Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2017_0057

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/NL/2013/116 from Dow AgroSciences LLC under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/NL/2013/116 was submitted by Dow AgroSciences LLC on 8 May 2013 for the marketing of genetically modified (GM) soybean DAS-81419-2 for food and feed uses, import and processing within the framework of Regulation (EC) No. 1829/2003[1].

Soybean DAS-81419-2 was developed by *Agrobacterium tumefaciens* –mediated transformation. It expresses the Cry1F and Cry1Ac proteins to confer resistance to certain lepidopteran species and the PAT protein that confers tolerance to glufosinate ammonium-based herbicides that was used as a selectable marker gene.

The application was officially acknowledged by EFSA on 7 February 2014. On 27 February 2014 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). Twelve 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 23 May 2014.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 26 October 2016 (EFSA Journal 2016; 14(12):4642[2]), and published together with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period.

On 11 January 2017 the opinion of EFSA was forwarded to the Belgian experts who were still on the common list of experts of the BAC and the SBB. 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

[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://www.efsa.europa.eu/en/efsajournal/pub/4642

EFSA including the answers of the EFSA GMO Panel form the basis of the advice of the Biosafety Advisory Council given below.

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

2. Molecular characterisation

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

3. Assessment of food/feed safety and nutritional value

3.1. Assessment of compositional analysis

The Biosafety Advisory Council agrees with the GMO panel of EFSA that the compositional data of GM soybean DAS-81419-2, in comparison with its conventional counterpart, do not raise safety concerns.

3.2. Assessment of toxicity

Soybean DAS-81419-2 was developed to express the Cry1F, Cry1Ac and PAT proteins. Based on previous positive assessments and taking into account the information provided by the applicant, the Biosafety Advisory Council is of the opinion that in the context of its intended uses GM soybean DAS-81419-2 does not raise safety concerns regarding toxicity.

3.3. Assessment of allergenicity

The Biosafety Advisory Council agrees with the EFSA GMO Panel that there are no indications that GM soybean DAS-81419-2 would have an allergenic profile that would be significantly altered in comparison with its conventional counterpart.

3.4. Nutritional value

The Biosafety Advisory Council is of the opinion that there are no indications that the GM soybean DAS-81419-2 would be less nutritious than 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 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.

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 DAS-81419-2 is unlikely to pose any risk to human and animal health.

Given the scope of the application of this GM soybean (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 soybean DAS-81419-2 is unlikely to pose any threat to the European environment.

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

Annex I: Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2013/116 and comments submitted on the EFSAnet on mandate of the Biosafety Council (ref. BAC_2014_0330)

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Bioveiligheidsraad
Conseil de Biosécurité

23-05-2014

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC_2014_0330
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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2013/116
and
Comments submitted on the EFSAnet on mandate of the Biosafety Council

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 17 March 2014
Coordinator: René Custers.
Experts: Eddy Decuypere (KUL), Jacques Dommes (ULg), Leo Fiems (ILVO), Rony Geers (KUL), Godelieve Gheysen (UGent), Johan Grooten (UGent), André Huyghebaert (UGent), Peter Smet (Consultant), Frank Van Breusegem (VIB), Jan Van Doorselaere (KATHO), Michel Van Koninckxloo (Hainaut Développement Territorial - CARAH), 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/NL/2013/116** concerns an application submitted by the company **Dow AgroSciences** for authorisation to place on the market genetically modified **Soybean DAS-81419-2** 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 7 February 2014.

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 received from the experts

GENERAL COMMENTS

Comments/Questions of the experts:

Comment 1
No comments

Comment 2
No comment, appropriate information is provided.

Comment 3
DAS-81419-2 soybeans contain Cry1F, Cry1Ac and PAT proteins in various tissues, and analyses confirm the expression of these proteins. However, it is amazing that other proteins were expressed in DAS-81419-2 than in its parent strains (AAD-12 and CP4 EPSPS ?). Obviously, there is some confusing information between the Summary and the main text of the Technical Dossier.
Most composition analyses of DAS-81419-2 soybeans are within the ranges reported in the literature for soybeans, but some composites fall outside the equivalence limits for the references. This may not be detrimental for human or animal health if the compositional analysis is taken into account.
Even if DAS-81419-2 soybean is not the result of conventional breeding of DAS-68416-4 soybean and MON-89788-1 soybean, there may be no significant risk for human or animal health, because of the history of safe use of Cry1Ac, Cry1F and PAT proteins.

