Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on application EFSA-GMO-DE-2012-111 (soybean SYHT0H2) from Syngenta under Regulation (EC) No. 1829/2003

10 March 2020
Ref. SC/1510/BAC/2020_0265

Context

Application EFSA-GMO-DE-2012-111 was submitted by Syngenta for the authorisation for the marketing of genetically modified (GM) soybean SYHT0H2 for food and feed uses, import and processing (excluding cultivation) within the European Union, within the framework of Regulation (EC) No. 1829/2003[1].

Soybean SYHT0H2 expresses the AvHPPD-03 and PAT proteins, conferring resistance to mesotrione and other p-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides, and glufosinate ammonium respectively.

The application was validated by EFSA on 9 January 2013 and a formal three-month consultation period of the Member States was started, lasting until 10 April 2013, 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. See Annex I for an overview of all the comments and the comments sent to EFSA on 8 April 2013.

The opinion of the EFSA Scientific Panel on GMOs was published on 20 January 2020 (EFSA Journal 2020;18(1):5946[2]) together with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period.
On 21 January 2020 these two documents were forwarded to the Belgian experts. They were invited to give comments and to react if needed.

In delivering the present advice the BAC considered in particular the information below:
- The comments formulated by the experts on application EFSA-GMO-DE-2012-111;
- The opinion of EFSA.

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (OJ L 268, 18.10.2003, p.1).
[2] See https://doi.org/10.2903/j.efsa.2020.5946

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Scientific evaluation

1. Environmental risk assessment

The Biosafety Advisory Council is of the opinion that it is unlikely that the accidental release of soybean SYHT0H2 (i.e. during transport and/or processing) into the European environment[3] will lead to environmental harm.

2. Molecular characterisation

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

3. Assessment of food/feed safety and nutritional value

3.1. Assessment of compositional analysis

The Biosafety Advisory Council agrees with the GMO panel of EFSA that the compositional data of GM soybean SYHT0H2, in comparison with its conventional counterpart, do not raise safety concerns.

3.2. Assessment of toxicity

The Biosafety Advisory Council agrees with the GMO panel of EFSA that the available data on the toxicity of GM soybean SYHT0H2, in comparison with its conventional counterpart, does not raise safety concerns. Taking the origin of the protein into account (*Avena sativa*), as well as the fact that the significant differences are minimal and the absence of dose-response effect, the BAC considers it unlikely that the AvHPPD-03 protein would have toxic properties.

3.3. Assessment of allergenicity

The Biosafety Advisory Council has evaluated the safety of the newly expressed PAT protein in the context of previous applications, and no concerns were identified. Since no new information on allergenicity of these proteins has become available, the Council is of the opinion that its previous conclusions remain valid. The Biosafety Advisory Council agrees with the GMO panel of EFSA that the available data on the allergenicity of GM soybean SYHT0H2, in comparison with its conventional counterpart, does not raise safety concerns.

3.4. Nutritional value

The Biosafety Advisory Council is of the opinion that the information provided is sufficient to conclude that the nutritional characteristics of soybean SYHT0H2-derived food and feed are not expected to differ from those of conventional soybean varieties.

4. Monitoring

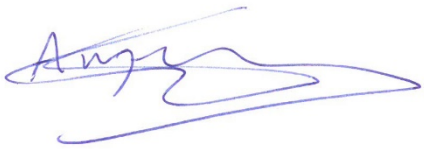
With regard to monitoring, the Biosafety Advisory Council is of the opinion that the information provided is sufficient.

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

Conclusion

Based on the whole set of data on soybean SYHT0H2 provided by the applicant, the scientific assessment of the dossier done by the Belgian experts, the opinion of EFSA, and the answers of the EFSA GMO panel to the questions raised by the Belgian experts, the Biosafety Advisory Council:

1) Agrees with the GMO panel of EFSA that the potential environmental release of soybean SYHT0H2 is unlikely to pose any threat to the European environment;
2) Agrees with the GMO panel of EFSA that in the context of its proposed uses, soybean SYHT0H2 is unlikely to pose any risk to human and animal health;



Dr. Geert Angenon
President of the Belgian Biosafety Advisory Council

Annex I: Compilation of comments of experts in charge of evaluating the application EFSA-GMO-DE-2012-111 (ref. BAC_2013_0224)

