Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2017_0261

Advice of the Belgian Biosafety Advisory Council on application EFSA-GMO-NL-2013-120 from Bayer CropScience under Regulation (EC) No. 1829/2003

Context

Application EFSA-GMO-NL-2013-120 was submitted by Bayer CropScience on 10 December 2013 for the marketing of genetically modified (GM) soybean FG72 x A5547-127 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].

The two-event stack soybean FG72 x A5547-127 was obtained by conventional crossing (no new genetic modification involved) of the corresponding single events:
- FG72, expressing HPPD W336 for tolerance to isoxaflutole, and 2mEPSPS for glyphosate tolerance;
- A5547-127, expressing phosphinothricin acetyltransferase (PAT) protein for tolerance to glufosinate ammonium-based herbicides.

The application was officially acknowledged by EFSA on 23 February 2015. On 26 August 2015 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). Nine experts answered positively to this request, and formulated a number of comments to the dossier. See Annex I for an overview of all the comments.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 1st March 2017 (EFSA Journal EFSA Journal 2017;15(4):4744[2]), and published on 6 April 2017 together with the responses from the EFSA GMO Panel to comments submitted by the Member States during the three-month consultation period.
On 13 April 2017 the opinion of EFSA was forwarded to the Belgian experts. They were invited to give comments and to react if needed.

[1] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (OJ L 268, 18.10.2003, p.1).
[2] See https://www.efsa.europa.eu/en/efsajournal/pub/4744

In delivering the present advice the Biosafety Advisory Council considered in particular the information below:
- The comments formulated by the experts on application EFSA-GMO-NL-2013-120;
- The opinion of EFSA;
- The advices already adopted by the BAC on the single events. The conclusions of the BAC were as follows:

Event	Application number	BAC advice	Conclusions
FG72	EFSA-GMO-BE-2011-98	BAC/2015/0597 (8-09-2015)	No major risks for human and animal health or concerning the environment were identified. A minority declaration was issued by a member regarding the substantial equivalence of the event.
A5547-127	EFSA-GMO-NL-2008-52	BAC/2011/0553 (16-06-2011)	No major risks for human and animal health or concerning the environment were identified.

Both GM soybean mentioned in the table above are all authorised in the EU for food and feed uses[3].

Scientific evaluation

1. Environmental risk assessment

The Biosafety Advisory Council is of the opinion that it is unlikely that the accidental release of soybean FG72 x A5547-127 seeds (i.e. during transport and/or processing) into the European environment[4] 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

Taking into account the previous assessment of the single events and the new data on compositional analysis provided by the applicant for the three-stacked event, the Biosafety Advisory Council agrees with the GMO panel of EFSA that the compositional data of GM soybean FG72 x A5547-127, in comparison with its conventional counterpart, do not raise safety concerns.

3.2. Assessment of toxicity

The Biosafety Advisory Council has evaluated the safety of the newly expressed HPPD W336, 2mEPSPS and PAT proteins in the context of previous applications, and no safety concerns were identified. Taking into account the updated information considered in the current application, the Council is of the opinion that its previous conclusions remain valid.

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

The Biosafety Advisory Council is also of the opinion that the combined expression of the newly expressed proteins in the stacked event should not raise toxicological concerns.

3.3. Assessment of allergenicity

The Biosafety Advisory Council has evaluated the safety of the newly expressed HPPD W336, 2mEPSPS and PAT proteins in the context of previous applications, and no concerns were identified. Since no new information on allergenicity of these proteins has become available, the Council is of the opinion that its previous conclusions remain valid.
The Biosafety Advisory Council is also of the opinion that the combined expression of the newly expressed proteins in the stacked event does not raise concerns regarding the allergenicity.

3.4. Nutritional value

The Biosafety Advisory Council is of the opinion that the information provided is sufficient to conclude that the nutritional characteristics of soybean FG72 x A5547-127-derived food and feed are not expected to differ from those of conventional soybean varieties.