Comment 4
Although unrelated to the molecular analysis and of no influence on the quality of the information provided part, I think caution should be taken in adequately referring to publications and not to use Wikipedia based information in regulatory dossiers (page 18).

Comment 5
The information provided in the application is sufficient.

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 questions

Comment 2
No comment, appropriate information is provided.

Comment 3
DAS-81419-2 has been obtained by conventional breeding of DAS-68416-4 and MON-89788-1 (Summary 3.1b). DAS-68416-4 expresses AAD-12 and PAT proteins (Herman et al., 2011b), whereas

CP4 EPSPS protein is expressed by MON-89788-1 (EFSA, 2008). If DAS-81419-2 has been obtained by conventional breeding, as mentioned in the Summary, it is amazing that other proteins were expressed in DAS-81419-2 than in the parent strains. Moreover, this information has not been mentioned in Part II of the Technical Dossier (Section 2.1.2; pp.23-24). Please clarify.

Comment 3 rewritten by the coordinator

There must be a mistake in the summary of the dossier in 3.1.b (nature and source of the vector used). It states that DAS-81419-2 has been obtained by conventional breeding of DAS-68416-4 (containing 2,4 D herbicide tolerance) and MON-89788-1 (containing CP4 EPSPS). While in the remainder of the dossier it is stated that DAS-81419-2 has been obtained by A.tumefaciens mediated genetic modification introducing the Cry1F, Cry1Ac and PAT proteins.

Comment 4

It is mentioned that the average human consumption of soybean is low. However, consumption may be higher in vegetarians and vegans and certainly is higher in specific populations such as Japanese people.

Comment 5

No comment.

Comment 6

The information provided in the application is sufficient.

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

No comments or questions

Comment 2

No comment, appropriate information is provided.

Comment 3

It would be useful to indicate if border A corresponds the left T-DNA border or the right border.

Comment 4

No comment.

Comment 5

P 25: Integration of the T-DNA is said to be “semi-random”. What is the reference for this statement? Has this been shown in literature? Modify to: random

Comment 6
The information provided in the application is sufficient.

A.2.2. INFORMATION RELATING TO THE GM PLANT Including:
- Description of the trait(s) and characteristics which have been introduced or modified
- Information on the sequences actually inserted or deleted
- Information on the expression of the insert
- Genetic stability of the inserted/modified sequence and phenotypic stability of the GM plant

Comment 1
No questions or comments; it is a clear description of the molecular characterisation and confirmation of the insertion of a single intact copy of the Cry1Fv3, cry1Ac and pat expression from the T-DNA insert of pDAB9582

Comment 2
No comment, appropriate information is provided.

Comment 3
Table 1 contains a typing error: the length of the cry1Ac is 3470 bp and not 34717 bp.
The southern blot hybridisations are generally very well done and described. However I have two small remarks.
1. Fig. 18B has been exposed longer to reveal the presence of a weaker band ca. 4 kb. Have other blots also been exposed for longer times to check for weaker bands?
2. Fig. 23 has a weak band at >9.5 kb that is referred to as most likely being due to partial digest (probably 5.4 + 4.5kb). The fact that it is also seen in the plasmid control would confirm this. It seems to be hybridizing to the common part in the cry1A3' and cry1F3' because it is also seen in some lanes in Fig. 20A such as lanes 2, 9, 10, 11. However it is not seen in the other blots with the same digest. If it is the partial band corresponding to most of the T-DNA, it should also be visible on the other blots, certainly if they come from the same gel such as appears to be the case for Figure 21A. Most likely a longer exposure of this blot will reveal the same bands (it is already faintly visible in Figure 21A lane 2 which is the plasmid control). I only make this comment to be sure that there is no negligence of extra T-DNA pieces inserted in the soybean genome that -although no problem for safety- could cause discussion later on, as has previously happened for soybean MON40-3-2.

Comment 4
No comment.

