Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2014_0142

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/NL/2007/45 from Pioneer under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/NL/2007/45 was submitted by Pioneer on 18 June 2007 for the marketing of genetically modified soybean 305423 for food and feed uses, import and processing within the framework of Regulation (EC) No. 1829/2003[1]. Soybean 305423 contains a *gm-fad2-1* expression cassette, resulting in a high oleic acid oil profile. The *gm-fad2-1* does not give rise to the formation of a protein, but is designed to suppress the expression of the native *fad2-1* gene. A *gm-hra* gene was also introduced as a selectable marker conferring tolerance to acetolactate synthase (ALS)-inhibiting herbicides.
The application was officially acknowledged by EFSA on 22 October 2007. 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). Six 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 21 January 2008.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 4 December 2013 (EFSA Journal 2013; 11(12):3499[2], and published together with the responses from the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period.

On 7 January 2014 the opinion of EFSA was forwarded to the Belgian experts. They were invited to give comments and to react if needed to the answers given by the EFSA GMO Panel, in particular in case the comments formulated in their initial assessment of the dossier were not taken into account in the opinion of EFSA. The comments formulated by the experts together with the opinion of EFSA including the answers of the EFSA GMO Panel 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://www.efsa.europa.eu/en/efsajournal/pub/3499.htm

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Scientific evaluation

1. Environmental risk assessment

According to the Biosafety Advisory Council no major risks were identified concerning the European environment[3].

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 composition of the GM soybean 305423 shows biologically relevant differences with conventional soybean in its fatty acid profile, as intended: increase in oleic acid at the expense of polyunsaturated fatty acids. But also changes in the levels of odd chain fatty acids (saturated fatty acids) are observed. This unintended effect is likely to be related to the introduction of the ALS enzyme, and is not expected to affect the conclusions on health and nutrition.

The Biosafety Advisory Council also considers that, although not required by the OECD Document on compositional considerations for new varieties of soybean (OECD, 2001), it lacks the analysis on dietary fibre. The Biosafety Advisory Council recommends the analysis on dietary fibre since this concept is widely accepted in human food studies and recommends the adaptation of the OECD consensus document accordingly.

3.2. Assessment of toxicity

In earlier dossiers acute toxicity studies with the GM-HRA protein showed that this protein is not toxic. The applicant provided additional data to substantiate that food and feed derived from GM soybean 30543 that contains the GM-HRA protein is not toxic. These data include in particular results from a subchronic (90-day) toxicity study in rats, two chicken feeding studies and one pig feeding study. The studies confirm that consumption of food and feed derived from GM soybean 30543 at the studied doses does not lead to any signs of toxicity.

Therefore, with regard to toxicity the Biosafety Advisory Council is of the opinion that the information provided is sufficient and does not raise safety concerns.

3.3. Assessment of allergenicity

The potential allergenicity of GM-HRA the only newly expressed proteins in soybean 30543 has been assessed as well as the allergenicity of the whole GM soybean. It is unlikely that the new expressed protein changed the allergenicity of the whole crop.

3.4. Nutritional value

The Biosafety Advisory Council is of the opinion that soybean 305423 does not raise nutritional concerns in the context of the intended use, i.e. commercial frying and spraying. General use is contra-indicated for humans due to the potential decreased intake of linolenic

[3] 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.

acid. The applicant did not provide an estimation of intake via unintended uses but the Council agrees with EFSA that even in case of full replacement of vegetable oils with oil derived from soybean 305423 the changes in the intakes of fatty acids are small and should have no consequences on human health and nutrition.

The results of the feeding studies in chicken and pigs indicate that the soybean is safe and as nutritious as conventional soybean.

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, GM soybean 305423 is unlikely to pose any risk to human and animal health.

Given the scope of the application of this herbicide tolerant soybean with altered fatty-acid profile (no cultivation in EU) and the fact that the establishment of volunteer plants would be unlikely (soybean cannot survive without human assistance), the unintended environmental release of soybean 305423 is unlikely to pose any threat to the environment.

p.o Dr. Philippe HERMAN, SBB

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

Annex I: Full comments of experts in charge of evaluating application EFSA/GMO/NL/2007/45 *(ref. BAC_2008_PT_632)*
Annex II: Comments submitted on the EFSAnet (ref. BAC_2008_PT_633)

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January 2008

Secretariaat
Secrétariat

N./réf. : WIV-ISP/BAC/2008/PT_632
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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/NL/2007/45

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 10 December 2007
Coordinator: René Custers
Experts: Pascal Cadot (Consultant), Armand Christophe (UGent), Eddy Decuypere (KUL), Peter Smet (Consultant), Nancy Terryn (UGent), Michel Van Koninckxloo (HEPHO)
Domains of expertise of experts involved: Genetic engineering, genome analysis, transgene expression, toxicology, immunology, alimentary allergology, human nutrition, biochemistry and analysis of food/feed, animal nutrition, agronomy, crop protection management, agro-ecology, herbicide tolerance, soybean
Secretariat: Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/GMO/NL/2007/45** concerns an application of the company **Pioneer Hi-Bred International** for the marketing of the genetically modified **soybean 305423** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 19 October 2007.
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).