4. Monitoring

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

Conclusion

Based on the scientific assessment of the dossier done by the Belgian experts, taking into account the opinion of EFSA, the advices already adopted by the BAC on the three single events and one of the possible subcombinations, and considering the data presently available, the Biosafety Advisory Council:

1) Agrees with the GMO panel of EFSA that the potential environmental release of soybean FG72 x A5547-127 is unlikely to pose any threat to the European environment;
2) Agrees with the GMO panel of EFSA that there is no reason to expect interactions between the newly expressed proteins that could impact on the food or feed safety;
3) Agrees with the GMO panel of EFSA that in the context of its proposed uses, soybean FG72 x A5547-127 is unlikely to pose any risk to human and animal health;
4) Considers that the conclusions of the Biosafety Advisory Council on the single events that have been assessed previously (FG72 and A5547-127 – see table on page 2) remain unchanged.

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

Annex I: Minority declaration.
Annex II: Compilation of comments of experts in charge of evaluating the application EFSA-GMO-NL-2013-120 and compilation of comments from the experts who evaluated the dossier on mandate of the Biosafety Council (ref. BAC_2015_0767)

Minority declaration of P. Baret

The following declaration, issued for application EFSA/GMO/BE/2011/98 (soybean FG72), is reiterated in the context of this advice on soybean FG72 x A5547-127:
“The compositional comparison of GM soybean FG72 and control plants is significantly different for several endpoints. In consequence, it is impossible to demonstrate substantial equivalence and a full toxicological analysis should have been performed. As such analysis is not provided by the notifier, a toxicological risk cannot be fully excluded. In consequence, my advice is negative on the authorisation of GM soybean FG72.”

Bioveiligheidsraad
Conseil de Biosécurité

19/11/2015

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC_2015_0767
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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2013/120
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 8 September 2015.
Coordinator: René Custers
Experts: Eddy Decuypere (KUL), Jacques Dommes (ULg), Leo Fiems (ILVO), Johan Grooten (UGent), André Huyghebaert (UGent), Peter Smet (Consultant), Jan Van Doorsselaere (KATO), Bart Van Droogenbroeck (ILVO), Hadewijch Vanhooren (KUL)
Domains of expertise of experts involved: Molecular characterisation, DNA/RNA/protein analysis, herbicide tolerance, animal and human nutrition, food/feed processing, toxicology, general biochemistry, statistics, immunology, alimentary allergology, plant allergens, soybean, breeding techniques, plant biology.
SBB: Didier Breyer, Fanny Coppens, Martine Goossens, Katia Pauwels

- **INTRODUCTION**

Dossier **EFSA/GMO/NL/2013/120** concerns an application submitted by the company **Bayer CropScience** for authorisation to place on the market genetically modified **Soybean FG72 x A5547-127** 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 19 August 2015.

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. No comments were placed on EFSAnet for the present application.

List of comments/questions received from the experts

GENERAL COMMENTS

Comment 1
Single events dealing with 2mEPSPS, hppdPfW336 and PAT proteins have already been assessed and EFSA concluded that they are safe for human and animal health. It is assumed that there is no plausible or testable hypothesis for an interaction of the newly-inserted proteins. Consequently, the genetic modification of FG72 x A5547-127 soybean is no reason to prohibit its import and processing in the EU.

No direct effect of the genetic modification of FG72 x A5547-127 soybean, but an indirect effect may be an increased use of the herbicides (glyphosate, isoxaflutole and glufosinate). Some health concerns about glyphosate, isoxaflutole and glufosinate have been reported. FG72 x A5547-127 soybean is not intended for cultivation in the EU. Nevertheless, introduction of the FG72 x A5547-127 soybean elsewhere may increase the use of these herbicides. As a consequence, imported soybean, destined for food and feed use, may contain residues of these herbicides and their metabolites. Therefore, cultivation of FG72 x A5547-127 soybean should meet the restrictions specific to herbicide-treated crops. Bayer CropScience should make efforts to guarantee that these restrictions are complied.