Comment 5
P 71: it is said that '… the insert is integrated in the genome as confirmed by sequence analysis…'
The results of the different bio-informatic analyses are not given. Reference is made to Guttikonda and Richey, 2012; Richey 2013;
It would be appropriate to mention some of the Blast results using the 5' and 3' flanking sequences. Add: '… the insert was located on chromosome 2 and there is no evidence that disruption or deletion of an endogenous gene or regulatory element occurred at the insertion locus for DAS-81419-2 soybean.'

A.3. COMPARATIVE ASSESSMENT

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

Comments/Questions of the expert

Comment 1
No comments

Comment 2
Soybean DAS-81419-2 is further referred to as soybean DAS.
As a comparator a non-transgenic near-isogenic control was used for the safety evaluation of soybean DAS.
In addition six commercial non transgenic soybean varieties, cultivated at ten sites, were included in the evaluation.
This approach is very often used in safety evaluations of transgenic plants.
No particular comments.

Comment 3
The information provided in the application is sufficient.

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

Comments/Questions of the expert

Comment 1
OK, no comments or questions

Comment 2
No comments on the experimental design, the location of field testing sites and the statistical analysis.

Comment 3
The information provided in the application is sufficient.

A.3.3. COMPOSITIONAL ANALYSIS

Comments/Questions of the experts:

Comment 1

- On p112 it is stated that mean values of the essential amino acids – histidine, threonine and valine – differed significantly from the Isoline (table 22). However, looking at table 22, it seems that DAS-81419-2 differed rather from the reference varieties for all 3 amino acids , and not from the Isoline; instead both Isoline and DAS – 81419 are somewhat different from REF.
- Nevertheless threonine is categorized under cat4 type 7 in table 18, while valine is under cat2 (similar to isoline). There seem to be some contradictions between table 18, table 22 and text on p112. Can this be clarified?

P126: …"it was determined that both transgenic soybean and non-transgenic soybean did not provide sufficient Thr to meet daily requirements. The deficiency is corrected by the addition of synthetic Thr to the ration".
However, Thr is also provided by other raw materials (as in fig 31) and only then the possible deficiencies are corrected by synthetic Thr. Therefore, a more correct formulation should be as formulated for valine (p129) and the practice of synthetic Val supplementation (similar as for Thr, Met,Lys……)

Comment 2
Compositional analyses of the grain revealed significant differences between soybean DAS-81419-2 and the control soybean for moisture, fat, ash, arginine, phenylalanine, palmitic acid, vitamin B5, γ-, δ- and total tocopherol and total glycitein, but values were within the range of literature data for soybeans. However, eicosenoic acid, threonine, stearic and linoleic acid fell outside the equivalence limits for the references. Berman et al (2011) reported that regional differences and growing season may be more important for compositional variability of fatty acids and vitamins in soybean than transgene insertion.

Comment 3
The OECD 2001 document was followed for the selection of the constituents tested. From the 88 analytes, 17 were excluded from the statistical analysis as most results are lower than the LOQ (limit of quantification). Results of 71 analytes are discussed in detail. The analysis covers important groups of analytes: proximates, fiber constituents, minerals, amino acids, fatty acids, vitamins and bioactives (or antinutrients).

Analytes are classified according to the conclusion as:
- equivalence likely,
- equivalence more likely than not,
- non-equivalence more likely than not,
- non-equivalence.

This is an original approach used for the different comparisons. To my knowledge it is the first time this approach is presented in this type of dossiers.

The biological significance of those analytes, classified as type 3 or higher or "non-categorised", is discussed.
I limit my comments to human nutrition as animal nutrition is not my field of expertise.

The calculation is based upon data of soybean intake and dietary reference values (DRV's).
Soybean mean intake was estimated for several EU countries between 0,20 and 2,52 % of the total diet. A conservative estimate of 2,5 % was proposed. A mean daily consumption of 44 g/d for soybean consumers is considered as a conservative upper limit. An analysis of specific soybean foods showed that soybean consumption is dominated by soymilk, drink, oil and sauce.

