Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on application EFSA-GMO-NL-2016-132 (genetically modified soybean DAS-81419-2 × DAS-44406-6) from Dow AgroSciences LLC under Regulation (EC) No. 1829/2003

22 January 2021
Ref. SC/1510/BAC/2021_0067

Context

Application EFSA-GMO-NL-2016-132 was submitted by Dow AgroSciences LLC for the marketing of genetically modified (GM) soybean DAS-81419-2 × DAS-44406-6 (Unique Identifier DAS-81419-2 × DAS-444Ø6-6), for food and feed uses, import and processing (excluding cultivation) within the European Union, within the framework of Regulation (EC) No. 1829/2003[1].

The two-event stack soybean DAS-81419-2 × DAS-44406-6 was obtained by conventional crossing (no new genetic modification involved) of the corresponding single events:
- DAS-81419-2, expressing the Cry1F and Cry1Ac proteins to confer resistance to certain lepidopteran species and the PAT protein that confers tolerance to glufosinate ammonium-based herbicides;
- DAS-44406-6, expressing the AAD-12, 2mEPSPS and PAT proteins, which confer tolerance to 2,4-D, glyphosate-based and glufosinate ammonium-containing herbicides;

The application was validated by EFSA on 9 August 2016. A formal three-month consultation period of the Member States was started, lasting from 3 March until 17 June 2017, in accordance with Articles 6.4 and 18.4 of Regulation (EC) No. 1829/2003 (consultation of national Competent Authorities within the meaning of Directive 2001/18/EC designated by each Member State in the case of genetically modified organisms being part of the products).

Within the framework of this consultation, the Belgian Biosafety Advisory Council (BAC), under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier, chosen from the common list of experts drawn up by the BAC and the Service Biosafety and Biotechnology (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 forwarded to EFSA on 15 June 2017.

The opinion of the EFSA Scientific Panel on GMOs was published on 20 November 2020 (EFSA Journal 2020;18(11):6302[2]), along with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period. On 27 November 2020 these two documents were forwarded to the Belgian experts. They were invited to give comments and to react if needed.

[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://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2020.6302

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In delivering the present advice the BAC considered in particular the following information:
- The comments formulated by the experts on application EFSA-GMO-NL-2016-132;
- The opinion of EFSA;
- The advices already adopted by the BAC on the single events. The conclusions of the BAC for the most recent applications for the single events were as follows:

Event	Application number	BAC advice	Conclusions
DAS-81419-2	EFSA-GMO-NL-2013-116	BAC/2017/0057 (31/01/2017)	Unlikely to pose any risk to human and animal health. No risk identified for the European environment.
DAS-44406-6	EFSA-GMO-NL-2012-106	BAC/2017/0438 (20/06/2017)	Unlikely to pose any risk to human and animal health. No risk identified for the European environment.

Soybean DAS-44406-6 is authorised in the EU for food and feed uses[3].

Scientific evaluation

1. Environmental risk assessment

The Biosafety Advisory Council is of the opinion that it is unlikely that the accidental release of soybean DAS-81419-2 × DAS-44406-6 (i.e. during transport and/or processing) into the European environment[4] 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

Taking into account the previous assessment of the single events and the new data on compositional analysis provided by the applicant for the two-stacked event, the Biosafety Advisory Council agrees with the GMO panel of EFSA that the compositional data of GM soybean DAS-81419-2 × DAS-44406-6, in comparison with its conventional counterpart, do not raise safety concerns.

3.2. Assessment of toxicity

The Biosafety Advisory Council has evaluated the safety of the newly expressed Cry1F, Cry1Ac, AAD-12, 2mEPSPS and PAT proteins in the context of previous applications, and no safety concerns were identified. Taking into account the updated information considered in the current application, the Council is of the opinion that its previous conclusions remain valid.
The Biosafety Advisory Council is also of the opinion that the combined expression of the newly expressed proteins in the stacked event does not raise toxicological concerns.

3.3. Assessment of allergenicity

The Biosafety Advisory Council has evaluated the safety of the newly expressed Cry1F, Cry1Ac, AAD-12, 2mEPSPS and PAT proteins 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 is also of the opinion that the combined expression of the newly expressed proteins in the stacked event does not raise concerns regarding the allergenicity.

[3] See EU register of GM food and feed: http://ec.europa.eu/food/dyna/gm_register/index_en.cfm
[4] As the application doesn't imply cultivation of the GM crop in the EU, a full environmental assessment is as in the case of an application for cultivation is not warranted.

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 DAS-81419-2 × DAS-44406-6-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.

