Bioveiligheidsraad
Conseil de Biosécurité

20-06-2017

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2017_0437

Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/NL/2011/91 from Dow AgroSciences LLC under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/NL/2011/91 was submitted by Dow AgroSciences LLC on 25 January 2011 for the marketing of genetically modified (GM) soybean DAS-68416-4 for food and feed uses, import and processing within the framework of Regulation (EC) No. 1829/2003[1].

Soybean DAS-68416-4 was developed by *Agrobacterium tumefaciens* –mediated transformation. It expresses the AAD-12 protein to confer tolerance to 2,4-dichlorophenoxyacetic acid (2,4-D) and other related phenoxy herbicides, and the PAT protein that confers tolerance to glufosinate ammonium-based herbicides.

The application was officially acknowledged by EFSA on 8 September 2011. On the same date EFSA started the formal three-month consultation period of the Member States, in accordance with Articles 6.4 and 18.4 of Regulation (EC) No. 1829/2003 (consultation of national Competent Authorities within the meaning of Directive 2001/18/EC designated by each Member State in the case of genetically modified organisms being part of the products).

Within the framework of this consultation, the Belgian Biosafety Advisory Council (BAC), under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier, chosen from the common list of experts drawn up by the BAC and the Biosafety and Biotechnology Unit (SBB). Eight experts answered positively to this request, and formulated a number of comments to the dossier, which were edited by the coordinator. See Annex I for an overview of all the comments and for the list of comments actually placed on the EFSAnet on 8 December 2011.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 26 January 2017 (EFSA Journal 2017;15(3):4719[2]), and published on 16 March 2017 together with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period.

On 27 March 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.

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (OJ L 268, 18.10.2003, p.1).
[2] See http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2017.4719/full

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The comments formulated by the experts together with the opinion of EFSA including the answers of the EFSA GMO Panel, form the basis of the advice of the Biosafety Advisory Council given below.

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-68416-4 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-68416-4, in comparison with its conventional counterpart, do not raise safety concerns.

3.2. Assessment of toxicity

Soybean DAS-68416-4 was developed to express the AAD-12 and PAT proteins. Based on previous positive assessments of the PAT protein and taking into account the information provided by the applicant including the additional information about the toxicity of the AAD-12 protein, the Biosafety Advisory Council is of the opinion that in the context of its intended uses GM soybean DAS-68416-4 does not raise safety concerns regarding toxicity.

The evaluation of the safety of the herbicides (or their breakdown products) to which GM soybean DAS-68416-4 is tolerant is not within the remit of the Biosafety Advisory Council. The Council notes however that it has verified that parallel to this import request for the GM soybean, an application has also been submitted under Regulation (EC) No 1107/2009 in which the safety of 2,4-D residue and its breakdown product 2,4-Dichlorophenol will be evaluated and a maximum residue level on soybean will be set.

3.3. Assessment of allergenicity

The Biosafety Advisory Council agrees with the EFSA GMO Panel that there are no indications that GM soybean DAS-68416-4 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-68416-4 would be less nutritious than conventional soybean varieties.

[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.

4. Monitoring

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

Conclusion

Based on the scientific assessment of the dossier done by the Belgian experts, taking into account the opinion of EFSA, the answers of the EFSA GMO Panel to the questions raised by the Belgian experts, the answers of the applicant to the EFSA GMO Panel questions and considering the data presently available, the Biosafety Advisory Council is of the opinion that in the context of its proposed uses, soybean DAS-68416-4 is unlikely to pose any risk to human and animal health.

The Biosafety Advisory Council notes that it has verified that parallel to this import request for the GM soybean, an application has also been submitted under Regulation (EC) No 1107/2009 in which the safety of 2,4-D residue and its breakdown product 2,4-Dichlorophenol will be evaluated and a maximum residue level on soybean will be set.