The biological relevance of nutrients was assessed. The approach was applied for particular amino acids. It was demonstrated that the observed differences for arginine, glutamic acid, glycine, histidine, threonine and valine were not biologically relevant to human nutrition.
A similar approach was followed for particular fatty acids such as palmitic, stearic, linoleic and eicosenoic acid.
Vitamin C was also discussed. I agree with the observation that soybeans are not a significant source of vitamin C in human nutrition.

Lectins are also discussed. DAS soybeans show somewhat higher levels of lectins, known to be antinutrients. Heat processing however is generally applied before human consumption. It has been shown that heat processing reduced the lectin levels to acceptable and safe levels.

With respect to analytes not categorized- different from Isoline two cases are considered: aspartic acid and lysine. The magnitude of change in aspartic acid is negligible.
Lysine is an indispensible amino acid. The lysine level in DAS soybeans is somewhat higher than in Isoline. However the observed increase poses a negligible risk.

The applicant concludes that the observed differences between DAS and Isoline soybeans have no biologically relevant impact on human nutrition.

I agree with this conclusion.

Comment 4
Concerning the antinutrients, the recombinant soybean seems to be comparable to the comparator and the reference lines.
The pat protein which confers tolerance to glufosinate-ammonium was – for the moment - used as a selectable marker and not as a trait to confer tolerance to this herbicide on the field, commercially. Consequently, the compositional analyses did not include herbicide spraying treatments with glufosinate-ammonium herbicide. As far as I understand, the trait is present and can be used commercially at any time, so spraying treatments should be included.

Comment 4 rewritten by the coordinator
The pat gene confers resistance to glufosinate-based herbicides. In this particular case the pat gene was used as a selectable marker and the GM crop will not be marketed as a herbicide tolerant crop. In case the event is going to be used in practice as a glufosinate tolerant crop, then the event sprayed with the herbicide should be included in the analysis.

Comment 5
The information provided in the application is sufficient.

Additional comment from the SBB
It is generally recognized that soybean is an important source of vitamins in the human diet, in particular vitamin E and vitamin K. The applicant only provided data for 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.

Comment from the SBB rewritten by the coordinator
It is generally recognized that soybean is an important source of vitamins in the human diet, in particular vitamins E and K. The applicant only provided data for vitamin E. Vitamin K is now also mentioned in the revised OECD Consensus document on Compositional Considerations for New Varieties of Soybean which is still under discussion at the OECD level. It is recommended to include vitamin K in the analysis.

A.3.4. AGRONOMIC AND PHENOTYPIC CHARACTERISTICS

Comments/Questions of the experts/

Comment 1

P137 and table 42: is the insect damage as mentioned, all damage, or other than resistance to target insect pests damage??
If it is total insect damage, then why no difference between DAS-81419-2 and Isoline for insect damage by the non-parametric analysis in table 45(p141)?

Comment 2

The information provided in the application is sufficient.

A.3.5. EFFECTS OF PROCESSING

Comments/Questions of the experts:

Comment 1

No comments

Comment 2

Soybeans are processed into a range of foods. An overview of processed applied and products obtained is given.
The applicant concludes that taking into account that no significant differences in nutritional composition were found, that the Cry1Ac, Cry1F and PAT proteins are heat labile and that processing temperatures are well above the denaturing conditions of these proteins, processing conditions will be equivalent for DAS soybeans as for other soybeans.
I agree with this conclusion

Comment 3

The information provided in the application is sufficient.

A.4. TOXICOLOGICAL ASSESSMENT

A.4.1. METHODOLOGY USED FOR TOXICITY TESTS

Comments/Questions of the experts:

Comment 1

No comments or questions; the mode of action of Cry proteins and PAT proteins is well described and clear; I would add on p153

Last sentence for the sake of clarity: "…… and thereby prevents autotoxicity in the producing organism by inhibiting the inhibitor of glutamine synthetase and consequently inhibiting the accumulation of nh3 in plant cells."

Comment 2

No comments

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
No comments

Comment 2
Repeated dose toxicity studies using laboratory animals:
Reference is made to four studies, i.e. Brooks and Andrus (1999), Brooks and Yano (2001a,b) and Jeong and Golden (2013). The same protocol was applied, i.e. 10 mice (5 female, 5 male), which within each study received the same dose of the tested product. Growth, mortality and pathological lesions were observed. No abnormalities were reported. However, instead of using information from the literature as reference material, it would have been improved the conclusion in case a control group was used in each study. It would then also be possible to calculate whether or not the number of animals used has been sufficient to support the conclusions made.