Bioveiligheidsraad
Conseil de Biosécurité

08-04-2013

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC_2013_0224
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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/DE/2012/111
and
Comments submitted on the EFSAnet on mandate of the Biosafety Council

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 21 January 2013
Coordinator: Dr. René Custers
Experts: Armand Christophe (UGent), Eddy Decuypere (KUL), Leo Fiems (ILVO), Johan Grooten (UGent), André Huyghebaert (UGent), Peter Smet (Consultant), Frank Van Breusegem (VIB), Jan Van Doorsselaere (KATHO), Hadewijch Vanhooren (KUL)
Domains of expertise of experts involved: Molecular characterisation, genome analysis, transgene expression, DNA/RNA/protein analysis, genetic engineering, human and animal nutrition, biochemistry of food/feed, analysis of food/feed, industrial processing, toxicology in vivo & in vitro, immunology, alimentary allergology , plant allergens, herbicide tolerance, soybean.
SBB: Didier Breyer, Fanny Collard, Adinda De Schrijver, Martine Goossens, Philippe Herman, Katia Pauwels

♦ INTRODUCTION

Dossier **EFSA/GMO/DE/2012/111** concerns an application submitted by the company **Syngenta** for authorisation to place on the market genetically modified **Soybean SYHT0H2** in the European Union, according to Regulation (EC) No 1829/2003 on genetically modified food and feed.
The application has been officially acknowledged by EFSA on 10 January 2013.
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 experts:

Comment 1

OK, in general this document gives very complete information.

Comment 2

Based on this dossier, it is assumed that the use of SYHT0H2 soybean will pose a neglectable risk to human and animal health or the environment.

A. HAZARD IDENTIFICATION AND CHARACTERISATION

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

Comments/Questions of the experts:

Comment 1

No comments

Comment 2

No comments

A.2. MOLECULAR CHARACTERISATION

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

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

Comments/Questions of the experts:

Comment 1

The gene avhppd-03 encodes for the hydroxyl-phenyl pyruvate dioxygenase enzyme that catalyzes the formation of homogentisic acid, the aromatic precursor for all vitE biosynthesis (α,β,γ,δ-tocopherols and –tocotrienols), as well as for plastoquinone, an essential electron acceptor in the phytoene desaturation reaction of carotenoid biosynthesis.
The oat–derived AvHPPD-03 is more tolerant to HPPD-inhibiting herbicides (such as mesotrione) than soybean, since it has a lower (but no absence of binding!!) affinity for HPPD-inhibiting herbicides.

Therefore, sufficient attention should be given to comparison between GM-plant and its conventional counterpart for Vit E components and Vit A.
-The gene pat encodes for the enzyme phosphinothricin acetyl-transferase, which inactivates the herbicide glufosinate ammonium, itself an inhibitor of glutamine synthetase, essential in the nitrogen assimilation pathway.

Comment 2

No comments

Comment 3

No comments

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 experts:

Comment 1

No questions

Comment 2

No comments

Comment 3

For sake of completeness of the general dossier, the AA substitution in the introduced AvHPPD gene could be indicated (pg. 18). Now there is only mentioned a 99.7% AA homology.

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

The choice of soybean variety “Jack” as conventional counterpart is logical.

Comment 3

As it is usually the case, the conventional counterpart was included in the comparative analysis. Six conventional non-GMO reference soybean varieties were also included.
No further comments as this approach is in accordance with the EFSA guidance document.

Comment 4

No comments

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

Comments/Questions of the expert

Comment 1

No questions; experimental design and statistical analysis is OK.

Comment 2

Also in accordance with the EFSA guidance document.
No particular comments.

A.3.3. COMPOSITIONAL ANALYSIS

Comments/Questions of the experts:

Comment 1

Four groups are included:
- untreated GM plant
- treated GM plant
- conventional counterpart
- reference varieties

In table A.3.3.3 and A.3.3.4 I miss the comparisons for β tocopherol and for the tocotrienols

Why lower levels for α tocopherol and higher for γ tocopherol in the GM soybean, while both would be expected to have the same trend as the GM-plant is presumed to change the precursor, homogentisic acid, for both (or all) tocopherols and tocotrienols?

