the evaluation of dossier EFSA/GMO/NL/2010/78 (MON 87705) (a comment that is also consistent with other previous dossiers):
"It is noted that the carbohydrates are reported in values 'by difference'. This way of reporting is no longer accepted for the inclusion in nutrient labels. We recommend to alter this into reporting in the form of 'available carbohydrates'

Comment 4

Concerning the antinutrient composition, there seems to be no problematic difference between MON 87705 × MON 89788 and its control.

Comment 5

Ratios of SFA/PUFA and n-6/n-3 for soybean MON87705 x MON89788 are not presented.

Additional remark of the SBB:
See also comment 3 in section A.5.2. on the differences observed in the composition of certain amino acids (cysteine, arginine, methionine) between the untreated as well as herbicide treated MON 87705 × MON 89788 and its conventional comparator

A.3.4. AGRONOMIC AND PHENOTYPIC CHARACTERISTICS

Comments/Questions of the expert

Comment 1

No questions

Comment 2

No comments

A.3.5. EFFECTS OF PROCESSING

Comments/Questions of the expert

Comment 1

No questions

Comment 2

Soy lecithin is one of the processed products of crude soybean oil which is widely used in foods including infant formulas.

Question:

One of the aims of the genetic modifications was to obtain a soybean oil of which the fatty acid composition is changed. This might result as **unintended effect** in a change in fatty acid composition of the phosphatides. Is this the case?

Additional comment from SBB:

For consistency with previous dossiers, we inform that in dossier EFSA/GMO/NL/2010/78 (MON 87705) the applicant analyzed the four phosphatides (α-phosphatidic acid, α-phosphatidylcholine, α-phosphatidylethanolamine, and α- hosphatidylinositiol.) which form the major fraction of the lecithin (65 – 75%). For Soybean MON 87705 x 89788, no information is provided as regard these phosphatides nor on the composition of crude lecithin despite the fact this information is recommended according to the OECD consensus document on "Compositional considerations for new varieties of soybean: key food and feed nutrients and anti-nutrients"

For MON 87705 x MON 89788, in contrast to MON 87705, no information is given on the amounts of the major phosphatides (α-phosphatidic acid, α-phosphatidylcholine, α-phosphatidylethanolamine, and α- hosphatidylinositiol.).

See also comment 2 in section A.6.2.

Comment 3

The applicant states that processing of soybean MON 87705 x 89788 is not expected to be different from that of conventional soybean. An overview of the different processing methods of soybean is given including solvent extraction, full fat processing, expelling, extrusion and others.
I agree with this conclusion.

Comment 4

The shift towards more oleic acid can be an advantage for frying processes. Double bonds in the fatty acids of vegetable oils subjected to high temperatures during frying can undergo geometrical isomerization from the cis to trans configuration, but oleic acid is hardly affected. There is sufficient evidence to suggest a positive association between trans fatty acids and coronary heart disease. Therefore, a recommendation for a reduction in intake of trans fatty acids is justified (Booker and Mann, 2008).

A.4. TOXICOLOGICAL ASSESSMENT

A.4.1. METHODOLOGY USED FOR TOXICITY TESTS

Comments/Questions of the expert

Comment 1

No questions

Comment 2

No toxicity studies on the processed MON87705 x MON89788 soy oil were submitted.

Data gap: Information is lacking on the levels and fate of herbicide residues in crop tissues.
Although the effect of herbicides residues on human and animal health falls under Directive 91/414/EC, it is the duty and responsibility of the toxicologist assessing the risk of the genetic modification to evaluate and discuss the complete picture of the genetic modification.
Rationale: The GM soybean plant is developed to be able to use the glyphosate-based herbicide formulations. Data concerning the use (over-the-top applications) of the herbicide in the field trials is available. However, no data is made available concerning the herbicide formulation and metabolites residues in the GM plants and grain used for food/feed. As the use of these herbicides is linked to the genetic modification, the applicant should make the residue data available and make an estimation of the anticipated intake (food/feed).