Comment 3
7a) Degradation of the PAT protein in simulated gastric fluid (Embrey and Gardner, 2013b).
The PAT protein was readily digested by pepsin (not detectable at 1 minute) in simulated gastric fluid (pH 1.2, 37 °C) as demonstrated by both SDS-PAGE and western blot analyses.

7b) Degradation of the Cry1F protein in simulated gastric fluid (Korjagin, 2001b).
The Cry1F protein degraded rapidly (less than 1 minute) upon exposure to SGF as demonstrated by both SDS-PAGE and western blot analyses.

7c) Degradation of the Cry1Ac protein in simulated intestinal fluid (Korjagin, 2001a).
The Cry1Ac protein degraded rapidly (less than 1 minute) upon exposure to SGF as demonstrated by both SDS-PAGE and western blot analyses.

7d) Degradation of the proteins in simulated intestinal fluid ().
Is this information missing?

7e) PAT: Acute Oral Toxicity Study in Mice (Jeong and Golden, 2013).
Under the conditions of this study, the acute oral LD50 of PAT protein was determined to be greater than 2000 mg/kg body weight (5882 mg/kg body weight of the test substance containing 34% PAT protein) in both male and female Crl:CD1(ICR) mice.

7f) Cry1F: Acute Oral Toxicity Study in Mice (Brooks and Andrus, 1999).
Under the conditions of this study, the acute oral LD50 of Cry1F microbial protein (FL) in male and female CD-l mice was greater than 600 mg/kg

7g) Cry1Ac: Acute Oral Toxicity Study in Mice ((Brooks and Yano, 2001a).

Under the conditions of this study, the acute oral LDso of CrylAc microbial protein in male and female CD-l mice was greater than 700 mg/kg

7h) 28 Day Repeat Dose Toxicity Study by Oral Gavage in Rats. ().
Not performed. No further testing is needed.

7i) Cry1F: Sequence homology with known toxins (Gao, 2013b)
The results indicate that the Cry1F protein expressed in transgenic crops contains no significant sequence similarity with any known toxic proteins that are harmful to humans or animals.

7j) PAT: Sequence homology with known toxins (Richey, 2013b)
The BLASTp search results indicated that the PAT protein contains no significant sequence similarity with any known toxic proteins that are harmful to humans or animals.

7k) Cry1Ac: Sequence homology with known toxins (Rapier, 2013c)
The BLASTp search results indicated that the Cry1Ac protein expressed in transgenic crops contains no significant sequence similarity with known toxic proteins that are harmful to humans or animals.

Conclusion: taking into account the above mentioned tests, there seem to be no concerns with respect to toxicological effects of the events.

Comment 4
No comment (a,b).

Comment 5
No comments on the biochemical characterisation and toxicity assessment.
An up-to-date bioinformatic search for homology was provided. However, PartII-main text p195: The references are correct but the text for the Cry1F and Cry1Ac proteins is handling the allergen data instead of the toxins data. Copy/paste error?

A.4.3. ASSESSMENT OF NEW CONSTITUENTS OTHER THAN PROTEINS

Comment 1
No comments

Comment 2
No new constituents are expected. However, no poultry and/or rodent study is performed that can confirm that Soybean DAS-81419-2 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
Not applicable

Comment 2
No further comments. On the basis of the results of the compositional analysis the forage and seed of Soybean DAS-81419-2 was found compositionally equivalent to conventional soybean, except for the presence of the Cry1Ac and Cry1F proteins, and PAT enzyme.

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

Comments/Questions of the expert

Comment 1
Not applicable

Comment 2
a) 42-day poultry feeding study ()
Not performed.
b) 90-Day rat feeding study (author).
Not performed.
No further testing is needed at this moment.