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-68416-4 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/2011/91 and comments submitted on the EFSAnet on mandate of the Biosafety Council (ref. BAC_2011_1027)

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08-12-2011

Secretariaat
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N./réf. : WIV-ISP/41/BAC_2011_1027
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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2011/91 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 23 September 2011
Coordinator: Dr. René Custers
Experts: Armand Christophe (UGent), Leo Fiems(ILVO), Johan Grooten (UGent), André Huyghebaert (UGent), Peter Smet (Consultant), Wim Stevens (UIA), Jan Van Doorsselaere (KATHO), Hadewijch Vanhooren (KUL)
Domains of expertise of experts involved: molecular characterisation, breeding techniques, human nutrition, animal Nutrition, analysis of food/feed, biochemistry of food/feed, industrial processing, toxicology in vivo & in vitro, immunology, alimentary allergology , plant allergens, herbicide tolerance
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman, Katia Pauwels

INTRODUCTION

Dossier **EFSA/GMO/NL/2011/91** concerns an application of the company **Dow AgroSciene** for the renewal of the marketing authorisation of the genetically modified **soybean DAS-68416-4** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 08 September 2011.
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 expert(s)

Comment 1

There is only a low risk that the use of soybean event DAS-68416-4 will be detrimental for animal and human health, based on a series of studies, including:

- Characterization of the mode of action of PAT and AAD-12 proteins
- Acute oral mouse toxicity studies
- Molecular and biochemical characterization
- Searches for homology to known toxins and allergens that affect human or animal health
- Stability during processing.

Furthermore, the safety of PAT protein has already been assessed (EFSA, 2007; EFSA, 2011).
However, a limited number of in vivo tests have been conducted to evaluate soybean event DAS-68416-4:

- Only 10 mice were used to test the acute toxicity of aad-12 protein
- As far as known, only one experiment was conducted, using 120 broilers per treatment fed a diet containing soybean event DAS-68416-4
- Only these 2 tests with living animals are mentioned in this dossier.

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

Information given is sufficient.

Comment 2

No comments

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

Comments/Questions of the expert(s)

Comment 1

Editorial comment: Part I, page 6, line 3: *full stop after* soybean varieties.

Comment 2

No comments

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

P16: What is "efficacy analysis"? Does it mean "herbicide resistance"?

D. INFORMATION RELATING TO THE GM PLANT

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

Comments/Questions of the expert(s)

Comment 1

No comments

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

Comments/Questions of the expert(s)

Comment 1

Point e) mention that “BlastN analysis did not reveal significant homologies with genes in Genbank”; has there been a thorough analysis of ORFs in the whole flanking 5’ and 3’ region DNA in order to look for insertion in gene(s)?
Additional comment from coordinator
It is required to look whether new ORFs have been created. It is not required to check at molecular level whether or not a plant gene has been disrupted by the insertion. The analysis of the composition, morphology and behaviour of the GM plant is there to pick up any indications of unwanted changes.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

No comments

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

Comments/Questions of the expert(s)

Comment 1

No comments

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

Comments/Questions of the expert(s)

Comment 1

Fig 35 is missing; to be added.

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

Comments/Questions of the expert(s)

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

D.7.1 Comparative assessment

Comments/Questions of the expert(s)

Comment 1

Although some statistically significant differences between the DAS-68416-4 soybean and its non transgenic comparator for the secondary metabolite and anti-nutrient composition in grain occurred, these differences seem to be of no major importance (mean values are always within the literature and reference range).

Comment 2

The genetically modified soybean DAS-68416-4 will be further referred to as DAS soybean.
The comparative analysis consists of an analysis of the composition of DAS soybean, non-GM control soybean with comparable genetic background and six conventional soybean varieties. Published data were also taken into consideration.
No particular remark on the general approach.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

Question: Is DAS-68416-4 meant with “a soybean line containing aryloxyalkanoate dioxygenase”? (Technical Dossier part I, page 66, first paragraph). If this is the case, why not say so?
Comment: It would be better to mention in Table 8 as First entry: Non-ADD-12 **non-PAT** control (Technical Dossier, part I page 67).

The following comment was finally sent to EFSA

We assume that on page 66, first paragraph of the Technical Dossier, the “soybean line containing aryloxyalkanoate dioxygenase” is the DAS-68416-4 line. And we assume the in table 8 on page 67, the first entry should read "Non-ADD-12 **non-PAT** control".