Additional comments from the SBB:
The assessment of the safety of the herbicide and its residues is outside the remit of the Biosafety Council.

From the Scientific Opinion of the EFSA panel on GMO "Guidance for the risk assessment of food and feed from genetically modified plants" (EFSA Journal (2011), 9(5): 2150) we can read on page 20: "Toxicological tests with the processed products are not required if the GM plant (or relevant parts of it) is demonstrated to be safe and there are no indications that the processed products would be any different from their non-GM counterparts. The applicant should provide adequate justification in this regard.
A genetic modification targeting metabolic pathways may result in changes in the concentration of plant constituents and lead to the production of new metabolites (e.g. nutritionally enhanced foods/feeds, functional foods). Processed products derived from such GM plants may require specific approaches for their risk assessment. The applicant should provide a scientific rationale for the selected approach. On a case-by-case basis, experimental data may be required. This may include information on the composition, on the level of undesirable substances, or on the nutritional value."

We inform a 90-day study in rats has been conducted within the context of the application EFSA/GMO/NL/2010/78 (MON 87705), the rats were fed with defatted soybean meal.
See also comment 1 under A.6.2 and comment 5 under A. 4.2.

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

Comments/Questions of the expert

Comment 1

No questions

Comment 2

No further questions.

Comment 3

Question (not related to safety): can MON 87705 expressing both the CP4 EPSPS protein and the FAD2-1A/FATB1-A suppression cassette be considered as a **single trait** product as mentioned on page 80, last paragraph?

Comment 4

The amount of CP4 EPSPS in MON 87705 × MON 89788 is comparable to the amount found in MON 87705.
It has been demonstrated earlier that the CP4 EPSPS protein excerts no acute toxicity to mice, is readily degradable in SGF and SIF and shows no similarity to known toxins.

Comment 5

A series of test have been conducted to evaluate health aspects, and most tests do not show a detrimental effect. Therefore, and also because of the safe history of the parent lines, it is assumed that it is unlikely that the newly expressed proteins will be unsafe for human or animal health.
This is confirmed by a study of Qi et al. (2012). Sprague–Dawley rats were fed dietary concentrations (7.5%, 15% and 30%) of 3Ø5423 x 40-3-2 or non-genetically-modified soybeans for 90 days. Soybean 3Ø5423 x 40-3-2 also expresses FAD2, resulting in higher concentrations of oleic acid relative to linoleic acid, compared with non-genetically-modified soybeans. These results demonstrated that the genetically-modified soybean 3Ø5423 x 40-3-2, expressing FAD2, is as safe as non-genetically-modified soybeans.

Additional remark from the SBB.
See also comment 2 in section A.6.2.
In the study of Qi et al (2012) it is mentioned that "the seven groups of rats were fed with balanced diets with 7.5%, 15%, 30% (wt/wt) transgenic ***soybean (****T1, T2, T3) or traditional soybean. » . In the discussion it is also mentioned that "****Processed fractions from HOA-HT soybean** *were formulated into rodent diet and Sprague–Dawley rats for 90 days to test for nutritional and safety effects compared with the non-GM isogenic control line. As with many subchronic feeding studies conducted with maize grain or processed fractions of soybeans from GM crops a number of statistical differences*

were observed between animals consuming diets containing the non-GM isoline when compared with the HOA-HT soybean. However, the differences were not dose-related and individual animal data fell within the normal ranges of the control group with commercial diet."

Hence it is not clear whether a processed fraction (which one?) has been administered to the rats during the study.

A.4.3. ASSESSMENT OF NEW CONSTITUENTS OTHER THAN PROTEINS

Comments/Questions of the expert

Comment 1

No questions

Comment 2

No further questions.

Comment 3

Question: Is MON 87705 x MON 89788 more tolerant to glyphosate than MON 87705 also expressing the EPSPS protein? (if so it could lead to higher use of glyphosate resulting in higher levels of glyphosate or its breakdown products in foods or feeds).