Comment 2

Some minor components show a statistical difference between SYHT0H2 soybean and its conventional counterpart (P<0.05). However, these differences may not be biologically relevant, with no further consequences for food and feed safety and a health perspective.

Comment 3

The differences found between SYHTOH2 and it conventional comparator have no nutritional relevance.
The increase of gamma-tocopherol can be considered as an unintentional effect of the genetic modification but a tentative explanation is given (part II, page 48). Note however that its biosynthetic product, alpha-tocopherol, is decreased.
Question: has it been excluded that the genetic modification does not result in a reduction of gamma-tocopherol methyltransferase activity? (If so this would be an unintentional effect; possible interaction with the gamma-tocopherol methyltransferase gene?).

Comment 4

There seem to be no major differences between the GMO and its control and references for the antinutrients and secondary metabolites.

Comment 5

Key nutrients in soybeans and forage were selected according to the OECD guidance document.
Components analysed in forage include the well known proximates, important in animal feeding.
Soybeans were analyzed in detail:

- Proximates,
- Minerals: no remarks
- Vitamins: relevant vitamins in soybeans are included,
- Vitamin E is analyzed in detail; in addition to the well known tocopherols in soybeans, tocotrienols are also analyzed; there is growing interest in these nutrients due to their anti-oxidative properties; they are present in significant amounts in soybeans,
- Amino acids: essential amino acids are included in addition to the non essential amino acids,
- Fatty acids: the analyses cover the significant fatty acids in soybeans,
- Anti-nutrients: known anti-nutrients like phytoestrogens and other anti-nutrients are included in the comparative analysis.

The results of the comparative analysis and the statistical evaluation are presented in detail.

The applicants concludes that based on these results, there is no difference in the compositional characteristics of soybean SYHT0H2, the conventional counterpart and the non-GMO soybean reference varieties, except for the induced trait. Natural variability, reported in the ILSI database is taken into consideration. Some minor differences are observed with no biological significance.

I have no particular remarks on the selection of the nutrients, the statistical evaluation and the results obtained.
I agree with the conclusion of the applicant.

A.3.4. AGRONOMIC AND PHENOTYPIC CHARACTERISTICS

Comments/Questions of the experts/

Comment 1

Table A.3.4.3 arguments on plant height in the text on p 53: if this argument is used, then a difference between the GM plant and its conventional counterpart for whatever substance is then compared to a set of reference varieties; if it is within the range of values of these, it is concluded that there is no biological relevance.
But the argument used here as to the limitation in representativeness of the reference varieties undermines the often used reasoning above.
I am unhappy about the wording of the sentence in the table, but I agree with the conclusion.

A.3.5. EFFECTS OF PROCESSING

Comments/Questions of the experts:

Comment 1

No questions

Comment 2

Note that the claim "that it is unlikely that the processing…normally applied to soybean would cause any modification to the processed products obtained from SYHTOH2 soybean compared with their non-GM counterparts" (Part II, page 56, 2nd paragraph) is not entirely justified as the proteins PAT and AvHPPD-03 were found in some processed fractions (Part II, page 55, lines 33-34).

Comment 3

Processes applied to soybeans are described in detail, including the composition of the products obtained. As there is no compositional difference between soybean SYHT0H2, the conventional counterpart and reference soybean varieties, no particular effects due to processing are to be expected.
In a series of processing tests on a laboratory scale, equivalent to industrial processing, the applicant demonstrated that the newly expressed protein AvHPPD-03 is readily degraded at high temperatures. It is concluded that temperatures of 65°C and higher would be expected to result in negligible exposure to this native protein.

Temperatures of 65°C and above are indeed generally applied in soybean processing.

With respect to heat stability of PAT protein the applicant refers to literature data and to laboratory processing studies .

It is concluded that it is unlikely that the processing technology applied to soybean SYHT0H2 would cause any modification of the processed products compared with their conventional non-GMO counterpart.

I agree with this conclusion.
No further questions.

A.4. TOXICOLOGICAL ASSESSMENT

A.4.1. METHODOLOGY USED FOR TOXICITY TESTS

Comments/Questions of the experts:

Comment 1

No questions

Comment 2

A series of tests has been conducted, presenting no indication of toxicity of SYHT0H2 soybean.