Comment 2

The production of material for comparative analysis is similar to previous applications.
No remark on the number of locations, growing seasons, geographical spreading and number of replicates. The field trial is well designed.
No remark on the statistical model used.
Baseline data used are obtained from commercial soybean, six conventional soybean varieties and published data. This is also in line with previous applications.

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

It is my opinion that metabolites that are/ could be formed as a consequence of applying pesticides of which the use is made permissible by the genetic modification should be analysed, especially in case they are known to be toxic. This is the case for instance for 2,4 dichlorophenol when 2,4-D is used as herbicide (e.g. Li et al.; 2010).
Additional comment from coordinator
Indeed, these metabolites should be analysed, but they are the consequence of the application of the herbicide, not the consequence of the genetic modification (see also at the end of section D.7.8 the general comment from the coordinator and the comment sent to EFSA about the possible presence of 2,4-D and its breakdown products on imported DAS-68416-4 soybean).

The OECD guidelines for comparative assessment of soybean suggest the phosphatides to be analysed. This is not done in this application. Soy “lecithin” is used in several food applications and in some food supplements.
As for the components analysed, I agree with the applicant that grain of DAS-68416-4 is compositionally equivalent to grain of non–transformed soybean.

Comment 2

Nutrients selected for analysis and demonstration of substantial equivalence are:

- Proximates: ash, moisture, protein, carbohydrates and fat. Analysis of fibre constituents consists of ADF, NDF and dietary fibre. As far as I remember this is the first application with data on dietary fibre.
- Amino acid composition; the application contains data for all essential amino acids.
- Fatty acid composition; relevant fatty acids have been studied.
- Minerals: relevant minerals are included
- Vitamins: relevant water soluble and fat soluble vitamins are included. With respect to tocopherols the analysis is not limited to α-tocopherol or vitamin E, but other tocopherols like β-, γ- and ∂-tocopherol are also considered. These tocopherols are nutritionally important antioxidants, particularly for soybean.
- Isoflavones and anti-nutrients: relevant constituents are considered.

From the results obtained the applicant concludes as follows:

- Proximate and fibre: no significant differences were found for most constituents: some effects were found for protein but the difference was not considered to be compositionally meaningful.
- Minerals: no significant differences were found and if observed, values are within reported literature data.
- Amino acids: all values are within literature and reference values
- Fatty acids: some effects were found for oleic, linoleic and linolenic acids but all values are within literature and reference values.
- Vitamins: some effects were observed, among others for folic acid, but all values are within literature ranges.
- Isoflavones: the applicant concludes in the same way as in previous cases.
- Anti-nutrients: similar conclusions.

The applicant concludes that DAS soybean composition samples were statically indistinguishable from the control soybean and /or within literature or reference ranges for conventional soybean.

Comment:
This chapter is well documented. The composition of DAS and other soybeans has been studied in detail. I agree with the overall conclusion.

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

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

No particular comment.

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

What is meant by “no new genetic modification has been introduced in DAS-68416-4 soybean”? (Technical dossier, part I, page 108, second line).

Comment 2

No particular comment.

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

Why is the PAT protein not mentioned? (Technical dossier, part I, page 108, paragraph 2 under 7.6).

Comment 2

The applicant states that DAS soybean is substantially equivalent to conventional soybean and will undergo conventional production processes. No novel process is envisaged.
No further comment.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

Both PAT and AAD-12 proteins are expressed in DAS68416-4 soybean grain. Only the safety of AAD-12 is assessed because the PAT protein has been recognized as posing no health risk (Technical dossier, part I, page 109). **Question:** Can an interactive effect between the PAT protein and the AAD-12 protein be excluded when both are fed simultaneously?
Additional comment from coordinator
I don’t see how the PAT protein can have interaction with the AAD12 protein, as it has a specificity for phosphinotrycin.

Comment 2

No further comment.