Additional remark from the SBB:
Table 4, pg 39 shows that CP4 EPSPS protein levels in MON 87705 × MON 89788 were higher compared to MON 87705 and even higher compared to MON 89788. The difference was mainly apparent in forage, and less (but still apparent) in seeds.
The presence of higher levels of CP4 EPSPS do not necessarily imply the use of higher concentration of glyphosate. The assessment of the use of glyphosate regimens is outside the remit of the Biosafety Council.

Comment 4

No comments

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

Comments/Questions of the expert

Comment 1

No questions

Comment 2

A compositional analysis on forage and seed was provided but not on processed oil.

Comment 3

Altered levels of food and feed constituents were sufficiently assessed.

Comment 4

I agree with the conclusion particularly in relation to the modification of the fatty acid composition and the use of this soybean oil for the replacement of saturated and trans fatty acids in the diet.

Comment 5

Ratios of SFA/PUFA and n-6/n-3 for soybean MON87705 x MON89788 are not presented.

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

Comments/Questions of the expert

Comment 1

No questions

Comment 2

Food: processed oil.
A compositional analysis on forage and seed was provided but not on processed oil. In addition, a broiler study (poultry fed defatted soybean meal, < 1.5% crude fat) was submitted for the nutritional assessment of the whole feed. However, no whole food study with processed oil is submitted: soybean oil constitutes approx. 29% of global consumption of vegetable oil. As the fatty acid profile of the oil is changed, at least a 90-day repeated dose rat toxicity study should be performed with processed soybean oil (gavage, heated and not-heated). Because of the modified fatty acid profile (due to RNA-based suppression) and of the high percentage of oleic acid in the oil a toxicological assessment should be performed to exclude any unintended adverse health effects: extra attention should be paid for the endpoints immunotoxicity, atopic sensitisation, lung toxicity, reproduction parameters. Because of the high percentage of oleic acid, additionally an inhalation study should be performed with the processed MON87705 x MON89788 soybean oil and flour. Oleic acid-induced pulmonary injury is a well-described animal model of human acute adult respiratory distress syndrome (ARDS) (Beilman, 1995).

Additional comment from the coordinator:
The safety of oil with an altered fatty acid profile has been substantiated by the applicant by referring to different studies, which are described in more detail in application 2010/78 (MON 87705). No specific toxicological or nutritional study has been done using non-defatted meal or processed oil,

even though these fractions would truly represent the alteration in the genetically modified soybean. Do the different studies referred to in the application also present a good substantiation of the safety from an immunotoxicological point of view?

Additional comments from the SBB:
Considerations as regards the use of defatted soybean meal in the 42-day broiler study have also been addressed in comment 1 of section A.6.2.

Acute respiratory distress (ARD) and oleic acid :
Olives and rapeseed are not processed in the same way as soya, the question here might be whether for example, flour whose particles in the air would pose particular safety concern, and whether it would justify specific conditions or instructions when MON 87705 x MON89788 is stored, packaged, transported, used and handled than those that are proposed by the applicant.
The effects of inhalation into the lungs of sensitive individuals of material high in oleic acid are unknown. However, oleic acid instillation provides a good model to study ventilatory strategies, lung mechanics, and V/Q distribution during lung injury in large and small animals provided that oleic acid administration occurs intravenously (Matute-Bello et al., 2008). In the paper of Matute-Bello G et al. it is also questioned to which extent this type of injury actually occurs in humans, because few cases of ARDS are associated with long bone trauma or lipid injury, and there is no evidence that the pathophysiology of the injury caused by oleic acid (direct toxicity to endothelial cells) is similar to that underlying ARDS associated with sepsis and aspiration. Authors of the paper concludes that the oleic acid model is probably not as appropriate for studying the pathophysiology of ALI due to sepsis, or therapeutic strategies aimed at modifying host inflammatory responses to reduce the severity of lung injury.

Comment 3

The whole food and feed were sufficiently assessed. Moreover a broiler chickens feeding study with soybean meal has been performed.