Conclusion

Based on the whole set of data on soybean DAS-81419-2 × DAS-44406-6 provided by the applicant, the scientific assessment of the dossier done by the Belgian experts, the opinion of EFSA, the answers of the EFSA GMO panel to the questions raised by the Belgian experts, and the advices already adopted by the BAC on the two single events, the Biosafety Advisory Council:

1) Agrees with the GMO panel of EFSA that the potential environmental release of soybean DAS-81419-2 × DAS-44406-6 is unlikely to pose any threat to the European environment;
2) Agrees with the GMO panel of EFSA that there is no reason to expect interactions between the newly expressed proteins that could impact on the food or feed safety;
3) Agrees with the GMO panel of EFSA that in the context of its proposed uses, soybean DAS-81419-2 × DAS-44406-6 is unlikely to pose any risk to human and animal health;

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

Annex I: Compilation of comments of experts in charge of evaluating the application EFSA-GMO-NL-2016-132 and comments submitted to EFSA on mandate of the Biosafety Council (ref. BAC_2017_0408)

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Secrétariat

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15/06/2017

Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2016/132
and
Comments submitted on the EFSAnet on mandate of the Biosafety Council

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 21 March 2017.
Coordinator: Marc De Loose
Experts: Eddy Decuypere (KUL), Jacques Dommes (ULg), Patrick du Jardin (Ulg), Leo Fiems (ILVO), Johan Grooten (UGent), André Huyghebaert (UGent), Peter Smet (Consultant), Frank Van Breusegem (UGent), Jan Van Doorsselaere (KATO)
SBB: Didier Breyer, Fanny Coppens, Katia Pauwels.

♦ INTRODUCTION

Dossier **EFSA/GMO/NL/2016/132** concerns an application submitted by the company **Dow AgroSciences LLC** for authorisation to place on the market genetically modified **soybean DAS-81419-2 × DAS-44406-6** 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 16 February 2016.

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

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

information is lacking, the expert was asked to indicate which information should be provided and what the scientifically reasoning is behind this demand.

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

List of comments/questions received from the experts

GENERAL COMMENTS

Comment 1
DAS-81419-2 × DAS-44406-6 soybean may be as safe for human and animal health and the environment as conventional soybean based on the results of the compositional analysis and the toxicological and allergenicity assessment.
However, the risk assessment should also take the potential for accumulation of residues and metabolites of the herbicides into account, against which DAS-81419-2 × DAS-44406-6 soybean is tolerant. Furthermore, a combined margin of exposure is lacking.

SBB Comment: the risk assessment of pesticides is not within the remits of the BAC.

Comment 2
No questions.

Comment 3
No comment, adequate information was provided.

Comment 4
No comments.

A. HAZARD IDENTIFICATION AND CHARACTERISATION

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

Comment 1
No comment, adequate information was provided.

Comment 2
No comments.

Comment 3
No comments.

A.2. MOLECULAR CHARACTERISATION

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

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

Comment 1

No new genetic modifications in the stacked product that was produced through traditional crossing between DAS-81419-2 expressing cry1A and cry1Fv3 and pat, and DAS-44406-6 expressing aad-12 , pat and 2mepsps.

Comment 2
No comment, adequate information was provided.

Comment 3
No comments.

Comment 4
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

Comment 1
The expression of the proteins in the single events are similar to the expression in the stacked event, with a combined effect on the expression of PAT, since it occurred in both single events, hence its concentration is double in the stacked event (table 8, p53).

Coordinator Comment: correct observation, but does not pose a risk.

Comment 2
No comment, adequate information was provided. Southern blot hybridisation and sequencing of the inserts in DAS-81419-2 x DAS-44406-6 show that these inserts are identical to those present in the single events.

Comment 3
1. In the bioinformatic search for similarities with allergens and toxins, one of the the putative peptides translated from the new ORFs of the transformation event DAS-81419-2 showed hits with two glutenin allergens (Guttikonda 2015d p.16 and Main dossier p. 47). The applicant concludes on the absence of safety concern based on the fact that : (i) the global similarity is low and the 8-AA match is outside the known allergen epitopes of the glutenin proteins ; (ii) there are no regulatory elements upstream of the ORF displaying the similarity. According to the Implementing regulation (EU) No 503/2013 in article 1.2.2.3, the applicant is expected to *"characterise the potential unintended expression of new ORFs identified under point 1.2.2.2(f) as raising a safety concern."* In practice, both bioinformatic and experimental data are acceptable in order to rule out the possibility of expression of the ORF of concern. In this dossier, no experimental data are presented and the applicant just indicates that *there are no regulatory elements upstream of RF_-1-81."* I consider that this proposal needs a more convincing bioinformatic argumentation, since no experimental data are produced.