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

Data gap: Information is lacking on the levels and fate of herbicide residues in crop tissues.
Although the effect of herbicides residues on human and animal health falls under Directive 91/414/EC, it is the duty and responsibility of the toxicologist assessing the risk of the genetic modification to evaluate and discuss the complete picture of the genetic modification.
Rationale: The GM soybean plant is developed to be able to use the herbicides 2,4dichlorophenoxyacetic acid (2,4-D) and glufosinate. Data concerning the use (over-the-top applications) of the herbicides in the field trials is available (Phillips and Lepping studies, 2010a, b, c). However, no data is made available concerning the herbicides and metabolites residues in the GM plants and grain used for food/feed. As the use of these herbicides is linked to the genetic modification, the applicant should make the residue data available and make an estimation of the anticipated intake (food/feed).
Additional comment from coordinator:
See at the end of section D.7.8 the general comment from the coordinator and the comment sent to EFSA about the possible presence of 2,4-D and its breakdown products on imported DAS-68416-4 soybean.

Comment 2 **(Smet)**

The concentration of the proteins in grain.

	PAT ng/mg Tissue Dry Weight			AAD-12 ng/mg Tissue Dry Weight		
Description	Mean	Std. Dev. (n=7)	Min/Max Range	Mean	Std. Dev. (n=7)	Min/Max Range
Control	ND	NA	ND-ND	ND	NA	ND-ND
DAS-68416-4	2.66	0.46	1.80-3.71	22.92	4.17	16.29-32.18
DAS-68416-4Gluf	2.66	0.37	1.81-3.52	21.67	4.47	14.21-31.59
DAS-68416-4 2,4-D	2.57	0.4	1.91-3.34	20.19	4.16	12.14-29.77
DAS-68416-4 Both	2.62	0.44	1.55-3.41	21.16	4.63	11.51-31.97
Overall Mean	2.63			21.49		

Comment 3

Has the combined toxicological effect of AAD-12 and PAT ever been tested, since both proteins were expressed in soybean event DAS-68416-4?
PAT protein has already been assessed by EFSA at the occasion of other dossiers and may not be a risk for human and animal health (EFSA, 2007; EFSA, 2011).
AAD-12 protein was tested in vivo, but:
- only 5 male and 5 female mice were involved, which is a limited number of animals (Séralini et al., 2009). OECD (1998) recommends to use at least 10 females and 10 males.

AAD-12 protein was microbially-produced using *Pseudomonas fluorescens*. Freese and Schubert (2004) mentioned that testing bacterial surrogate proteins should not substitute for testing the plant-expressed proteins.

Additional comment from coordinator
Microbially produced protein is an accepted practice in the assessment, on the condition that different data have shown that the proteins are identical.

Comment 4

In my opinion, compositional equivalence and no toxic effects of the newly expressed proteins does not prove that the whole food/feed is not toxic (for instance see my comments under D.7 and 8.1). Therefore I suggest to perform an animal toxicity test using the whole GM food.
Additional comment from coordinator:
Performing an animal toxicity test would in this case go beyond the EFSA Guidelines. Indeed, according to these Guidelines, a 90-day toxicity study in rodents is required only when the compositional analysis has shown that the composition of the food and/or feed derived from GM plant is substantially modified, or if there are any indications for the potential occurrence of unintended effects based on the preceding molecular, compositional or phenotypic analyses.

Comment 5

It has been documented that exposure to large amounts of DCP, the AAD-12 catalyzed conversion product of the 2,4-D herbicide, is lethal (Kintz P, Tracqui A, Mangin P (1992). "Accidental death caused by the absorption of 2,4-dichlorophenol through the skin". Arch. Toxicol. 66 (4): 298–9. doi:10.1007/BF02307178. PMID 1514931). Toxicology and feeding studies were however performed using non-sprayed GM soybean. In order to assess the potential for a toxic exposure to DCP following spraying of the GM, the rate of clearance of the conversion product and “safe” lag time after herbicide spraying for minimal DCP residue should be specified.