General comment with regard to the robustness of this dossier: referring to the incomplete data provided previously in other dossiers by other applicants (Windels et al., 2001; EFSA, 2015b), the robustness of the evaluation of this dossier is based on the reliability and the integrity of the applicant in question. I do hope the applicant will respect his promise (P.133 of the technical dossier; 4.5. Reporting) and inform the European Commission if information that confirms an adverse effect of FG72 x A5547-127 soybean and that alters the existing risk assessment becomes available.

SBB and Coordinator comment:
The assessment of the use and safety of pesticides is not within the remits of the BAC.

Comment 2
No comments.

Comment 3
Typing error on pg. 9, third paragraph, 'resistan**t' –** 't' in the end of the word is missing.

Comment 4
Soybean FG72 x A5547-127 is obtained by crossing single parental lines. No new genetic modification is applied.
In this particular case significant changes in the composition are not expected.

A. HAZARD IDENTIFICATION AND CHARACTERISATION

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

Comment 1

No comment, adequate information was provided.

Comment 2
No comments.

Comment 3
No comments.

Comment 4
- The link provided on page 18 of the dossier, http://www.nsrl.uiuc.edu/aboutsoy/soyprocessing.html is broken.
- The same is true for the link provided on page 19 of the dossier, http://www.nsrl.uiuc.edu/aboutsoy/images/processing_diagram.gif

Coordinator comment:
These comments were sent informally (by e-mail) to EFSA.

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 comment, adequate information was provided.

Comment 2
No comments.

Comment 3
No comments.

Comment 4
- No comments or questions. Information provided is adequate. The applicant has summarized the information that was provided in previous applications related to both single events.
- On page 25 the applicant is describing a digest with restriction enzyme *Pvu*I to disrupt gene *bla*. Brief description of the function of this gene as selection marker in the cloning procedure and inclusion of the abbreviation in the list of 'ACRONYMS AND SCIENTIFIC TERMS' would make it easier to read.

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
Southern blot analyses confirmed the presence of intact FG72 and A5547-127 DNA inserts in FG72 x A5547-127 soybean. New bioinformatics analyses of the junction regions for the single events FG72

and A5547-127 were carried out. These analyses confirmed that no endogenous genes were interrupted and that no new transcription unit was created. Expression of the three introduced genes was assayed at the protein level through validated ELISA assays. Data showed similar levels as those reported for the parental lines. This suggested the absence of interactions between the single events. I reached the conclusion that le molecular characterization of FG72 x A5547-127 soybean was adequately carried out and that the results do not rise any safety concern.

Comment 2
FG72 gives tolerance to glyphosate and to isoxaflutole by expressing respectively 2mEPSPS and HPPD-W336 protein, while A5547-127 gives tolerance to glufosinate by expressing PAT-protein; FG72xA5547-127 has comparable levels of the 3 proteins with no indications of potential interactions. No further questions.

Comment 3
The individual events FG72 and A5547-127, when combined in one plant, are stably maintained and the expression of the transgenes is comparable with the parents (carrying the individual events).

No comments.

Comment 4
- The information provided by the applicant is adequate. From the southern blot analysis the intactness and stability of the inserts was proven. Bioinformatics analysis did not provide any evidence that functional endogenous genes or ORFs were interrupted. From the *in silico* analysis of the inserted sequences it is clear that there are neither allergenic nor toxicological findings associated with the presence of the putative ORF polypeptides or putative products of predicted genes. Based on this information, no unintended changes and no indications of potential interactions between the single events or between the newly expressed proteins were identified. Therefore it can be concluded that the molecular characterization of FG72 x A5547-127 soybean does not indicate safety concerns.

Remark in relation to the protein expression level:
- On pg. 45 the expression levels of the different proteins from the field trial data in Brazil are discussed and compared with those observed in the parental lines. For the 2mEPSPS protein and the PAT protein expression levels are similar in the stacked event compared to those observed in the single event. For the HPPD W336 protein, the range was 0.538 to 2.14 µg/g (d.w.) in seed of FG72 soybean and 0.160 to 0.291 µg/g (d.w.) in seed of FG72 X A5547-127 soybean. So it is a ten-fold lower in the stacked event compared to the single event. What could be the explanation for this ten-fold difference observed? Could the lower expression level result in a reduced tolerance to the herbicide? This is only discussed in relation to the LLOQ and by stating that the expression level is low and comparable in both the single and stacked event, and hence not of any biological relevance. Discussing this observation a bit more in depth focussing on the (absence of) potential impact on the targeted phenotype, i.e. herbicide tolerance, could have been appropriate in my opinion.