2. In the study of the levels of expression of the newly expressed proteins (Theoaris 2013), the described methods do not mention if and how the protein extraction efficiencies were estimated and

used in the calculation of the tissue concentrations of the newly expressed proteins. Could the applicant clarify this? The applicant is cautious by using the wording “soluble extractable proteins” but which proportion of the total protein content of the tissue is actually extractable? However, for such a dossier assessing a stack, the main purpose is to compare the protein levels between the stack and the corresponding single events and it can reasonably be said that the extraction efficiencies for a given tissue are expected to be similar, whatever the genotype.

Coordinator comment: I agree with the reasoning and the conclusion for a dossier assessing a stack, the main purpose is to compare the protein levels between the stack and the corresponding single events and it can reasonably be said that the extraction efficiencies for a given tissue are expected to be similar, whatever the genotype.

3. The study of the protein levels (Theoharis 2013 and main dossier page 51) concludes: *Expression levels of the Cry1Ac, Cry1F, PAT, AAD-12 and 2mEPSPS proteins in the stacked event, DAS-81419-2 x DAS-444Ø6-6, were consistent with those in the parental events, DAS-81419-2 and DAS-444Ø6-6.* However, when looking to the detailed results, large differences can be observed in some tissues when comparing the stack with the corresponding singles. As an example, table 5 on page 22 gives a Mean across site of 25.36 (ng / mg DW) for the single event (unsprayed) and of 8.68 for the unsprayed stack. Could the applicant comment on this difference? It is worth noting that the imported material corresponding to the scope of this application (“grain”) does not show such high differences, raising no concern. It is also unclear whether the herbicide regimes could have had any impact on the protein levels (no such impact is expected). A further observation is that the ranges (minimum and maximum values for a given tissue and stage) tend to be quite high. In conclusion, it is uneasy to conclude on the protein levels and on whether or not the stack and the corresponding single events show the same expression levels. Similar expression levels are used as an indication of the absence of interaction between the events in the stack and it is accordingly difficult to conclude on the absence of such interactions in this case.

Coordinator comment: Nice to know. Lower expression of a newly inserted gene does in this case pose no risk.

Comment 4

- In the 1.2.2.2 e) section, I think that the information on the PCR detection methods for the insert is mispositioned. This part deals with sequences inserted and the junction sequences.
- P45: No further discussion on this somehow surprising homology with a Teramnus labialis sequence? Why no homology with other legumes? **(*)**
- P45 bottom: A similar level of details in the description of the BLAST results as provided for the DAS-81419-2 5' and 3' insert was expected.
- 1.2.2.2. f) “Bioinformatic searches using up to date databases – assembled as follows (Song, 2016) were used in order to identify potential ORFs within the insert and spanning the junction sites.” It’s somehow discrepant that the Guttikonda reports (in the non C.I. appendices) in which these bioinformatics analysis are presented in more detail are all dated in 2015.
- P47. Typo: lucine should be leucine.
- P47 top: Why are the results obtained with the 12 junction-ORFs of both inserts not described and discussed as was done for the ORFs within the insert? **(**)**
- For clarity: indicate why only 10 RFs instead of the expected 12 were subjected to BLASTp. **(**)**

Coordinator comments:
(*)Hypothesis could be formulated but I do not think they contribute to the risk assessment.
(**) I do not find what the problem is – see Guttikonda 2015 c: identified, 6 RFs (≥ 29 amino acids) were subject to searches against the allergen database, using Dow AgroSciences LLC Study ID: 150090 Page 15 the FASTA program. The remaining 6 RFs were less than 29 amino acids and thereby incapable of meeting the "> 35% identity over 80 aa" threshold. No over threshold identities (> 35% identity over ≥ 80 aa) were detected in the FASTA search outputs when the peptide sequences deduced from the four RFs were used as query sequences. When the amino acid sequences of the 12 RFs were compared with the FARRP allergen database, no matches of eight or greater contiguous amino acids were observed in any of the translated sequences (Table 1, Appendix 1). The allergenicity positive control returned hits as expected.
When the 12 RFs were subjected to BLASTp search against the NCBI nr protein database to look for homologies to known toxins, no alignments with E-values less than 1 were returned (Table 1, Appendix 2). The toxicity positive control returned hits as expected.

Comment 5
No comments. The plant is a combination of approved transformation events. The T-DNAs are stably inherited.

A.3. COMPARATIVE ASSESSMENT

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

Comment 1
No questions.

Comment 2
No comments.

Comment 3
Soybean DAS-81419-2 × DAS-44406-6 was compared with the non-GM control and six commercially available non-transgenic soybean varieties, representative for soybeans grown in the areas of production.