Comment 2
No animal studies (90-day rat study, broiler chicken study) are performed. However, no additional animal studied are considered necessary as in earlier whole feed studies with GM cotton (cotton 281-24-236 x 3006-210-23, EFSA/GMO/NL/2005/16) expressing these proteins no indications of adverse effects or potential synergistic effects were detected. In addition, on the basis of the results of the compositional analysis the forage and seed of Soybean DAS-81419-2 was found compositionally equivalent to conventional soybean, except for the presence of the Cry1Ac and Cry1F proteins, and PAT enzyme.

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
Conform the requirements from EFSA, the applicants performed an updated analysis of aa sequence homology with known allergens, resulting in no hits. Also, the Cry1F and Cry1Ac proteins were found to be readily digested by pepsin in less than a minute under simulated gastric conditions. Previous dossiers also found the PAT protein to be readily degradable under simulated gastric conditions. Finally, the newly expressed proteins are heat labile, resulting in loss of enzymatic activity upon

processing, and lack glycosylation. On this basis the newly expressed Cry1Ac, Cry1F and PAT proteins are considered to have a low risk of allergenic potential.

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

Comments/Questions of the experts:

Comment 1
No questions

Comment 2
In line with soybean being a prominent allergenic plant, the applicants verified whether the DAS-81419-2 soybean has an altered endogenous allergenicity potential compared with its non-transgenic counterpart. Protein extracts derived from transgenic and non-transgenic seed were evaluated for IgE reactivity using sera from individuals allergic to soybean (n=10). One-dimensional immunoblotting with pooled sera and ELISA-inhibition assays did not reveal increases in immunoreactivity. However, these assays are relatively insensitive. The most sensitive and hence relevant readout is immunoblotting of individual sera on 2-dimensional PAGE blots. The applicants conclude from these analyses that the genetic modifications used to generate DAS-81419-2 soybean did not alter endogenous allergenicity compared with the non-transgenic control. However, I do not feel confident with this conclusion. In my opinion the data do not support this conclusion and to the contrary may point towards an increased allergenicity. Thus, only 6 sera from soybean allergic patients have been used in the 2D-immunoblotting. This constitutes a very small cohort. Furthermore, from these 6 sera, three showed altered/increased spots on the 2D immunoblots (serum 20770-MH, 23736-AM, 23450-SM). On this basis, I find it difficult to conclude that the whole GM plant does not pose a risk for increased allergenic potential. Further testing using a larger sample cohort seems imperative before any conclusions on allergenicity potential of the whole GM plant can be made.

A.5.3. ADJUVANTICITY

Comments/Questions of the experts:

Comment 1
No questions

Comment 2
There are no indications for the Cry1Ac, Cry1F and PAT proteins to exert adjuvant activity.

A.6. NUTRITIONAL ASSESSMENT

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

Comment 1
No comments or questions

Comment 2
The information provided in the application is sufficient.

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

Comment 1
No comments

Comment 2
Some composites (eicosenoic acid, threonine, stearic and linoleic acid) fall outside the equivalence limits for the references.
A 6-week broiler study, conducted with diets containing 40, 36 and 32% toasted DAS-68416-4 soybean meal during the starter, grower and finisher period, respectively, to evaluate nutritional wholesomeness and safety compared with the non-genetically modified counterpart, showed no signs of toxicity or anti-nutrients (Herman et al., 2011a). DAS-81419-2 may have been obtained by conventional breeding of DAS-68416-4 and MON-89788-1, according to the Summary, although this aspect should be clarified.

Comment 3
The information provided in the application is sufficient.

B. EXPOSURE ASSESSMENT - ANTICIPATED INTAKE/EXTENT OF USE

Comments/Questions of the expert

Comment 1
No comments

Comment 2
Although cattle diet composition may vary across the EU, the maximum potential exposure to soybean may be higher for double-muscled Belgian Blue calves (Fiems et al., 2013) than the 20% for beef cattle, as proposed in Table 50. This is due to the extreme meatiness of double-muscled beef cattle, so that a higher dietary protein concentration is required.
The safety assessments of Cry1F, Cry1Ac and PAT proteins were based on digestibility studies in simulated gastric fluid, the lack of homology to known allergens and protein toxins, and the lack of mammalian toxicity as demonstrated by acute oral mouse gavage studies.

Comment 3
The information provided in the application is sufficient.