Coordinator comment:
These questions don't pertain to potential concerns for the environment or human/animal health in the case of marketing of the product, they were not sent to EFSA.

Other minor remarks:

- On pg. 29 a schematic drawing of the FG72 soybean insert with indication of the restriction enzymes and probes used for Southern blot analysis is given. It is confusing for the reader to notice that the FG72 T-DNA probe is depicted two times in this scheme, but referring to different molecular sizes. In the upper panel it covers one copy of the elements in the pSF10 vector, while in the lower panel it stretches along a the genomic FG72 area that is harbouring two copies of the elements of the pSF10 vector. Indicating that the probe is matching two times in the FG72 genomic DNA could simply solve this issue.
- Pg. 31: Typing error "faint **p**and" – should be "faint band".
- Pg. 31: The applicant states "Fragment sizes were determined by a different method than applied in the study" without providing reference to a specific study" – so it is hard to check this statement.
- Pg. 32: Typing errors in last line of third column 'secondary structure f the ScaI – digested pSF10 vector' should be 'secondary structure **o**f the ScaI – digested pSF10 vector' and also in footnote 2: 'The overlap between the prove and the fragment was too small to be visualized' s hould be 'The overlap between the pro**b**e and the fragment was too small to be visualized'

A.3. COMPARATIVE ASSESSMENT

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

Comment 1
No comment, adequate comparators were selected.

Comment 2
- FG72xA5547-127, both treated and non-treated (or conventionally treated)
- Suitable conventional counterpart (conventionally treated)
- 6 non-GM reference varieties (conventionally treated)

This is a correct set-up.

Comment 3
As it is usually the case in this type of comparisons, soybean FG72 x A5547-127 was compared to its conventional counterpart and to other non-GMO reference varieties.

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

Comment 1
No comment.

Comment 2
No questions.

Comment 3
In this trial a comparative assessment took place in nine field trials including soybean FG72 x A5547-127 treated with the intended herbicides, soybean FG72 x A5547-127 treated with conventional herbicides, the conventional counterpart treated with conventional herbicides and six non-GMO reference varieties.

No further remarks.

A.3.3. COMPOSITIONAL ANALYSIS

Comment 1
Most of the composition parameters analysed in FG72 x A5547-127 soybean were equivalent to the set of non-GM soybean references, and mean values are within the range of soybean references and the absolute differences between them are minor. These results confirm the compositional equivalence of FG72 x A5547-127 soybean to conventional soybean varieties.

Comment 2
No comment, the results do not rise any safety concern.

Comment 3
No questions.

Comment 4
The constituents studied were selected according to the OECD guidelines and include:

- proximates: moisture, protein, fat, ash and carbohydrates by difference,
- fiber fractions: acid detergent, neutral detergent fiber
- amino acids: relevant amino acids are included
- fatty acids: the whole range of fatty acids was studied
- minerals: important minerals are included
- vitamins: vit A, B1, B2, K, folic acid, tocopherols
- anti-nutrients: trypsin inhibitor, lectins, phytic acid, raffinose and stachyose
- isoflavones: relevant isoflavones are included

The constituents were studied in detail. I have a comment, as has been the case in previous assessments on the approach for fiber and for carbohydrates by calculation. At least for human nutrition this is no longer accepted. Up to date methods are widely applied for the determination of fiber constituents (soluble and isoluble fiber) and for carbohydrates.

SBB and coordinator comment:
This comment was already transmitted to EFSA. In the instance of application 2011/98 (Soybean FG72), EFSA replied that "Analytical methodologies for specific endpoints are not defined in EFSA guidance documents". This comment was therefore not sent to EFSA.