Comment 3

No further comments and questions. The information provided is satisfactory.

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 experts:

Comment 1

Oral gavage is using 2,10 or 51 mg/g/day for AvHPP-03 protein, each time 5x which is OK; moreover, the lowest dose is 50 times higher than “dietary intake” (p78) or approximately 30 times in case of children; OK

Since there is loss of functional activity at high t° (processing t°) and complete digestion/proteolytic degradation in SGF/SIF, no further questions.

Comment 2

EFSA (2011) concluded that there is no reason for concerns regarding the potential toxicity of PAT protein. With regard to HPPD, humans have a HPPD (Moran, 2005), where it plays a central role in the metabolism of aromatic amino acids, so that they are exposed to HPPD enzymes and their metabolites directly. HPPD has also been found in meat-producing animals and Endo et al. (1992) reported a 88.6% homology of the nucleotide sequences between the porcine and human HPPD cDNAs.

Comment 3

Note that the plant and micro-organism derived PAT (Part II, page 58) and AvHPPD-03 (Part II, page 57) proteins are not chemically identical (differ in number of N-terminal amino-acids).
Question: could this chemical (primary structure) difference affect the conformation of the protein (and affect amongst others the allergenic potential)?

Comment 4

Comment on avHPPD-03 protein animal toxicity studies:
Robertson, 2012. 28-day oral toxicity study, rats, doses: 2, 5, 51 mg/kg bw/d
♂, *10 mg avHPPD-03 mg/kg bw/d group*: ↓ body weight, ↓ absolute brain weight, ↓ absolute liver weight, ↓ absolute spleen weight, shorter flick time, ↑ body temperature, ↑ red blood cells count, ↓ mean cell volume, ↓ reticulocytes, ↓ white blood cells count, ↑ glucose
However, no statistically significant changes were observed in ♀ and in the higher dose group.
There is no clear scientific explanation provided for the observations made in the male 10 mg/kg bw/d group.

Eapen, 2012. Single dose oral toxicity study, mice, doses: 500, 1500, 2000 mg/kg bw
No effects seen in this study (no organ weights measured in this study).

Comment 5

The amount of PAT protein in soybean seems to be comparable to other equivalent products. For the AvHPPD-03 protein no comparable data were available.

7a) Degradation of the PAT protein in simulated gastric fluid (**From CBI:** Appendix A.4-7)

The PAT protein was shown to completely digest in SGF within 0.5 minutes, in a pepsin solution at pH 1.2 at a temperature of 37°C

7a) Degradation of the AvHPPD-03 protein in simulated gastric fluid (Appendix A.4-6).

The AvHPPD-03 protein degraded rapidly upon exposure to SGF. No intact AvHPPD-03 or AvHPPD-03 derived fragments were detected following its incubation in SGF for 1 minute.

7b) Degradation of the PAT protein in simulated intestinal fluid (Hérouet et al. 2005).

Western blot analysis showed degradation of the intact PAT protein and all lower molecular weight fragments by 0.5 minute.

7b) Degradation of the AvHPPD-03 protein in simulated intestinal fluid (Appendix A.4-12).

The AvHPPD-03 protein degraded rapidly upon exposure to SIF. No intact AvHPPD-03 was detected by SDS-PAGE or Western blot analyses after digestion in SIF for 1 minute. Furthermore, no AvHPPD-03 derived fragments were detected after incubation for five minutes.

7c) PAT: Acute Oral Toxicity Study in Mice ().

The results demonstrated that PAT was not acutely toxic as there were no systemic effects or mortality observed at either dose.

7c) AvHPPD-03: 28 Day Repeat Dose Toxicity Study by Oral Gavage in Rats. (Appendix A.4-3).

No signs of toxicity were observed. The No Observed Effect Level (NOEL) for this study was greater than 51 mg AvHPPD-03/kg/day (the highest dose tested).

7d) PAT: Sequence homology with known toxins (**From CBI:** Appendix A.4-2)

The PAT protein shows a high degree of homology with other proteins of its respective family.
No records were found on potential hazard associated with this protein family. In addition, no biologically significant similarities were found with any toxic protein from the Bayer Toxin database.