Comment 4

a) 42-day feeding study in broiler chickens (MSL0022972, 2011)
No test substance-related effect was observed.

b) 90-Day rat feeding study ().
Not performed. No further testing is needed.

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

Comments/Questions of the expert

Comment 1

No questions

Comment 2

The potential for eliciting allergic responses of the CP4 EPSPS protein expressed in either MON 87705 and MON 89788 parental lines has been the subject of a prior assessment by the EFSA and was found to be unlikely to be allergenic. As the present MON 87705 × MON 89788 was derived by conventional crossing of both parental GM plants, I agree with the applicants that CP4 EPSPS protein expressed in MON 87705 × MON 89788 is unlikely to be allergenic.

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

Comments/Questions of the expert

Comment 1

No questions

Comment 2

Note that the allergenicity of the whole MON 87705 x MON 89788 plant was not determined but assumed not to be different from conventional soybean on basis of the findings that both parental lines were not different in allergenicity.

Comment 3

The main arguments put forward by the applicant for NOT performing additional allergenicity testing of the whole GM plant and rather base the assessment on the prior 'safe' evaluations of the parental MON 87705 and MON 89788 GM plants essentially are:
1. The derivation of MON 87705 × MON 89788 by traditional breeding of MON 87705 and MON 89788, thus without the introduction of new genetic traits;
2. The absence of (obvious) interactions between the newly combined events, i.e. the suppression cassette and CP4 EPSPS protein;

3. The lack of evidence for an interaction between both copies of the cp4 epsps gene based on CP4 EPSPS expression levels;
4. The high substrate specificity of CP4 EPSPS, thus rendering highly unlikely endogenous plant constituents to serve as substrates for the enzyme and to be modified in the process.

Whereas this reasoning clearly makes sense, certain features of the analysis not considered by the applicant do raise some concern regarding potential allergenicity of the whole GM plant.
First, CP4 EPSPS expression values are not stochastic in the hybrid with respect to the parental lines, showing values in the forage of 260-420 µg/g DW for MON 87705 × MON 89788 as opposed to 160-270 and 120-180 for the parental lines. This difference may point to some level of interaction between both gene copies. Second and to me most worrisome, the untreated as well as herbicide treated MON 87705 × MON 89788 consistently differs from its conventional comparators in the composition of certain amino acids (cysteine, arginine, methionine). With unaltered total protein levels, such differences in amino acid levels may be indicative of altered expression levels of certain endogenous proteins.
Expression increments of endogenous allergenic proteins can therefore not be excluded and should be verified by the applicant as a prerequisite for assessing the allergenicity of the whole plant. As allergens from soybean are not well defined, quantitative proteomic approaches may not be conclusive (unless unaltered expression levels would be observed). Allergenicity testing using human IgE from soybean allergic individuals on 2D SDS-PAGE quantitative immunoblots seems the most appropriate approach for verifying whether whole plant protein extracts contain modified levels of endogenous allergens.

Comment from the coordinator:
According to the text no significant differences were found in methionine levels of non-treated MON87705xMON89788 compared to controls.
And when we look at table 8 we don't see big changes in the percentages of the amino acids in the different treated and non treated soybeans.

Comment 4

A series of test have been conducted to evaluate risks of allergenicity, and most tests do not show a detrimental effect. Therefore, and also because of the safe history of the parent lines, it is assumed that it is unlikely that any interaction between the newly expressed proteins would alter the pattern of expression of endogenous proteins/potential allergens.

A.5.3. ADJUVANTICITY

Comments/Questions of the expert

Comment 1

No questions

Comment 2

I agree with the applicant's conclusion that no indications for adjuvanticity of the MON 87705 × MON 89788 GM plant are present.