With respect to vitamins, important vitamins were studied including vit K and the individual tocopherols. On the other hand tocotrienols, important antioxidants, were not analysed.

Coordinator comment:
Tocotrienols are not included for analysis according to the OECD recommendations, therefore this question was not sent to EFSA.

The statistical evaluation is described in detail.

The results of the comparison confirm the conclusion of equivalence between soybean FG72 x A5547-127 and the non-GMO references. Taking into account natural variations no biological relevant differences were observed between soybean FG72 x A5547-127 and its comparator.

I agree with the overall conclusion.

Comment 5
Seems to be normal. No comments.

A.3.4. AGRONOMIC AND PHENOTYPIC CHARACTERISTICS

Comment 1
No comment, the results do not rise any safety concern.

Comment 2
No questions.

Comment 3
No remarks.

A.3.5. EFFECTS OF PROCESSING

Comment 1
No comment.

Comment 2
No questions; I agree with the conclusion of no difference between FG72 soybean and A5547-127, as well as combined FG72xA5547-127 soybean and their conventional counterpart from the comparative assessment.

Comment 3
According to the applicant the effects of processing on soybean FG72 x A5547-127 are not expected to be different from the effects on conventional soybeans. Processing into flour, grits, concentrates, isolates, meal, soybean oil and lecithin is reviewed.

I agree with this conclusion.

A.4. TOXICOLOGICAL ASSESSMENT

A.4.1. METHODOLOGY USED FOR TOXICITY TESTS

Comment 1
No questions.

A.4.2. ASSESSMENT OF NEWLY EXPRESSED PROTEINS including:

- Molecular and biochemical characterisation of the newly expressed proteins
- Up-to-date bioinformatic search for homology

- Information on the stability of the protein under the relevant processing and storage conditions for the food and feed derived from the GM plant
- Data concerning the resistance of the newly expressed protein to proteolytic enzymes
- Repeated dose toxicity studies using laboratory animals

Comment 1
Based on the weight of evidence in this dossier it is unlikely that FG72 x A5547-127 soybean will pose serious risks for toxicity. Single events FG72 soybean (EFSA, 2015a) and A5547-127 soybean (EFSA, 2011) are as safe as their conventional counterparts and non-GM reference varieties with respect to potential effects on human and animal health and the environment.
It is assumed that there is no biological pathway in which the newly-inserted genes would directly or indirectly interact safety (Kok et al., 2014; Zdziarski et al., 2014). There is no plausible or testable hypothesis for the interaction of 2mEPSPS, hppdPfW336 and PAT proteins in FG72 x A5547-127 soybean (Steiner et al., 2013).

Comment 2
New bioinformatics search for sequence homology between the amino acid sequence of the 2mEPSPS, HPPD W336 and PAT proteins with known toxins were carried out. The results confirmed the conclusion reached for the single events: there is no relevant homology with known toxins.

Comment 3
No questions.

Comment 4
The three proteins, 2mEPSPS, HPPD W336, and PAT, were already assessed during the toxicological assessment of the single parental lines FG72 and A5547-127. For both parental lines a scientific opinion from EFSA is available: EFSA Journal 2015; 13(7):4167 and EFSA Journal 2011; 9(5):2147.
Concerning the HPPD W336 protein (my comments during the assessment of soybean FG72, EFSA/GMO/BE/2011/98):
The by gavage administration of the limit dose of 2000 mg HPPD W336 protein/kg bw in female OF1 mice (n=5) in the acute toxicity study (Rascle, 2009) resulted in decreased spleen weight (absolute and relative) although without gross or histopathological related findings. In contrast, in the 28-day oral toxicity study (Kennel, 2010) the administration of HPPD W336 protein given by gavage (limit dose, 1000 mg/kg bw/d) to male and female mice (5/sex/group) did not result in an effect on the spleen (absolute and relative spleen weight, histopathology). Nevertheless, a decrease in aspartate (22%) and in alanine aminotransferase (31%) was observed in male mice although without related histopathological findings and only in this sex.
An additional up-to-date bioinformatic search for homology for the three proteins was performed.
No further comments or questions.