List of comments received from the experts

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

The dossier states that the product described in this application also consists of soybean products from progeny, containing the genetic modification, as derived from conventional breeding between 305423 soybean and traditionally bred soybeans.
Is this generally so? It was my understanding that every trait, when in a new genetic background should be seen as a new event. I believed biosafety works at the whole GM plant level, not just the inserted trait. If the genetic background through breeding would become very different from the one used for obtaining the BC1F5 and F6 generation that have been used for most analysis, it might have to be re-evaluated, since levels of the altered fatty acids might be different per soybean variety. There should be uniform guidelines for this?

In part II summary point 7: " Yes, notifications concerning all uses of 305423 soybean, including cultivation of 305423 soybean seed products, have been submitted in the US and Canada. Applications for authorization to import for all uses of 305423 soybean have also been submitted in Mexico and are being prepared for other countries around the world."
I guess that by now there might be update on this, maybe it would be nice in the future if applicants provide a link maybe to web pages that would give update news on other applications.

Comment 2

305423 soybean contains a gm-fad2-1 gene fragment and the gm-hra gene.

- gm-fad 2-1 gene fragment is part of the coding region for omega-6-desaturase gene 1, but does not code for a functional protein and at the same time suppresses transcription of endogenous omega-6-desaturase, resulting in high oleic acid levels since this fatty acid is normally further transformed by the enzyme in linoleic acid.
- Gm-hra gene encodes for GM-HRA, an acetolactate synthase (ALS), but tolerant for ALS-inhibiting herbicides. The enzyme will catalyse the synthesis of acetolactate from 2 pyruvate, and this is the basis for synthesis of leucine and valine, and will catalyse the synthesis of acetohydroxyl-butyrate from pyruvate and 2-ketobutyrate, and this is the basis for synthesis of isoleucine.

Hence particular attention has to be drawn on the composition of the branched amino acids leucine, isoleucine and valine.

Comment 3

Sufficient

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

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Sufficient

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

No comments

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

Comment 2

Sufficient

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

Comments/Questions of the expert(s)

Comment 1

It is clear that this event is not of the type that we always take as an example of what an elite event should look like, a single copy and no vector backbone. The complex nature of the 4 insertion sites has however been very clearly documented and followed as for their stability. Molecular analysis has been very carefully done. If recombination is to occur and would affect the phenotype the applicant

would suffer from this, so I suppose that they will monitor stability once in a while during years of seed multiplication.

One issue is not clear to me. The four inserts behave like one locus, so they should be more or less close together. However not so close that they could be found on the same cosmid clone. Somehow I feel that it would be easy to include a PFGE Southern with some rare cutting enzymes to have a rough idea on what scale we are taking here. Or even soybean genomic data which I assume the applicants could have access to, could give an idea of what distance is covered by the 4 inserts, thus the whole locus.

Comment 2

No comments

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

In text: " In addition, comparable transcript levels of the endogenous and constitutively expressed *FAD2-2* and *FAD3* genes (Figures 24 and 25, respectively) were observed in leaves of 305423 and non-GM control soybean plants as expected".

According to me FAD2-2 in fig 24 is clearly lower, for figure 28 it is correctly mentioned that it is lower.

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 biologically significant differences compared to non-GM control soybean.

Comment 2

Sufficient

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

Comments/Questions of the expert(s)

Comment 1

See D.2

Comment 2

No comments

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

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Sufficient

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)

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

Sufficient

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

Saponins are present in soy in relatively high quantities (Berhow et al, 2006) and although poorly absorbed in humans (Hu et al., 2004), they can cause bloat in ruminants (Van Haver et al., 2003) and induce enteritis in salmon (Knudsen et al., 2007). **Q: Would it not be indicated to determine the level of saponins in products derived from 305423 soybeans and from regular or near isogenic control soybeans?**

Comment 2

Comparisons are made with non-GM control soybean with comparable genetic background, across different locations, but also comparisons are made with 4 commercial soybean varieties as well as publicly available literature reference data. Attention could also be drawn a bit more on similarities or differences of the comparisons between GM and non-GM soybean in US/Canada on the one hand and Chili/Argentina on the other hand.

In US/Canada comparisons:

- in grain, crude fat, ash and calcium were significantly lower in 305423 soybean compared in non-GM soybean, but within the combined literature range. Oleic acid was much higher and linoleic and linolenic acid much lower in 305423 soybean grain as expected due to variation of the gm-fad2-1 gene fragment; but levels of myristic acid (14:0), palmitic acid (16:0) were lower in 305423 soybean (compared to non-GM soybean as well as to the literature range for these fatty acids), while heptadecanoic acid (17:0), heptadecenoic acid (17:1), arachidic acid (20:0), eicosenoic acid (20:1) and lignoceric acid (24:0) were higher in 305423 soybean compared to non GM. No possible explanation or hypothesis is given why this could be the case; it is just stated that it causes no problem for the consumption of 305423 grain.
- No differences were found for amino acids.
- Vit B1 was significantly lower in 305423 soybean compared to non-GM as well as compared to the literature range of 4 commercial breeds. Also trypsin inhibitor was lower.