General comment from coordinator:
Genetically modified, herbicide tolerant crops and their accompanying herbicides need two types of authorization. The GM crop needs to be safety assessed within the scope of the GMO legislation and the active ingredient of the herbicide needs to be safety assessed within the framework of the European pesticide legislation (Regulation (EC) No 1107/2009 repealing Directive 91/414/EEC). Both glufosinate and 2,4-D have been safety assessed under Regulation 1107/2009 and are authorized for use in herbicides. The member states authorize the use of formulated herbicides. It may be the case that herbicides based on glufosinate and 2,4-D may not be authorized in all EU countries. And it is very unlikely that 2,4-D is authorized for on-crop use in soybean, as this type of application is not within the scope of the European authorization for 2,4-D.
It is very important that in the case of herbicide tolerant GM crops all aspects of the crop-herbicide combination are assessed. In some cases the use of a herbicide on a herbicide tolerant GM crop may result in the formation of other types of breakdown products than in the classical situation. If not looked at this specifically this point might be overlooked. In my opinion this should be looked at within the scope of the application for the on-crop use of the herbicide on soybean.
In this particular case the most important breakdown product of 2,4-D in the GM crop is 2,4-Dichlorophenol. This is also the major breakdown product of 2,4-D in the classical, non-GM situation. In other words, there are no specific new breakdown products of 2,4-D in the herbicide tolerant GM soybean. So in this particular case, when one safety assesses the herbicide tolerant GM soybean separately and the 2,4-D separately (within the scope of Regulation 1107/2009), no assessment points will be missed. 2,4-D is safety assessed, and maximum residue levels (MRLs)

have been established. Any 2,4-D treated DAS-68416-4 soybean that is imported into the EU should not contain 2,4-D residue amounts that exceed the allowed maximum MRL levels.
We understand the worries from many of the experts about the use of 2,4-D and especially about the likely presence of 2,4-Dichlorophenol. DAS-68416-4 soybean that is treated with 2,4-D for sure is likely to be more toxic than 2,4-D untreated DAS-68416-4 soybean. But in principle it should not raise more concerns than those already considered within the framework of Regulation 1107/2009, and which have resulted in the establishment of an MRL for 2,4-D.
There is one particular situation that may create a problematic situation. That is the case when in the GM crop specific breakdown products arise, that do not arise in the classical non-GM situation, combined with the situation that the crop is only imported into the EU, and there will never by an application within the framework of Regulation 1107/2009 for the on-crop use of the herbicide. In that case certain important assessment points may be missed if one does not look at the toxicity of the GM crop products on which the herbicide has been used.

Additional comment from coordinator sent to EFSA:
A number of experts have voiced concerns about the possible presence of 2,4-D and its breakdown products on imported DAS-68416-4 soybean. In particular they have raised concerns about the possible presence of the breakdown product 2,4-Dichlorophenol on imported soybeans. 2,4-Dichlorophenol is known to be an endocrine disruptor with reproductive toxicity (Aoyama et al.; 2005). It also induces lipid peroxidation and insufficient natural antioxidant intake has been described in Central Europe, especially during the winter period (Clerhata et al.; 1996). Due to its high lipophilicity (Clerhata et al.; 1996) 2,4-Dichlorophenol is expected to be accumulated during soy processing in the oil. The major soy product used by humans is soy oil and soy oil is incorporated in some infant formulas (Ponders et al.; 1992).
Any presence of 2,4-Dichlorophenol is the result of the application of 2,4-D on the soybeans, and not a direct result of the genetic modification. Where the current dossier focuses on the safety of the DAS-68416-4 soybean as such, this is not the way the soybeans are going to be imported. The experts therefore wish that due consideration is being given to the properties of 2,4-Dichlorophenol in the knowledge that it is going to be applied on-crop on DAS-68416-4 soybeans, within the appropriate regulatory framework, whether this is the GM food and feed Regulation (EC) No 1829/2003 or the pesticides Regulation (EC) No 1107/2009.
We should be careful not to double any work, but should also be careful that no important points are being overlooked. The latter could be the case when in the GM crop specific breakdown products arise, that do not arise in the classical non-GM situation, combined with the situation that the crop is only imported into the EU, and there will never by an application within the framework of Regulation 1107/2009 for the on-crop use of the herbicide. In that case certain important assessment points may be missed if one does not look at the toxicity of the GM crop products on which the herbicide has been used. In this particular case of the DAS-68416-4 soybeans no specific breakdown products arise in the GM crop, which do not arise in the classical situation. Even though no specific breakdown products arise, there is a genuine concern that in the GM soybeans considerable higher amounts of 2,4-Dichlorophenol are being formed than in the classical situation. This point has not been addressed in the dossier.
2,4-D has been assessed within the framework of Regulation 1107/2009, and Maximum Residue Levels (MRL) have been set for this herbicide. We would expect that this MRL has been set in the knowledge that 2,4-Dichlorophenol is the most important breakdown product of 2,4-D. It may be possible that the amount of 2,4-Dichlorophenol in a product is higher than the amount of 2,4-D residue. This automatically triggers the question whether or not a specific MRL has been set for 2,4-Dichlorophenol.