SBB and coordinator comment:
This comment was sent to EFSA for application 2011/98, and EFSA took it into account in its opinion for that application. The EFSA GMO Panel concluded that there is no safety concern with regard to the HPPD W336 protein expressed in soybean FG72. The final advice of the BAC on application 2011/98 (Soybean FG72) did not mention any toxicity concerns. Therefore this comment was not sent to EFSA for the present application.

Comment 5

The amounts of newly expressed proteins are similar to the levels in the parental lines.

2mEPSPS: Sequence homology with known toxins (Capt, A., 2013)

The results of the overall homology search with general protein databases showed only main similarities with other enzymes from the EPSPS family from various organisms.

HPPD W336: Sequence homology with known toxins (Capt, A., 2013)

The results of the overall homology search with general protein databases showed only main similarities with other HPPD proteins from various origins.

PAT: Sequence homology with known toxins (Mirsky, 2014)

The results of the overall homology search with general protein databases showed only main similarities with other acetyltransferases from various bacterial origins.

A.4.3. ASSESSMENT OF NEW CONSTITUENTS OTHER THAN PROTEINS

Comment 1
Not applicable.

Comment 2
No further comments/questions.

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

Comment 1
Not relevant.

Comment 2
No relevant compositional changes were observed in the comparative analysis of the composition study. No particular natural constituents of FG72 x A5547-127 soybean are considered to be of significant concern to require additional information or further risk assessment. No further comments/questions.

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

Comment 1
Not applicable.

Comment 2
Soybean FG72 x A5547-127
No whole food/feed animal studies (90-day rat, 42-day poultry) were performed. I agree that it is unlikely that any interactions occur between the three proteins that could lead to adverse effects (different substrates, different pathways). The outcome of the comparative analysis of the composition study did not reveal indications of additive, synergistic or anatagonistic interactions. In addition the 42-day studies performed with the single parental lines did not reveal adverse effects. No rat study was performed with soybean A5547-127. According to EFSA, not enough animals were used in the rat study performed with soybean FG72 to be taken into account in the assessment.

Further comment: No data is made available concerning the herbicides glufosinate ammonium, glyphosate and isoxaflutole and their metabolites residues in the FG72 x A5547-127 soybean seed/meal used for food/feed.

Comment 3
90-Day rat feeding study (author).

Not performed. No further testing is needed.

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
Based on the weight of evidence in this dossier it is assumed that FG72 x A5547-127 soybean does not pose a serious allergenic risk, and that it is comparable with conventional maize with regard to allergenicity.

Comment 2
No questions.

Comment 3

- The FG72 x A5547-127 soybean was developed by conventional crossing of the parental lines FG72 and A5547-127. Both parental strains have been individually evaluated before by EFSA and BAC. These studies concluded that the endogenous allergen profile of FG72 and of A5547-127 soybean is equivalent to the endogenous allergen profile of the non-GM conventional counterparts and that there is no evidence that either soybean GMO would present any different risk of soybean allergy than the conventional lines already on the market.
- Expression data provided in the dossier showed that the mean 2mEPSPS, HPPD W336 and PAT protein levels in FG72 x A5547-127 soybean seeds are comparable to the protein levels in the parent FG72 and A5547-127 GMOs, thus excluding the occurrence of crosstalk between the introduced traits.
- The bioinformatics analyses of the 2mEPSPS, HPPD W336 and PAT proteins for amino acid sequence similarity with known allergens have been updated from the previous applications using a 2013 database. These *in silico* analyses did not reveal hits that might indicate a risk for allergenicity of the introduced traits.

Accordingly, there is no new information indicative of an increased risk of allergenicity from the individual traits nor of their combination in the hybrid FG72 x A5547-127 soybean.

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

Comment 1
No questions.

