Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2017_0438

Advice of the Belgian Biosafety Advisory Council on the application EFSA-GMO-NL-2012-106 from Dow AgroSciences LLC under Regulation (EC) No. 1829/2003

Context

The application EFSA-GMO-NL-2012-106 was submitted by Dow AgroSciences LLC on 16 February 2012 for the marketing of genetically modified (GM) soybean DAS-44406-6 for food and feed uses, import and processing (excluding cultivation) within the European Union (EU), within the framework of Regulation (EC) No. 1829/2003[1].

Soybean DAS-44406-6 was developed by *Agrobacterium tumefaciens* -mediated transformation. It expresses the AAD-12, 2mEPSPS and PAT proteins, which confer tolerance to 2,4-D, glyphosate-based and glufosinate ammonium-containing herbicides.

The application was officially acknowledged by EFSA on 15 April 2013. 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).

The Belgian Biosafety Advisory Council (BAC) did not participate in this consultation.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 17 February 2017 (EFSA Journal 2017;15(3):4738[2]), and published on 21 March 2017 together with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period.

In the frame of the preparation of this advice, the BAC, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts chosen from the common list drawn up by the BAC and the Biosafety and Biotechnology Unit (SBB). The experts were invited to evaluate the dossier, taking also into account the EFSA opinion.
Three 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 the comments.

The comments formulated by the experts together with the opinion of EFSA including the answer of the EFSA GMO Panel to the comments formulated by other Member States during the three-month consultation period, form the basis of the advice of the Biosafety Advisory Council given below.

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (OJ L 268, 18.10.2003, p.1).
[2] See http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2017.4738/full

Wetenschappelijk Instituut Volksgezondheid | Institut Scientifique de Santé Publique
Dienst Bioveiligheid en Biotechnologie | Service Biosécurité et Biotechnologie
Rue Juliette Wytsmanstraat 14 | B-1050 Brussels | Belgium
T + 32 2 642 52 11 | F + 32 2 642 52 92 | bac@wiv-isp.be | www.bio-council.be

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-44406-6 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-44406-6 do not raise safety concerns.

3.2. Assessment of toxicity

Soybean DAS-44406-6 was developed to express the the AAD-12, 2mEPSPS and PAT proteins. Based on previous positive assessments of the 2mEPSPS and PAT proteins and taking into account the information provided by the applicant in this application, the Biosafety Advisory Council is of the opinion that in the context of its intended uses GM soybean DAS-44406-6 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-44406-6 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-44406-6 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 and considering the data presently available, the Biosafety Advisory Council is of the opinion that in the context of its proposed uses, soybean DAS-44406-6 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-44406-6 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-2012-106 (ref. BAC_2017_0351)

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Secrétariat

O./ref.: WIV-ISP/41/BAC_2017_0351
Email. : bac@wiv-isp.be

Compilation of comments of experts in charge of evaluating the application EFSA-GMO-NL-2012-106

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 21 March 2017.
Coordinator: Dr. René Custers
Experts: Eddy Decuypere (KUL), André Huyghebaert (UGent), Johan Grooten (UGent)

Domains of expertise of experts involved: Animal and human nutrition, immunology, alimentary allergology, plant allergens.

SBB: Didier Breyer, Fanny Coppens, Katia Pauwels.

- **INTRODUCTION**

Dossier **EFSA-GMO-NL-2012-106** concerns an application submitted by **Dow AgroSciences** for authorisation to place on the market genetically modified soybean **DAS-444Ø6-6** 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 15 April 2013. The opinion of the EFSA Scientific Panel on GMOs was adopted on 17 February 2017 (EFSA Journal 2017;15(3):4738) and published on 21 March 2017.

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.
Since comments from experts were requested after the publication of the EFSA's opinion, they were not sent to EFSA but rather used directly by the Biosafety Council as a scientific basis to draft its final advice on this application.

List of comments/questions received from the experts

GENERAL COMMENTS

Comment 1

No comments.

A. HAZARD IDENTIFICATION AND CHARACTERISATION

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

Comment 1

No questions.

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 questions.

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

DAS-44406-6 was transformed by insertion of 2mepsps gene encoding for the protein 2mEPSPS providing tolerance to glyphosate, and insertion of aad-12 gene encoding for AAD-12 (aryloxyalkanoate dioxygenase-12 enzyme) conferring tolerance to 2,4D, and insertion of pat-gene encoding for PAT-protein conferring tolerance to glufosinate. The newly expressed proteins were comparable with and without herbicide treatments, indicating stable expression; a single intact copy of the three inserts and no additional DNA fragments from the expression cassettes were proven by Southern blot analysis.