7d) AvHPPD-03: Sequence homology with known toxins (Appendix A.4-8 and A.4-9)

The results of a comprehensive amino acid similarity search of the NCBI Entrez® Protein Database support the conclusion that the AvHPPD-03 amino acid sequence shows no significant similarity with any known or putative toxins.

A.4.3. ASSESSMENT OF NEW CONSTITUENTS OTHER THAN PROTEINS

Comments/Questions of the experts:

Comment 1

No questions

Comment 2

Question: does the PAT protein acetylate any endogenous soy constituents?

Comment 3

No new constituents are expected. However, no poultry and/or rodent study is performed that can confirm that SYHT0H2 soybean is safe for food/feed use.

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

Comments/Questions of the experts:

Comment 1

No questions

Comment 2

Note that alpha-tocopherol (reduced in SYHTOH2 soybean) is the most biological active vitamin E vitamer and that attempts have been made to increase in soybean the conversion of gamma-tocopherol (increased in SYHTOH2 soybean) into alpha-tocopherol (Tavva et al., 2007).

Comment 3

No further comments and questions. The information provided is satisfactory.

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

A detailed compositional analysis was carried out. However, no poultry and/or rodent study is performed that can confirm that SYHT0H2 soybean is safe for food/feed use.

Herbicide and herbicide metabolites: What are the residue levels in the grain (animal and human health)?
Data is lacking concerning the occurrence, levels and fate of residues of the herbicides (mesotrione, glufosinate ammonium) and their metabolites (MNBA, AMBA; NAG, MPP) in the plant tissues and the potential adverse health effects as indirect effects associated with the use on human and animal health. Although the effect of the herbicides 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. As the herbicides are used as integral parts of the biotechnology-based weed management strategy, the risk assessment must also consider the potential impact on human and animal health.

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

Comment 3

a) 42-day poultry feeding study
Not performed.
No further testing is needed at this moment.

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

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 experts:

Comment 1

No questions

Comment 2

See above (A.4.2).

Comment 3

I agree with the applicant's conclusion on the absence of an increased risk for allergenicity deriving from the newly expressed AvHPPD-03 and PAT proteins. Taking into consideration the AvHPPD-03 protein being derived from a recognized food allergen (oat), the *avhppd-03* gene was additionally screened for similarity to known gluten proteins responsible for IgE-independent gluten enteropathy. Also these additional searches did not indicate an increased risk.

I have no further questions or comments.

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

Comments/Questions of the experts:

Comment 1

No questions

Comment 2

EFSA (2011) concluded that there is no reason for concerns regarding the potential allergenicity of PAT protein.

Comment 3

The analyses performed show that there are no biological meaningful changes in soy endogenous allergen levels.

Comment 4

The main concern here derives from the fact that soybean-derived foods can elicit IgE food allergies. There is therefore the risk that as a result of the genetic modifications, the expression levels of (certain) soybean allergens are increased in the GM plant. In his dossier, the applicant acknowledges this risk and has in my opinion properly addressed this issue. Using 5 sera from human soybean allergic persons, the following parameters were addressed:
- Qualitative IgE binding patterns to soybean proteins by 1-D western blotting
- Qualitative IgE binding patterns to soybean proteins by 2-D western blotting
- Quantitative analysis of the concentration of 12 known soybean allergens by mass spectrometry analysis.

On this basis, the applicant states that all three assays do not indicate increased allergen levels in the GM plant as a result of the genetic modification nor a change in allergen expression pattern.

In my opinion the experimental data do not support this conclusion. Whereas the mass spectrometry analysis provides a clear-cut (negative) result, no firm conclusions can be drawn from both western blotting experiments with sera from allergic individuals. The reason for this is the suboptimal to near absent immunoreactivity of 2 out of the 5 sera assayed. Hence, conclusions are drawn on the results of merely 3 sera, which is insufficient to allow for firm conclusions. In addition, it is unclear to what extend the individual allergens quantified by mass spectrometry also are detected on the 2-D blots, or in other words to what extend are these individual allergens effectively recognized by IgE from the allergic individuals. Increasing the number of (immunoreactive) sera assayed and positioning on the 2-D blots the individual allergens quantified seems imperative for drawing firm conclusions on increased allergenicity or not of the whole GM plant.

A.5.3. ADJUVANTICITY

Comments/Questions of the experts:

Comment 1

No questions

Comment 2

No comments or questions.