Any soybean imported into the EU should fulfill the MRL for 2,4-D, and as stated above the MRL should take into account the knowledge we have about 2,4-Dichlorophenol.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

Newly expressed proteins: AAD-12 and PAT proteins
The assessment is adequate and acceptable. No further comments/questions.

Comment 2

a) Degradation of the aad-12 protein in simulated gastric fluid (Embrey and Shafer, 2008).

AAD-12 protein is readily digested by pepsin (not detectable at 30 seconds) in simulated gastric fluid as demonstrated by both SDS-PAGE and western blot analyses.

b) Degradation of the aad-12 protein in simulated intestinal fluid ().

Not performed.

c) Aad-12: Acute Oral Toxicity Study in Mice (Wiescinski and Golden, 2008).

Conclusion: Under the conditions of this study, the acute oral LD50 of AAD-12 in male and female mice is greater than 2000 mg/kg (5666 mg/kg of test substance at 35.3% purity).

No further comment.

d) Aad-12: Repeated dose oral toxicity (28-day feeding) study in mice (Thomas et al, 2010).

Conclusion: The no-observed-effect level (NOEL) for AAD-12 protein in Crl:CD1(ICR) mice of either sex following 28-days of dietary administration was 47 mg/kg/day.

No further comment.

e) Aad-12: Assessment of Amino Acid Sequence Homology with Known Toxins (Song, 2010b).

AAD-12 protein expressed in soybean event DAS-68416-4 contains no significant sequence similarity with any known toxic protein that is harmful to humans or
animals.

f) Degradation of the PAT protein in simulated gastric fluid ().

PAT protein is readily degradable in simulated digestive juice, (CFIA, 1998; EPA, 1995; EPA, 1997; OECD, 1999 and EFSA, 2007)

g) Degradation of the PAT protein in simulated intestinal fluid ().

PAT protein is readily degradable in simulated digestive juice, (CFIA, 1998; EPA, 1995; EPA, 1997; OECD, 1999 and EFSA, 2007)

h) PAT: Acute Oral Toxicity Study in Mice (Brooks, 2000).

Study not available in references.

Conclusion: earlier studies indeed demonstrated that the PAT protein shows no acute toxicity.

i) PAT: Assessment of Amino Acid Sequence Homology with Known Toxins (Song, 2010d).

The results indicated that the PAT protein expressed in transgenic events contains no significant sequence similarity with any known toxic proteins that are harmful to humans or animals.

Comment 3

Question: Should in the context of this application "expression of the PAT protein in *59122 maize"* (Technical Dossier page 127, line 31) not read "expression of the PAT protein in soybean DAS-68416-4". Was there a mixing up of 2 different applications?

Editorial remarks:
Technical Dossier page 121, first line after 28 days repeat dose study in mice: commiss**io**ned….
Technical Dossier page 131, first line after "Acute oral toxicity study in mice": reference to Brooks, 2000 is missing in the Reference lists.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

No further comments/questions

Comment 2

2,4 dichlorophenol is expected to be present in grain of soybean DAS-68416-4 (if the plants were treated with 2,4-D) as the aad-12 is expressed in grain. It is known that this compound is an endocrine disruptor with reproductive toxicity (Aoyama et al.; 2005). It also induces lipid peroxidation and insufficient natural antioxidant intake has been described in Central Europe, especially during the winter period (Clerhata et al.; 1996).
Additional comment from coordinator:
See at the end of section D.7.8 the general comment from the coordinator and the comment sent to EFSA about the possible presence of 2,4-D and its breakdown products on imported DAS-68416-4 soybean.