A.3. COMPARATIVE ASSESSMENT

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

Comment 1

For the safety evaluation DAS-44406-6 soybean was compared with a non-transgenic near-isogenic control. In addition several conventional soybean varieties were included. Unsprayed and sprayed events with 2,4-D, glufosinate and glyphosate were also investigated.
The selection is well described and motivated.
No particular remarks.

Comment 2

No questions.

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

Comment 1

No remarks.

Comment 2

A non-transgenic control soybean as isogenic control, DAS-44406-6 with and without treatments (5 entries) and 3 reference varieties (of the 6) were used: 9 entries for each location (of the 10 locations in total) in a balanced incomplete block design with 3 of the 6 reference lines at each site by randomizing across sites.
No questions; perfect design.

A.3.3. COMPOSITIONAL ANALYSIS

Comment 1

Some comments on the selection of constituents in soybean seeds. I limit my comments to seeds.

Proximates:
Fiber: dietary fiber is assessed in addition to acid and neutral detergent fiber; however there is no differentiation of fiber constituents; as a first step soluble and insoluble dietary fiber could be considered, taking into the account their role in human nutrition.
Carbohydrates are assessed as a group but not differentiated; carhohydrates have specific nutrition properties; this is illustrated by the ongoing debate about sugar in food.

Amino acids and fatty acids are analysed in detail; essential amino acids and polyunsaturated fatty acids are determined according to the actual know how of their prominent role in human nutrition.

Minerals are also studied in detail; minerals and some trace elements known for their positive role in human nutrition; it would be of interest to have information about the potential effect on trace elements with negative properties such as cadmium, arsenic and even aluminium (sometimes discussed in relation to soybeans).

Vitamins are determined according to their presence in soybeans; tocopherols are differentiated in α-β-, γ- and δ- tocopherols; attention is given to the antioxidative properties in addition to the vitamin activity of tocopherols; the important analogous constituents tocotrienols are not considered; they are known for their antioxidative potential.

Secondary metabolites and anti-nutrients: no remarks on the selection of constituents;

This in depth assessment could be improved, so additional information would become available for the nutrition properties.
As this is a safety assessment, undesirable constituents have to be considered: e.g. some heavy metals.

The analysis scheme is appropriate for comparative purposes of DAS-44406-6 soybean and the reference lines.

Results of the *equivalence tests* are graphically represented.

The applicant discusses results of the equivalence test for *proximates and fiber*.
A significant difference was found for total fat but the values were within ranges reported in literature.
From the results the applicant concludes that the proximate and the fiber composition in seeds of the transgenic lines are normal for soybean.

Minerals
Significant differences were observed for some elements but the values are within ranges reported in literature.

Amino acids
Significant differences were found for some amino acids. Among those, lysine, tryptophan, leucine and isoleucine are essential amino acids. These differences deserve attention but are within the ranges reported in literature.

Fatty acids
In a similar way significant differences were found for important fatty acids from a human nutrition point of view: palmitic, oleic, linoleic and linolenic acid. However the values are once again within the ranges reported in literature.

Vitamins
Similar observations and conclusions for some vitamins, among others folic acid and some tocopherols.

Anti-nutrients and bioactives
Similar observations and conclusions; Lectin levels were found higher in DAS-44406-6 soybean than in the reference. This is an important observation. The values are once again within the range reported in literature. The applicant reports that lectins are denatured during processing, particularly heat processing. Raw soybeans are indeed not consumed as such but only after an appropriate heat treatment.

Conclusion of the applicant
The applicant concludes that the compositional characteristics are in agreement with those of traditional analyses. Minor differences found are not biologically significant.

Comment
I basically agree with the conclusion.

Several GM soybeans have been introduced and studied for compositionally equivalences. During analysis some non-equivalence is found for particular constituents. Values are however within normal ranges in literature.
I wonder if a *meta analysis* of all these data is not worthwhile to consider in order to detect any shift in the composition of soybeans in particular a potential shift in essential nutrients for human nutrition or in toxicants.

Comment from the coordinator
With regards to the last comment above (suggestion for a meta-analysis), the coordinator is of the opinion that this suggestion is interesting from a scientific point of view, but goes beyond the evaluation of individual dossiers. It is also unclear why such a meta-analysis should be performed specifically for GM soybean and not for traditional soybean in general. This is an issue that could be kept in mind for further discussion in the Biosafety Council.

Comment 2

- Why in table 20 Vit E (alpha – tocopherol) is presented, while in table 19, alpha, gamma, delta and total tocopherol are mentioned?
- Also secondary metabolites and anti-nutrients do not completely correspond between table 19 and 20.