A.6. NUTRITIONAL ASSESSMENT

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

Comments/Questions of the experts:

Comment 1

No questions

Comment 2

It is my opinion that a 90-day rodent study should be done in case there is any uncertainty about the questions asked above in A.4.2. and in A.4.3.

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

Comments/Questions of the experts:

Comment 1

No questions

Comment 2

Jacobs et al. (2008) investigated stacked genetically modified maize expressing cry34Ab1, cry35Ab1 and PAT genes, and reported that the performances of laying hens were similar to that of hens fed diets formulated with near-isogenic grain.

Comment 3

Soy saponins have been demonstrated to be an anti-nutritional factor for fish (e.g. Wei et al., 2011; Yamamoto et al., 2012;). As these compounds were not measured, the effect on fish in aquaculture fed soy SYHTOH2 products is unclear. It is suggested that determination of saponins in soy would become mandatory in the future.

Additional remarks from the SBB:
The request for determination of saponins has been a recurrent demand for several soybean dossiers (EFSA/GMO/NL/2009/64, EFSA/GMO/NL/2009/73, EFSA/GMO/NL/2009/76, EFSA/GMO/NL/2009/78, EFSA/GMO/BE/2009/79). For consistency with previous dossiers the comment concerning saponins could be preceded with the following sentence:
"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, one expert has suggested to include saponins in the compositional analysis."

It could be noted that no comments have been made as regards the lack of analysis of phosphatides and dietary fibre in this dossier. However this has been put forward as a remark in other soybean dossiers (EFSA/GMO/NL/2009/73, EFSA/GMO/NL/2009/79). In this dossier, the compositional

analysis as performed by the notifier, has not included the analysis of phosphatides in lecithin, as recommended by the OECD consensus document on compositional considerations for new varieties of soybean (OECD, 2001).
Although not required by the OECD, the dossier lacks an 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.

B. EXPOSURE ASSESSMENT - ANTICIPATED INTAKE/EXTENT OF USE

Comments/Questions of the expert

Comment 1

No questions

Comment 2

Note that consumption data of soybean in Japan (Part II, page 77) are not relevant for Europe. Intake date for soy products in European member states have been published (Keinan-Boker et al., 2002).

Comment 3

Food: AvHPPD-03 protein
Using a conservative approach (all soybean consumed is SYHT0H2 soybean, unprocessed soybean seed, highest 'mean' protein concentration – fresh weight from the McDonald, 2012 study, highest soybean consumption globally), the daily dietary exposure (DDE) to avHPPD-03 is 0.038 mg/kg bw/d for adults and 0.0698 mg/kg bw/d for children.
As we discuss a life-time exposure, the NO(A)EL of the 28-d rat study of avHPPD-03 (NOAEL 51 mg/kg bw/d) should be used for estimating the MOE. MOE adults (using 28-d study) is 1342, MOE children (using 28-d study) is 739.
The MOE's are of no concern.

C. RISK CHARACTERISATION

Comments/Questions of the expert

Comment 1

Very good and synthetic overview, no further questions.

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, except that for compositional analysis no mentioning is made for tocotrienols or β-tocopherol; in other words, what about the total content of Vit E in the GM versus its conventional counterpart?
The formulation here on p 91, the second paragraph, for the difference in plant height, is better than on p 53 of the document.
I can agree with the conclusion on p 91.

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

Not relevant. 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

Not relevant.

E.3.6. EFFECTS ON BIOGEOCHEMICAL PROCESSES

Comments/Questions of the expert

Comment 1

Not relevant. No questions.

E.3.7. EFFECTS ON HUMAN AND ANIMAL HEALTH

Comments/Questions of the experts:

Comment 1

No questions

Comment 2

No health problems were reported for laying hens fed stacked genetically modified maize expressing cry34Ab1, cry35Ab1 and PAT genes (Jacobs et al., 2008).

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

E.4.2. CASE-SPECIFIC GM PLANT MONITORING

Comments/Questions of the expert

E.4.3. GENERAL SURVEILLANCE FOR UNANTICIPATED ADVERSE EFFECTS

Comments/Questions of the expert

E.4.4. REPORTING THE RESULTS OF MONITORING

Comments/Questions of the expert

References

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