No questions.

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

Comment 1
Beside the non-GM control soybean, unsprayed DAS-81419-2 × DAS-44406-6 soybean and 6 reference varieties, the experimental design also included a treatment with the combined application of 2,4-D, glyphosate and glufosinate, against which DAS-81419-2 × DAS-44406-6 soybean is tolerant. This is in agreement with the guidelines of EFSA (2010). Consequently, it is evident to take this factor into account for the comparative analysis, including the residue concentrations of 2,4-D, glyphosate and glufosinate in DAS-81419-2 × DAS-44406-6 soybean.

Comment 2
OK, no questions.

Comment 3
No questions.

A.3.3. COMPOSITIONAL ANALYSIS

Comment 1
Although some compounds of DAS-81419-2 × DAS-44406-6 soybean were significantly different, either from conventional soybean or from the reference varieties, differences are not considered as relevant. It may be recognized that if a compositional variation is detected, this should not be inferred as representing a de facto hazard. A natural variation in soybean composition is well known (Medic et al., 2014).
No residue concentrations were given for 2,4-D, glyphosate and glufosinate in case of DAS-81419-2 × DAS-44406-6 soybean sprayed with these herbicides. So, what is the objective of the inclusion of a spraying treatment with 2,4-D, glyphosate and glufosinate in the experimental design if part of the results is omitted? **(*)** OECD (2012) mentioned residue concentrations of herbicides and the analysis of toxicants, meaning those toxicologically significant compounds known to be inherently present in the species, whose toxic potency and levels may impact human and animal health. Recombinant DAS-81419-2 × DAS-44406-6 soybean exhibiting herbicide tolerance may indirectly result in the potential for accumulation of residues, altered metabolites of such residues, toxic metabolites, contaminants, or other substances that may be relevant to human health. The risk assessment should take this potential for accumulation into account (FAO, 2009). Therefore, it is desirable to analyse residues and metabolites of glyphosate and glufosinate in DAS-81419-2 × DAS-44406-6 soybean.

Coordinator comment: (*) Testing substantial equivalence under different conditions

Comment 2
P89: Why acid equivalent (ae) as unit for 2,4D and glyphosate, while active ingredient (ai) is used for glufosinate?

Comment 3
The OECD document was followed for the selection of the parameters.
I will comment on grain and not on forage.

Proximates and fiber: total dietary fiber is included; carbohydrates are assessed as a group with no differentiation; from a nutrition point of view a differentiation in carbohydrates is desirable due to their specific role in human nutrition. **(*)**

Amino acids and fatty acids are discussed in detail; data on important compounds like essential amino acids and unsaturated fatty acids are present; as an example polyunsaturated fatty acids are fully characterized e.g. a differentiation in linoleic, linolenic and γ-linolenic acid.

Minerals: important compounds are studied; there is no information on trace elements important from a safety point of view. **(**)**

Vitamins are studied in detail; it has to be emphasized that tocopherols are differentiated in α-,β-, γ- and δ-tocopherol; in the discussion it is mentioned that only α-tocopherol has vitamin E activity; it suggests that other tocopherols are not so relevant; other tocopherols play a major role in the antioxidative capacity of the oil; no data are given for the equivalent tocotrienols also important as antioxidants. **(**)**

Bioactives: no comment

Data are analyzed according to the EFSA guidelines and classified in tables.
Most constituents are classified in the group “equivalence likely” and even in the group “equivalence more likely than not”.
Some compounds with an outcome “non-equivalence more likely than not” and “ non-equivalence” deserve particular attention. Relevant compounds are palmitic acid, phosphorous, threonine, zinc and calcium.

The applicant discusses the biological relevance of these observations. Several sources of dietary soybean intake are used in the analysis. A mean daily consumption of 44 g/d soybean as a conservative upper limit was adopted.

As an example within the fatty acids, the palmitic acid value is somewhat higher in DAS-81419-2 x DAS -44406-6 soybean, sprayed and unsprayed, in comparison to the isoline and the reference values. Values for stearic acid show an opposite trend. It is accepted that the content of saturated fatty acids in human nutrition is to high but that a shift from stearic to palmitic acid is undesirable. Palmitic acid is more atherogenic than stearic acid.

With this comment I want to underline two things: are the differences observed in a positive or a negative direction and are the differences systematic. An in depth analysis of the data, also from previous applications could give an answer to this questions.

I agree with the applicant that soybean DAS-81419-2 × DAS-44406-6 is compositionally equivalent to the non-transgenic soybeans.