A.6. NUTRITIONAL ASSESSMENT

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

Comments/Questions of the expert

Comment 1

No questions

Comment 2

The ratios of SFA/PUFA and n-6/n-3 fatty acids were not presented.
Furthermore, the effect of soybean MON87705 x MON89788 on the quality of the end-product is not presented in the dossier. Based on previous experiments, we can assume a shift in the fatty acid composition of intramuscular fat content in pigs (Myer et al., 1992) and broilers (Ortis et al., 2006).

Additional comments from the SBB:
The applicant states (p6): Meat analysis results (fat, moisture and protein) for skinless breast and thigh meat samples collected during bird processing were not different ($p \geq 0.05$) for birds fed diets containing soybean meal produced from MON 87705 × MON 89788 versus those of birds fed diets containing control or reference meal based on individual diet comparisons or comparison to the population of control and reference meal diets (Table 14 and Table 15).

However, as addressed further in comment 1 of A.6.2, for the 42-day feeding study, broilers were fed with defatted meal. The question is whether results obtained using defatted soybean meal in the 42-feeding study with broilers contribute to the assessment of the nutrional wholesomeness of soybean with altered fatty acid composition.

The broiler feeding study shows that defatted soybean meal of MON87705xMON89788 is as nutritious as control soybean and does not result in differences in meat. However, such a study with defatted meal does not contribute to the assessment of the nutritional wholesomeness of whole soybean with an altered fatty acid composition.

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

Comments/Questions of the expert

Comment 1

-p93: was this full-fat soybean meal or soybean meal after oil extraction? This should be mentioned here

-p94:There are 18 pens / block, so 9 feed treatments for males and for females I suppose; were the birds sexed before the start of the experiment?
The nine feed treatments are in fact only 8 treatments in the table (14 & 15), as it is explained in a footnote (p94) that one test substance of which the results are excluded since it was not the subject of this study.
But then it could be questioned if the statistics were done for the 8 treatments or for the 9 treatments as this is not clear from the text??

-p96: I wonder what about the fat composition of the fat of the birds and why this is not analysed?
No differences would be expected if it was SBM after fat extraction, but with full-fat SBM, as this is sometimes used, a difference of fat composition of the feed and hence also in poultry fat could be expected.
The latter can influence characteristics of poultry fat such as softness, easiness of skin rupture during the defeathering process… (the so-called oily birds).
On p100 I then understood that the tests has been done with defatted soybean meal, but then what is the purpose here to use defatted soybean meal as the genetic modification just concerns the difference in fatty acids??
You make a genetic modification, here in fatty acid composition of the oil, you extract this, give the defatted meal, hence eliminating the proposed difference, to chickens to conclude that there is no difference from a feed nutritive perspective between the control and MON87705xMON89788 ?? This is a strange way of conducting such a feed trial.
I would rather compare the full-fat SBM, even if most of SBM is defatted, nevertheless sometimes full-fat grains after toasting are or may be used as feed source (as also stated on p 107 e.g.)

Additional comment from the SBB:
We note that the broiler chickens in the 42-day feeding study were fed with defatted meal from Soybean MON 87705 × MON 89788 in broiler chickens. We also inform that for the 42-day feeding study as well for the 90-day study in rats conducted within the context of the application EFSA/GMO/NL/2010/78 (MON 87705), both broiler chickens and rats were fed with defatted soybean meal.
According to the applicant, the finished meal, which is used as feed source and which is obtained from dehulled soybean contains less than 1.5% (w/w) crude fat and approximately 48% (w/w) protein, and is referred to as high protein meal (p15, Part II).

Comment 2

The fatty acid composition of the phosphatides in soy lecithin may have been changed (see above; A.3.5.). Note in this respect that the fatty acid composition of some soy phosphatides is of nutritional

(Yaguchi T et al., 2010; Gundermann KJ et al., 2011) and pharmacological (Gundermann KJ et al., 2011) importance.

Note that in its final report on MON 87705, EFSA mentioned that the oil derived from MON 87705 is not suited for commercial frying. Probably the same holds for MON 87705 x MON 89788 having the same fatty acid composition and levels of antioxidants as MON 87705 (the oxidative stability of the oil derived from seeds of MON 87705 x MON 89788 was not determined).