C. RISK CHARACTERISATION

Comments/Questions of the expert

Comment 1
No comments or questions

Comment 2
The information provided in the application is sufficient.

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

Comments/Questions of the expert

Comment 1
No comments

Comment 2
The information provided in the application is sufficient. PMM not necessary

E. ENVIRONMENTAL RISK ASSESSMENT

E.1. INTRODUCTION

Comments/Questions of the expert

Comment 1
No comments

Comment 2
The information provided in the application is sufficient.

E.2. GENERAL APPROACH OF THE ERA

Comments/Questions of the expert

Comment 1
No comments

Comment 2
The environmental risk of DAS-81419-2 soybean may be negligible, because DAS-81419-2 soybean will not be used for cultivation in the EU. Furthermore, PAT and Cry1F proteins are considered as environmentally safe (ILSI, 2011 and 2013).

Comment 3
The information provided in the application is sufficient.

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 comments

Comment 2
The information provided in the application is sufficient.

E.3.2. PLANT TO MICRO-ORGANISMS GENE TRANSFER

Comments/Questions of the expert

Comment 1
No comments

Comment 2
No comment, appropriate information is provided.

Comment 3
The information provided in the application is sufficient.

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

Comments/Questions of the expert

Comment 1
Not applicable.

Comment 2
The information provided in the application is sufficient.

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

Comments/Questions of the expert

Comment 1
Not applicable.

Comment 2
The information provided in the application is sufficient.

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

Comments/Questions of the expert

Comment 1
Not applicable.

Comment 2
Not applicable.

E.3.6. EFFECTS ON BIOGEOCHEMICAL PROCESSES

Comments/Questions of the expert

Comment 1
No comments

Comment 2
The information provided in the application is sufficient.

E.3.7. EFFECTS ON HUMAN AND ANIMAL HEALTH

Comments/Questions of the experts:

Comment 1
No questions or comments

Comment 2
Because most composites of DAS-81419-2 soybean are similar to conventional soybean, and Cry1Ac, Cry1F and PAT proteins have a history of safe use, no significant impact is expected on human or animal health. Dryzga et al. (2007) reported that genetically modified cottonseed, expressing Cry1F, Cry1Ac and PAT proteins, had no adverse effects in a 90-day feeding test involving Sprague-Dawley derived rats. These proteins are expressed in DAS-81419-2 soybean.

Comment 3
The information provided in the application is sufficient.

E.3.8. OVERALL RISK EVALUATION AND CONCLUSIONS

Comments/Questions of the expert

Comment 1
No comments

Comment 2
It has been mentioned in the Summary that DAS-81419-2 soybean has been obtained by conventional breeding of DAS-68416-4 soybean and MON-89788-1 soybean (?).
EFSA (2008) considered that soybean MON89788 is as safe as its non-genetically modified counterpart with respect to potential effects on human and animal health or the environment. A broiler study with DAS-68416-4 soybean meal (Herman et al., 2011a) reported no signs of toxicity or the presence of anti-nutrients.

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 DAS-81419-2 soybean on the nutritive value and animal and human health. Even if DAS-81419-2 soybean is not the result of conventional breeding of DAS-68416-4 soybean and MON-89788-1 soybean, there may be no significant risk for human or animal health, because of the history of safe use of Cry1Ac, Cry1F and PAT proteins.

Comment 3
The information provided in the application is sufficient.

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 comments

Comment 2
The information provided in the application is sufficient.

E.4.2. CASE-SPECIFIC GM PLANT MONITORING

Comments/Questions of the expert

Comment 1
No comments

Comment 2
Not needed.

E.4.3. GENERAL SURVEILLANCE FOR UNANTICIPATED ADVERSE EFFECTS

Comments/Questions of the expert

Comment 1
No comments

Comment 2
The information provided in the application is sufficient.

E.4.4. REPORTING THE RESULTS OF MONITORING

Comments/Questions of the expert

Comment 1

No comments

Comment 2

The information provided in the application is sufficient.

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Weber, N., Halpin, C., Hannah, L.C., Jez, J.M., Kough, J., Parrott, W., 2012. Crop genome plasticity and its relevance to food and feed safety of genetically engineered breeding stacks. Plant Physiol. 160, 1842-1853.