Coordinator comment: (*) This will be discussed internally by the Council.
(**) Discussed earlier in context of other dossiers

A.3.4. AGRONOMIC AND PHENOTYPIC CHARACTERISTICS

Comment 1
-p95: I can agree with the absence of biological relevance to human nutrition for all components showing non-equivalence of the stacked product with the reference varieties and/or isoline (cfr table 25 and 26). But if no explanation is given for the non-equivalence (or no hypothesis), and if it has no biological relevance, then why such an elaborate statistical analysis and why then the categorization of all analytes according to table 21&22? (e;g; for heptadecanoic acid, Ca, zinc, threonine)

-p103 if NDF and ADF are determined according to the Van Soest method, why then not proceed with oxidation of lignin with KMnO4, leaving cellulose and ash, in order to fractionate carbohydrates into

more realistic groups according to their utilization by animals and their resident microbial populations (see my earlier remarks regarding to the EFSA document about methodology and terminology)

-p104 Calcium and Phosphorus: both are higher in the stacked event than in isoline and than in reference varieties. The ratio remains unchanged. But again, as earlier remarks, why? And why bother about this categorization as non-equivalent if it has no relevance as to dietary intake?

-p107: again, why trypsin inhibitor and especially lectin are higher in the stacked event? For lectin, it is about double of the reference lines and also significantly higher than the non-GM isoline: why?

-p108 the text about health benefits and anti-nutritional properties associated with isoflavone consumption is rather vague; what are the positive or negative effects of glycitein consumption?

-p109: the biological relevance in poultry and livestock is discussed here, but since processing is always a necessary step, why then not giving the composition of the toasted soybean meal for all analytes instead of whole beans?

-p115: changes in positive direction for amino acids are highlighted, but changes in negative direction more or less discarded (e;g. last sentence under amino acids p 115)

-p116 : NDF: see earlier remarks.

-p117: It is well known that for most animals the Ca/P ratio approaches 2:1. Then, it is pointed to the high Ca-demands for egg and milk production, followed by the sentence that insufficient phosphorus in the diet will induce mobilization of bone phosphorus reserves while excess Ca has to be excreted;
In most cases it is the reverse that will occur: high requirements of Ca (e.g. for shell formation where Ca is deposited as CaCO3) above what can be ingested during periods of intense calcification of the shell will induce mobilization of Ca-reserve from medullary bone, but here Ca deposits are under form of Ca-phosphate crystals (appatite), so there will be an excess of phosphorus that has to be excreted. After egg formation this medullary bone has to be replenished with the need of a good Ca/P balance in the diet.
This is inverse as what is described on p 117!!! **(*)**

-p118 “there are no toxicity concerns as animals can tolerate high levels of zinc without adverse effects. However, the upper tolerable level for poultry and cattle has been set at 500 mg/kg diet (500ppm).
It has to be mentioned that from 2000 to 10.000 ppm zinc is used to decrease appetite very strongly in artificial egg-laying stop programmes for laying hens. Moreover, zinc has also direct effects (at hose levels) on follicle cells in the ovary.
Moreover, possible biological/toxic (?) effects of zinc depend on the nature of the zinc salts (ZnCO3, ZnSO4, ZnCl)

-p119: Vit K: which one? Vit K3 I presume?But this is not clearly explicated: it has to be mentioned that minor increase in the Vit K content of the stacked product poses no toxicity concerns as menadione levels 1000 times the requirement are tolerated. Menadione is a Vit K2 analogue (2-methyl-1,4naphtioquinone) known as Vit K3

Vit K1 is alpha-phylloquinone and VitK2 is beta-phylloquinone, mainly from animal (or bacterial) origin in feed/food.
All are involved in blood clotting, but there relative contribution is unknown to me.
Therefor, which one is analysed here??

-p120:first sentence of second paragraph (Maenz et al.,1999), and first sentence of third paragraph (the same report by the same author) is identical, but in second paragraph over 90% less trypsin inhibitor was found in toasted meal, and in third paragraph only 85% less trypsin inhibitor in the same toasted meal.
Why such an inconsistency??

Coordinator comment: (*) I think this statement by the expert is correct, but I do not think that has impact on the risk evaluation.

Comment 2
Differences between the stack GM soybean and the comparator isoline were identified by the field trials for the following agronomic characteristics: days to maturity, yield and seed weight. Nor could equivalence be concluded by comparing the stack GM soybean with the six reference varieties. The applicant concludes (page 128 of main dossier): *"Regardless of the cause, soybean varieties with lower days to maturity, yield, and seed weight are not known to have compromised safety".* This assumption is quite vague but considering the scope of the application, which excludes cultivation, and the absence of consistent differences in composition of the imported seeds, which would be more relevant, I consider that there is no safety issue arising from the comparative phenotypic analysis.