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

No further comments/questions

Comment 2

The analyses performed show that there is no change in soy allergen levels.

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

Broiler feeding study (Fletcher, 2010)
In this report it is not made clear under which herbicide regime the DAS-68416-4 grain was grown. No data is available concerning the herbicide residues in the DAS-68416-4 soybean meal.

Repeated-dose 90-day oral toxicity study in rodents
Not studied. According to Cicchillo et al. (2010) AAD-12 appears to be capable to oxidise both trans-cinnamic acid and the plant hormone indole-3-acetic acid (IAA) within the transgenic plant. However, according to the authors the poor catalytic efficiencies suggest that these transformations are likely to have no metabolic impact within transgenic plants. The broiler feeding study (Fletcher, 2010) conducted to evaluate the nutritional value of feed containing DAS-68416-4 soybean meal is not designed for assessing toxicological endpoints. In addition, no data was made available concerning the herbicide residues.
In conclusion, a repeated-dose 90-day oral toxicity study in rodents is needed for assessing toxicological endpoints and as such to exclude unintended effects. The herbicide treatment regime of the DAS-68416-4 soybean plant must be made available, just as the data concerning the herbicide (and metabolite) residues in the DAS-68416-4 soybean feed given to the rats.
Additional comment from coordinator:
See previous comment. According to EFSA a 90-day feeding study is required only when the molecular, compositional or phenotypic analyses have raised safety concerns.

Comment 2

a) 42-day feeding study in broiler chickens (Fletcher, 2010).

TREATMENTS				
Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Genetically modified (GM) (AAD-12, DAS-68416-4) soybean	Near-isogenic soybean (non-AAD-12 control)	Commercial soybean 1 (LG C3540)	Commercial soybean 2 (Pioneer 93B82)	Commercial soybean 3 (HiSoy 38C60)
TSN032920-0001	TSN032945-0001	TSN032947-0001	TSN032948-0001	TSN032949-0001

These results indicate that event DAS-68416-4 soybean is nutritionally equivalent to the non-transgenic near-isogenic control.
No further comment.

b) 90-Day rat feeding study ().

Not performed. No further testing is needed.

Comment 3

See above (D.7.8.2).

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

AAD-12 protein was readily digested in SGF. However, the rapid degradation of GM proteins is not a guarantee for the lack of an allergenic potential in novel foods (Meredith, 2005). Spök et al (2005) have shown that digestibility studies can not be considered as suitable tools to address the allergenic potential of a protein. Bannon et al. (2003) and Herman et al. (2006) concluded that the use of the SGF technique to predict the allergenic status of the proteins remains uncertain.

Comment 2

Endogenous allergen levels were qualitatively and quantitatively analysed. No differences were found between DAS- 68416-4 soybean and its comparators.

Comment 3

An exhaustive study of the potential for an increased risk for allergenicity of the genetically modified soybean has been performed, indicating a low risk. Because a *PAT* gene modified soybean has

already been evaluated and approved by EFSA (EFSA-GMO-NL-2005-18 for the placing on the market of the glufosinate tolerant soybean A2704-12, for food and feed uses, import and processing under Regulation (EC) No 1829/2003), this genetic modification is kept out from this opinion. The focus therefore is on the *AAD-12* genetic modification. Here a number of issues remain.
The AAD-12 protein was shown to have low thermal stability, resulting in denaturation of the protein upon heating. This heating denatured form of the protein is the one consumers will mostly be exposed to. The digestibility of heated AAD-12 protein in simulated gastric fluid was however not tested, making it difficult to draw conclusions on this important aspect regarding potential risk for allergenicity.
A second remark concerns the potential for AAD-12 and/or its catalytic products to increase the well known allergenicity of soybean. Changes in composition in endogenous soybean allergens were verified by immunoblotting using sera from allergic patients. This analysis did not indicate changes in the levels of endogenous allergens but does not exclude the occurrence of new allergens. AAD-12 protein being unlikely to act as an allergen (provided digestibility assays on heated protein are positive), the catalytic conversion product DCP and modification by DCP of endogenous soybean proteins following herbicide spraying remains a potential risk factor that was not properly addressed.