- Page 89: elevated mean levels for copper (and lower mean levels for potassium) were mentioned; these were respectively higher and lower than literature range. However, the lower zinc for seeds, even lower than the literature range (see fig 34, p.95) are not mentioned or discussed on p.89. Could copper antagonists such as molybdene and zinc be a potential reason: lower zinc, hence higher copper for uptake by plants? (in analogy with animals were a zinc –copper antagonism exists).
- Page 96: glutamic acid is lower (p.104) and tryptophane higher (p.106) in most or all of entries in this study, and are outside literature ranges (fig 36) for soybean (as % of dry weight). Why? Any hypothesis?
- Page 127: levels of Vit B1 (thiamine) and Vit B2 (riboflavin) are higher than literature range: this is not mentioned on p.123 in results of equivalence test of vitamins in seed. Any reason why? Any hypothesis why this is so for all entries?

Additional comment: The expert clarified by email that these questions are not critical from the safety viewpoint and do not challenge the conclusion from EFSA that none of the differences identified between soybean DAS-44406-6 and the conventional counterpart needs further assessment regarding food and feed safety.

A.3.4. AGRONOMIC AND PHENOTYPIC CHARACTERISTICS

Comment 1

No remarks.

Comment 2

No questions.

A.3.5. EFFECTS OF PROCESSING

Comment 1

No particular effects due to the compositional equivalence.

Comment 2

No questions.

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

Mode of action of the newly expressed proteins 1mEPSPS, AAD-12, and PAT is very well explained on p. 155-157.
All newly expressed proteins and their microbiologically-derived counterparts are equivalent and identical. All proteins are denatured by heat, digested is simulated gastric fluid and without any effect in repeated dose toxicity studies in mice.

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.

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

The risk assessment of the newly expressed 2mEPSPS, AAD-12 and PAT proteins has been performed in full compliance with EFSA regulation. No indications pointing towards an allergenic risk of the newly expressed proteins resulted from this analysis.
I have no further comments.

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

Comment 1

No questions.

Comment 2

Soybean being an important allergenic food, the applicant performed an appropriate analysis of the levels of endogenous allergens using sera (n=6) from soybean allergic individuals. This analysis did not reveal changes in the levels of endogenous allergens nor the appearance of additional allergenic proteins recognized by the human sera.
I have no further comments.

A.5.3. ADJUVANTICITY

Comment 1

No questions.

Comment 2

No comments.

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

- Page 206: no difference occurred between broilers fed DAS-44406-6 and control soybean except for a 7.6% difference in thigh weights (higher weight) in the DAS-44406-6 soybean meal group. How is this compensated and by what? Lower (but not significant) breast weight? This may be relevant, especially in view of the considerable proportion of thigh weight as a % of total body weight, and about half that of breast weight.
Can this be more specified here? Is this more explained in the document of Papinemi (2012) (study ID:101931. Dow Agrosciences, LLC). How can this study be made available? Reference is made to table 5 and table 7 of this study, but not given here.

Additional comment from the SBB: The study Papinemi (2012) has been made available to the expert, who therefore considered that the information provided was sufficient to clarify the issue raised above.

B. EXPOSURE ASSESSMENT - ANTICIPATED INTAKE/EXTENT OF USE

Comment 1

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 questions.

E. ENVIRONMENTAL RISK ASSESSMENT

E.1. INTRODUCTION

Comment 1

No questions.

E.2. GENERAL APPROACH OF THE ERA

Comment 1

No questions.

E.3. SPECIFIC AREAS OF RISK

As stated in the EFSA guidance on the environmental risk assessment of genetically modified plants (EFSA Journal 2010, 8(11):1879) the objective of the ERA is on a case-by-case basis to identify and evaluate potential adverse effects of the GM plant, direct and indirect, immediate or delayed (including cumulative long-term effects) on the receiving environment(s) where the GM plant will be released. For each specific risk the ERA consists of the six steps described in Directive 2001/18/EC:

1. Problem formulation including hazard identification,
2. Hazard characterisation,
3. Exposure characterisation,
4. Risk characterisation,
5. Risk management strategies,
6. Overall risk evaluation and conclusions.

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

Comment 1

No questions.

E.3.2. PLANT TO MICRO-ORGANISMS GENE TRANSFER

Comment 1

No questions.

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

Comment 1

Negligible.

E.3.4. Interaction between the GM plant and non-target organisms (NTOs)

Comment 1

Negligible.

E.3.5. Impacts of specific cultivation and management and harvesting techniques

Comment 1

Not applicable.

E.3.6. Effects on biogeochemical processes

Comment 1

Negligible.

E.3.7. Effects on human and animal health

Comment 1

No questions.

E.3.8. Overall risk evaluation and conclusions

Comment 1

No comments.

E.4. Post Market Environmental Monitoring plan

E.4.1. Interplay between environmental risk assessment and monitoring

Comment 1

No questions.

E.4.2. Case-specific GM plant monitoring

Comment 1

No questions.

E.4.3. General surveillance for unanticipated adverse effects

Comment 1

No questions.

E.4.4. REPORTING THE RESULTS OF MONITORING

Comment 1

No questions.