Comment 3
Data contain information on disease incidence and insect damage that could affect the safety of soybeans.

No further comments.

A.3.5. EFFECTS OF PROCESSING

Comment 1
No questions.

Comment 2
Processing of soybeans is reviewed. No particular effects are expected.

I agree with this conclusion.

A.4. TOXICOLOGICAL ASSESSMENT

A.4.1. METHODOLOGY USED FOR TOXICITY TESTS

Comment 1
No questions.

A.4.2. ASSESSMENT OF NEWLY EXPRESSED PROTEINS including:

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

Comment 1

The chance that the new proteins of DAS-81419-2 × DAS-44406-6 soybean (2MEPSPS, AAD-12 PAT, Cry1Ac and Cry1Fv3) will pose serious risks for toxicity is negligible. It is assumed that there is no biological pathway in which the newly-inserted genes would directly or indirectly interact with safety (Kok et al., 2014; Zdziarski et al., 2014). There is no plausible or testable hypothesis for an interaction of new proteins in DAS-81419-2 × DAS-44406-6 soybean (Steiner et al., 2013).

Apart from the new proteins 2MEPSPS, AAD-12, PAT, Cry1Ac and Cry1Fv3, the toxicity of DAS-81419-2 × DAS-44406-6 soybean is inseparable from the spraying of 2,4-D, glyphosate and glufosinate in DAS-81419-2 × DAS-44406-6 soybean: see the experimental design involving a combined application of 2,4-D, glyphosate, glufosinate in DAS-81419-2 × DAS-44406-6 soybean.
FAO (2009) and OECD (2012) mentioned the analysis of toxicants, meaning those toxicologically significant compounds known to be inherently present in the species, whose toxic potency and levels may impact human and animal health.
The main text and the appendix did not mention an adverse effect in a 90-day feeding study conducted with Crl:CD(SD) rats. However, deformed new-born pigs were reported when glyphosate tolerant GM soy products were used (Sørensen et al., 2014), so that some caution may be warranted.

SBB Comment: The safety assessment related to the pesticide use is not within the remit of the BAC.

Comment 2

All expressed proteins (Cry1Ac, Cry1F, AAS-12,PATn2mEPSPS) have a long history of commercial use, no structural or functional similarities with proteins adversely affecting human or animal health, no structural similarity to known toxins, and no acute toxic effects in mammals.
The mode of actions are well described on p 143-158.

-p150: when no synergistic effects are expected between Cry1f , Cry1Ac, PAT, and 2mEPSPS proteins, why AAD-12 is not mentioned here , as it is on P 146-147??

Comment 3

Evaluated. No safety concern.

Comment 4

A sound analysis of the possible interactions between the newly expressed proteins, based on the description of their activities and modes of action, is presented by the applicant.
No other comments.

Comment 5

The digestibility of the Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins, using simulated gastric fluid (SGF), has been discussed in detail in earlier dossiers.

Absence of acute oral toxicity for Cry1F, Cry1Ac and PAT has been demonstrated in dossier 116.

Regarding the AAD-12 protein, two 28 day repeated dose studies were carried on microbially produced AAD-12 protein. Under the conditions of these study, the no-observed-effect level (NOEL) of AAD-12 in CD1(ICR) mice of either sex were 47 mg/kg bw/day and 103.8 mg/kg bw/day, respectively, based on no treatment-related effects.

No sequence similarities with known toxins were found, for either of the proteins, but all reports date from 2015. Please provide more recent studies.

SBB Comment: If studies are outdated, EFSA always requests updated ones.

A.4.3. ASSESSMENT OF NEW CONSTITUENTS OTHER THAN PROTEINS

Comment 1
Not applicable.

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

Comment 1
No questions.

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

Comment 1
No questions.

Comment 2
Under the conditions of this study, the soybean breeding stack DAS-81419-2 x DAS-44406-6 (10% or 20%) did not cause any treatment-related effects in male or female Crl:CD(SD) rats following at least 90 days of dietary administration as compared to rats fed diets with 20% isoline control or commercial reference controls.

A.5. ALLERGENICITY ASSESSMENT

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

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

Comment 1
No questions.

Comment 2
Evaluated. No safety concern.

Comment 3
No comments.

Comment 4
The applicants have performed a weight-of-evidence approach as requested by EFSA. This involved analyses performed in the framework of previous EFSA applications as well as an updated bioinformatics analysis using the FARRP Allergen Database (v15, January, 2015). These analyses support the conclusion that the Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins are unlikely to have any allergenic potential.

I have no further remarks.