Comment 4

Soy is known as an allergenic food; in infants it is often used when cow milk allergy is present; cow milk allergic infants can become allergic to soy too.
The GM soy does not seem to behave different from the natural variant.
This product was also evaluated in Australia; there were no major comments

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

Compositional analysis has demonstrated that no unexpected alterations in nutrients and other food components have occurred and that no nutritional imbalances were introduced in soybean event DAS-68416-4.
Obviously, one animal performance experiment has been conducted, as far as published in the literature (Herman et al., 2011). As the number of animals involved, the chicken breed, the duration of the experiment and the inclusion rate of soybean event DAS-68416-4 are identical as in of the report of Fletcher (2010), it is assumed that both references deal with the same experiment. PAT protein was slightly lower in Fletcher et al. (2010): 1.6 in the Materials and methods section and 1.66 ng/mg in Table 1, vs. 1.7 ng/mg in Herman et al. (2011), but AAD-12 protein was similar (16.6 ng/mg) in soybean event DAS-68416-4
Furthermore, only 120 chickens per group were fed a diet containing soybean event DAS-68416-4. Was the involvement of 120 chickens based on a power analysis?
To my knowledge, no other experiments dealing with animal performance have been mentioned. On the other hand, high incorporation levels were used for soybean meal in the study of Herman et al. (2011) compared with Sterling et al. (2002), as all supplementary protein came from soybean meal.

Additional comment
It is also the understanding of the coordinator and the SBB that the study of Fletcher (2010) mentioned in the dossier and the study of Herman et al (2011) published in the literature both refer to the same set of data.

Comment 2

A 42 days broiler study was performed with soybean meal derived from DAS-68416-4 and controls. It is not specified in the application whether herbicides were used during plant growth, neither have I found this information in the GML Study No. 208-008-21 (reference Fletcher, 2010 in the application). This is essential to know in connection with the possible negative effects of 2,4 dichlorophenol, if present.
(PS: this information may be present in Herman et al.; 2011 which was published after the application was filled. I could not access this paper however).
Question: were the DAS-68416-4 soybean plants from which the meal was used in the bird study treated with 2,4-D?
Additional comment from SBB
This information is not available in Herman et al (2011).

In my opinion, a 2 generation rat study would be of value, if 2,4 dichlorophenol would be present in DAS-68416-4 soybean products from plants sprayed with 2,4-dichlorophenoxyacetic acid (the rationale is given in D.7.8.2;). Due to its high lipophilicity (Clerhata et al.; 1996) 2,4-dichlorophenol is expected to be accumulated during soy processing in the oil. The major soy product used by humans is soy oil and soy oil is incorporated in some infant formulas (Ponders et al.; 1992).
Additional comment from coordinator:
See at the end of this section D.7.8 the general comment that was sent to EFSA about the possible presence of 2,4-D and its breakdown products on imported DAS-68416-4 soybean.

Editorial comment: …hypothesis, **a**….(Technical dossier, part I, page 141, line 17).

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

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

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

D.9.1. Persistence and invasiveness

Comments/Questions of the expert(s)

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1

Although the number of animals involved in the toxicity studies and the animal performance experiment are restricted, soybean event DAS-68416-4 is considered to be safe for human health, based on a combination of tests.

Comment 2

In my mind there remains the uncertainty about the possible presence of 2,4 dichlorophenol in 2,4-D sprayed DAS-68416-4 products, which if present could lead to adverse effects (see above).
Additional comment from coordinator:
See at the end of section D.7.8 the general comment from the coordinator and the comment sent to EFSA about the possible presence of 2,4-D and its breakdown products on imported DAS-68416-4 soybean.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

Although the number of animals involved in the toxicity studies and the animal performance experiment are restricted, soybean event DAS-68416-4 is considered to be safe for animal health, based on a combination of tests.

Comment 2

No questions

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

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