Comment 2
Soybean being an allergenic food, the applicant correctly performed a screening of the protein levels of (37) known soybean allergens in order to verify to what extend expression levels may be altered in FG72 x A5547-127 soybean seeds as compared to reference conventional soybean seeds. This analysis was performed using 2D-SDS-PAGE and did not reveal biologically/statistically relevant differences between the GM plant and its conventional comparators. Combined with the above remarks, it is unlikely that the traits of the parental GMOs introduced in the hybrid FG72 x A5547-127 GMO have increased the allergenic potential of the plant or have resulted in mutual interactions between the FG72 and A5547-127 events, leading to an increased allergenicity risk.

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
Based on the compositional equivalence (see A.3.3), there is no reason to assume that the genetic modification has affected the nutritional value of food derived from FG72 x A5547-127 soybean.

Comment 2
No questions.

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

Comment 1
Based on the compositional equivalence (see A.3.3), there is no reason to assume that the genetic modification has affected the nutritional value of feed derived from FG72 x A5547-127 soybean.

Comment 2
No questions; no need to carry out further nutritional studies. This holds also for A.6.1.

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 comment.

Comment 2
No questions.

E.2. GENERAL APPROACH OF THE ERA

Comment 1
No comment.

Comment 2
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 comment, no safety concern.

Comment 2
No questions.

E.3.2. PLANT TO MICRO-ORGANISMS GENE TRANSFER

Comment 1
No comment, no safety concern.

Comment 2
No questions.

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

Comment 1
No comment, no safety concern.

Comment 2
Not applicable.

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

Comment 1
No comment, no safety concern.

Comment 2
Not applicable.

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

Comment 1
Herbicide use in the USA on soybean, corn and cotton declined slightly in the first years following introduction of herbicide resistant GM crops, but increased moderately in recent years (Fernandez-Cornejo et al., 2014), whereas Benbrook (2012) reported that herbicide-resistant crop technology has led to a 239 million kg increase in herbicide use in the USA between 1996 and 2011.

FG72 x A5547-127 soybean is not intended for cultivation in the EU. Nevertheless, an indirect effect of the approval of FG72 x A5547-127 soybean is that it may have consequences in countries where its cultivation is allowed. The continued application of the same herbicide in subsequent rotations may lead to increased selection pressure for herbicide resistant weed populations. Furthermore, the continued application of same herbicides may result in an increased accumulation of residues of herbicides and metabolites in plant tissues (Bøhn et al., 2014) and surface water (VMM, 2015). Health concerns with regard to the use of glyphosate (Guyton et al., 2015; Seneff et al., 2015) and glufosinate (Laugeray et al., 2014) have been reported. Food and feed that compromises human and animal health is unacceptable. FAO (2014) reported an increased incidence of adenomas and carcinomas of the liver in male and female rats at an intake of 500 mg/kg body weight per day. In male rats, an increase of thyroid follicular cell adenomas was also observed at 500 mg/kg body weight daily. It is concluded that isoxaflutole is carcinogenic in mice and rats.

Therefore, the application of these herbicides in weed management should meet the restrictions specific to herbicide-treated crops. Herbicide mixing exposes weeds to multiple mechanisms of action, which may delay resistance evolution. However, herbicide mixtures are not a permanent solution to the problem of herbicide resistance, as they do not prevent it on the long run (Evans et al., 2015).

SBB Comment:
The assessment of the use and safety of pesticides is not within the remits of the BAC.

Comment 2
Not relevant (no cultivation in the EU).

Comment 3
Not relevant or applicable.

E.3.6. EFFECTS ON BIOGEOCHEMICAL PROCESSES

Comment 1
No comment, no safety concern.

Comment 2
Not applicable.

E.3.7. EFFECTS ON HUMAN AND ANIMAL HEALTH

Comment 1
No new food or feed safety concerns are expected when stacked transgenes are not expressed in the same tissues or when their products are not translocated to the same tissues (Steiner et al., 2013).

Comment 2
No comment, no safety concern.

Comment 3
No questions.

E.3.8. OVERALL RISK EVALUATION AND CONCLUSIONS

Comment 1
No comment, no safety concern.

Comment 2
No questions.

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.

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