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

Comment 1
No questions.

Comment 2
Soybean being one of eight foods that account for a majority IgE-mediated food allergies, the applicants engaged in a quantification of 10 potential soy allergens. While some differences in expression levels, upwards as well as downwards, could be observed, the magnitude of these differences does not point towards a biologically significant increase in allergenic potential derived from these 10 endogenous soy allergens.

I would like to emphasize here that I do not agree with the applicant's conclusion "… *and no new allergenic proteins were expressed as a consequence of the genetic modification*…" (p. 162, section 1.5.4.b). The above mentioned analysis of 10 known soy allergens, while obviously being an appropriate approach, also is a biased approach that is limited to the 10 proteins studied. As a consequence, this analysis cannot reveal new or additional allergens in the stacked event! I would urge the applicants to perform hereto 2D-gel electrophoresis analysis along with Western blotting with sera from soy allergic individuals.

I have no further remarks.

Coordinator comment: In addition to being sent to EFSA, this will also be discussed internally by the Council.

A.5.3. ADJUVANTICITY

Comment 1
No questions.

Comment 2
I have no remarks.

A.6. NUTRITIONAL ASSESSMENT

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

Comment 1
No questions.

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

Comment 1
Some compounds of DAS-81419-2 × DAS-44406-6 soybean were significantly different from conventional soybean. Therefore, a general surveillance should be used to evaluate if the observed difference poses a risk to food and feed safety or the environment.
The main text and Appendix 11 did not mention an adverse effect in a 6-week broiler study, using diets containing 40, 36 and 33% DAS-81419-2 × DAS-44406-6 soybean meal during starter, grower and finisher phase, respectively. This is in agreement with a previous study of Herman et al. (2011), where diets containing 40, 36 and 32% of toasted DAS-68416-4 soybean meal, including AAD-12 and PAT proteins, did not show signs of toxicity or anti-nutrients.

Comment 2
No questions.

B. EXPOSURE ASSESSMENT - ANTICIPATED INTAKE/EXTENT OF USE

Comment 1
The Technical Dossier (P.167) only mentioned large margins of exposure (MOE) for Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins. Why did the applicant not calculate a combined MOE for these proteins? Furthermore, I miss an extended combined MOE, based on the new proteins (Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS) and the 3 herbicides (2,4-D, glyphosate, glufosinate), as proposed by Wilkinson et al. (2000) and Meek et al. (2011), because of the accumulation of risks with regard to human health of the new proteins and the herbicides.

Coordinator comment: Could be a general question to be send to EFSA, but leaving out the herbicides.

Comment 2
No questions.

C. RISK CHARACTERISATION

Comment 1
No questions.

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

Comment 1
No comments.

E. ENVIRONMENTAL RISK ASSESSMENT

E.1. INTRODUCTION

Comment 1
A drawback of the use of DAS-81419-2 × DAS-44406-6 soybean may be that it may not be sustainable with regard to the weed management, although there may be an agronomic advantage for this GM soybean when 2,4-D, glyphosate and glufosinate are used for weed management. Herbicide mixing (2,4-D, glyphosate and glufosinate) exposes weeds to multiple mechanisms of action, which may delay resistance evolution. However, herbicide mixtures are not a permanent solution to the problem of herbicide resistance, as they do not prevent it on the long run (Evans et al., 2015).

Comment 2
No questions.

Comment 3
No comment, adequate information was provided.

Comment 4
No comments.

E.2. GENERAL APPROACH OF THE ERA

Comment 1
No questions.

Comment 2
No comment, adequate information was provided.

Comment 3
No comments.

E.3. SPECIFIC AREAS OF RISK

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

E.3.1. PERSISTENCE AND INVASIVENESS INCLUDING PLANT-TO-PLANT GENE FLOW

Comment 1

No questions.

Comment 2
No safety concern.

Comment 3
No comments.

E.3.2. PLANT TO MICRO-ORGANISMS GENE TRANSFER

Comment 1
No questions.

Comment 2
No safety concern.

Comment 3
No comments.

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

Comment 1
No questions.

Comment 2
No target organism.

Comment 3
No comments.

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

Comment 1
No questions.

Comment 2
No safety concern.

Comment 3
No comments.

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

Comment 1
DAS-81419-2 × DAS-44406-6 soybean is tolerant against 2,4-D, glyphosate and glufosinate, which may result in an increased application of these herbicides, which may not prevent herbicide resistance

on the long run (Evans et al., 2015). Furthermore, adverse impacts of herbicide-resistant crops on biodiversity can be expected when they are widely adopted (Schütte et al., 2017).
So, the problem is not the genetic modification in itself, but rather the management and the governance of this innovation with regard to the use of some herbicides against which DAS-81419-2 × DAS-44406-6 soybean is tolerant, and that may pose some risk for human health.