Comment 3

The phytic acid in soybean MON87705 x MON89788 (average of treatments with or without glyphosate) was increased by 7.5% compared to control soybean ($P < 0.10$). Phytic acid is important, especially in monogastric nutrition, as it makes dietary phosphorus insoluble, resulting in an increased phosphorus emission into the environment.
Feeds with a reduced content of saturated fatty acids (SFA) are also interesting for monogastric animals, as their body fat is a largely a reflection of dietary fat. Dietary fatty acid profile is less relevant for ruminant animals, as lipids are largely hydrolysed in the rumen and subsequently bio-hydrogenated, so that 60 to 90% of the fatty acids is saturated.

B. EXPOSURE ASSESSMENT - ANTICIPATED INTAKE/EXTENT OF USE

Comments/Questions of the expert

Comment 1

No questions

Comment 2

No questions

C. RISK CHARACTERISATION

Comments/Questions of the expert

Comment 1

No questions

Comment 2

It is true that, when substituted for saturated fatty acids, both oleic acid and linoleic acid lower plasma cholesterol and LDL cholesterol (part II, page 114). However, what is not mentioned is that the cholesterol lowering effect of linoleic acid is higher than that of oleic acid. The important question in this respect is how oil derived from MON 87705 x MON 89788 compares to conventional soybean oil (somewhat higher in saturated fatty acids but much higher in linoleic acid) as to cholesterolemic effect.

Question: is the cholesterolemic effect of oil derived from MON 87705 x MON 89788 lower than that of conventional soybean oil? (This can be estimated based on formulas in literature).

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

Comments/Questions of the expert

Comment 1

No questions

E. ENVIRONMENTAL RISK ASSESSMENT

E.1. INTRODUCTION

Comments/Questions of the expert

Comment 1

No questions

E.2. GENERAL APPROACH OF THE ERA

Comments/Questions of the expert

Comment 1

No questions

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

Comments/Questions of the expert

Comment 1

No questions

E.3.2. PLANT TO MICRO-ORGANISMS GENE TRANSFER

Comments/Questions of the expert

Comment 1

No questions

E.3.3. INTERACTION BETWEEN THE GM PLANT AND TARGET ORGANISMS

Comments/Questions of the expert

Comment 1

No questions

E.3.4. INTERACTION BETWEEN THE GM PLANT AND NON-TARGET ORGANISMS (NTOS)

Comments/Questions of the expert

Comment 1

No questions

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

Comments/Questions of the expert

Comment 1

No questions

E.3.6. EFFECTS ON BIOGEOCHEMICAL PROCESSES

Comments/Questions of the expert

Comment 1

No questions

E.3.7. EFFECTS ON HUMAN AND ANIMAL HEALTH

Comments/Questions of the expert

Comment 1

No questions

Comment 2

Based on the arguments presented, I agree with the conclusion of the applicant that the likelihood for any adverse effects in humans as a result of their contact with MON 87705 x MON 89788 is probably not higher than that of contact with conventional soybean.

Comment 3

No allergenic and toxicological risks are expected. The increased concentration of unsaturated fatty acids may be an advantage for human health (Llor et al., 2003).
It is assumed that less trans fatty acids will be formed when oleic acid is used for frying (Warner and Knowlton, 1997). So, this is an advantage for soybean MON87705 x MON89788.

Additional comment from SBB:
As regards the use for frying, see also comment 2 in section 6.2

E.3.8. Overall risk evaluation and conclusions

Comments/Questions of the expert

Comment 1

No questions

E.4. Post Market Environmental Monitoring plan

E.4.1. Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert

Comment 1

No questions

E.4.2. Case-specific GM plant monitoring

Comments/Questions of the expert

Comment 1

No questions

E.4.3. General surveillance for unanticipated adverse effects

Comments/Questions of the expert

Comment 1

No questions

E.4.4. Reporting the results of monitoring

Comments/Questions of the expert

Comment 1

No questions

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