SBB Comment: the risk assessment of pesticides is not within the remits of the BAC.

Comment 2
No questions.

Comment 3
Not relevant as this application does not include cultivation of the GM plant in Europe.

Comment 4
Not applicable.

E.3.6. EFFECTS ON BIOGEOCHEMICAL PROCESSES

Comment 1
negligible

Comment 2
No safety concern.

Comment 3
No comments.

E.3.7. EFFECTS ON HUMAN AND ANIMAL HEALTH

Comment 1
In the case of genetically modified herbicide-tolerant crops, such as DAS-81419-2 × DAS-44406-6 soybean, the effect of the genetic modification cannot be isolated from the effect of 2,4-D, glyphosate and glufosinate. Deformed newborn pigs were reported when glyphosate tolerant GM soy was used (Krüger et al., 2014; Sørensen et al., 2014). High residue concentrations may inhibit rumen digestion, as shown by Reuter et al. (2007), using an in vitro experiment. Furthermore, glyphosate has been detected in the urine of dairy cows (Krüger et al., 2013).
DAS-81419-2 × DAS-44406-6 soybean may indirectly result in the potential for accumulation of residues and metabolites that may be relevant to human health. According to FAO (2009) and OECD (2012) the risk assessment should take this potential for accumulation into account.

Comment 2
No questions.

Comment 3
No safety concern.

E.3.8. OVERALL RISK EVALUATION AND CONCLUSIONS

Comment 1
Weber et al. (2012) reported that there is no readily identifiable biological reason why genomic changes occurring in the breeding of a GM stack would be different in nature, scale, or frequency from those taking place in conventional crops or in GM crops with a single event. Pilacinski et al. (2011) concluded that combined GM event plants, produced through conventional breeding, can be considered to be safe, given the expected safety of the parent plants. Therefore, we expect no detrimental effect of the new proteins in DAS-81419-2 × DAS-44406-6 soybean on the nutritive value and animal and human health.
Because health concerns with regard to 2,4-D have been reported (Loomis et al., 2015) and high doses of glyphosate may be toxic (Williams et al., 2016), the application doses during cultivation should be carefully respected. Even if DAS-81419-2 × DAS-44406-6 soybean is not intended for cultivation in the EU, the application high doses of glyphosate elsewhere may result in the presence of residues. Import of DAS-81419-2 × DAS-44406-6 soybean containing residues of 2,4-D and glyphosate may be detrimental for the health of the EU consumer.

SBB Comment: the risk assessment of pesticides is not within the remits of the BAC.

Comment 2
No comments.

Comment 3
I agree with the conclusion.
However I regret that the text contains several language problems, including incomplete sentences and mistakes. This is surprising in a dossier of this importance.
For instance, on page 193, it is written:
“Zea mays, donor of the 2mEPSPS protein, is the causal agent of crown gall disease (the formation of tumours) in over 140 species of dicotyledonous…” I agree that Zea mays is indeed the donor of the gene used to produce the 2mEPSPS protein in the stacked event. But the rest of the sentence concerns Agrobacterium tumafasciens and it has nothing to do with the 2mEPSPS protein.
Another example on page 197 where I read “Animals have been naturally exposed to the Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS proteins, originally obtained from Bacillus thuringiensis, Sphingobium herbicidovorans and Streptomyces viridochromogenes common soil organisms, which have no known toxic or pathogenic potential…” Here the source of the 2mEPSPS protein is missing.

Comment 4
No comments.

E.4. POST MARKET ENVIRONMENTAL MONITORING PLAN

E.4.1. INTERPLAY BETWEEN ENVIRONMENTAL RISK ASSESSMENT AND MONITORING

Comment 1
No questions.

Comment 2

No comments.

E.4.2. CASE-SPECIFIC GM PLANT MONITORING

Comment 1
No questions.

Comment 2
No comments.

E.4.3. GENERAL SURVEILLANCE FOR UNANTICIPATED ADVERSE EFFECTS

Comment 1
Because of some compounds of DAS-81419-2 × DAS-44406-6 soybean were significantly different from conventional soybean (See A.3.3), the general surveillance should check whether unexpected, adverse effects could occur. Furthermore, some vigilance is necessary until the effect of glyphosate on health in clarified.

SBB Comment: the risk assessment of pesticides is not within the remits of the BAC.

Comment 2
No questions.

Comment 3
No comments.

E.4.4. REPORTING THE RESULTS OF MONITORING

Comment 1
No questions.

Comment 2
No comments.

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