In Chili/Argentina comparisons:

- same observations as for fatty acid differences between 305423 soybean and non-GM soybean as found in US/Canada trials, and here also Behenic acid (22:0) was higher in 305423 soybean.
- Same observations also for total fat, ash, calcium and Vit B1 as for the US/Canada trials.

However, here valine, leucine, isoleucine were significantly lower in 305423 soybean compared to non-GM, and these are exactly the branched amino acids that could have been influenced by gm-hra gene. However, no effect of sprayed versus non-sprayed with ALS-inhibiting herbicides was found and levels of amino-acid remained within the literature range.
This striking difference between US/Canada and Chili/Argentina trials may question if possible genotype-environment interactions could occur for the introduced gm-hra gen ???

For the Chili/Argentina trials, the least square mean for crude protein in forage of sprayed 305423 soybean is not significantly different (table 25), the range is between 19.3-25.8 as % dry weight comparable to all other values of non-sprayed 305423 and non-GM soybean, but the mean is 39.9 which is almost double as non-sprayed 305423 or non-GM soybean: I think this must be an error: is it not the mean of grain that is erroneously typed here ??

- Genistin, malonylgenistin, daidzin, malonyl-daidzin, glycitin, malonylglycitin, are outside literature ranges both in US/Canada as in Chili/Argentina trials ? Although no differences between GM and non-GM soybean were found here, the question remains why such values for these isoflavones ? (table 22 & 31). Is this due to this type of soybean used in the trials ? Or is this due to the methodology of measurements used ? Anyway, no remarks are given on such observations. It is stated that they are within their respective tolerance intervals and/or literature ranges, but is this not a bit misleading ? The tolerance intervals are defined as the intervals for 4 commercial soybean varieties containing 99 % of observed values for each analysis; with a broad variance and without any data of skewness or kurtosis of the distribution, almost anything can then lay within such tolerance intervals. Moreover, the sentence "and/or" is in this sense misleading since a lot of these insoflavones do not lay within the literature ranges given.

Therefore, even if the overall conclusion may remain that it causes no problem for the consumption of 305423 grain, a number of points as were raised here could have been described and questioned in a bit more scientific and critical way, e.g. as for isoflavone levels, or e.g. to what happened to the equilibria in the fatty acid synthesis pathways by changing one of the pathways ?

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Sufficient, soybean is not cultivated in Belgium and the 305423 soybean will not be cultivated in the UE.

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Sufficient

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Sufficient.
Comments : this new high oleic phenotype of soybean allows to envisage novel methods of production of a highly stable vegetable oil that is suitable for frying applications without the need for hydrogenation and thus less expensive in energy.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

General comment
An argument given for the production of high oleic acid soy oil is that it can replace trans fats for certain applications. Trans fats have indeed nutritionally less desirable properties. However, this is only part of the truth. High oleic acid soy oil is lower than regular soy oil in alpha-linolenic acid which has beneficial properties and the consumption of which is lower in Belgium and in many European countries than recommended. Thus, from a nutritional point of view, the use of soybean oil from 305423 soy may have advantages when used for certain applications but general use would probably be contra-indicated.

It is stated that the oil derived from soybean will be used primarily to replace commercial frying and as a spray treatment on grain snacks (part I, page 74-75) and based on these presumptions estimations are made on changes in dietary intake of fatty acids in the US (not in Europe) (Part I, page76; Exponent study, Annex 18). **Q1: Are there arguments to expect that oil from 305423 soybean will not be used just as any other soybean oil? If not, what would the impact be on dietary intake of alpha-linolenic acid in European populations? Q2: What is the reason to seek approval for all applications and to give results on expected fatty intake changes assuming limited application?**

Comment 2

In text: "The 305423 soybean and all food, feed and processed products derived from 305423 soybean are expected to replace a portion of similar products from commercial soybean with total consumption of soybean products remaining unchanged. Therefore, the total anticipated intake/extent of use of soybean and all food, feed and processed products derived from soybean is expected to remain the same"

Can this be stated? The 305423 soybean oil is altered to be even better, so maybe more used?
In fact in 7.10.1 this issue is discussed but just as to replace common soybean oil with 305423 soybean oil, not an increase because of it has better qualities?

Comment 3

No comments

Comment 4

Sufficient

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment1

The 305423 soybean has been obtained by introducing the *gm-fad2-1* gene fragment and the *gm-hra* gene into the soybean genome.

The inserted *gm-fad2-1* gene fragment is part of the coding region of the soybean omega-6 desaturase gene 1 (*FAD2-1*) and does not code for a functional protein. Transcription of the *gm-fad2-1* gene fragment in 305423 soybean seeds acts to suppress transcription of endogenous omega-6 desaturase, resulting in the high oleic phenotype.

The *gm-hra* gene encodes the GM-HRA protein, an optimized version of the soybean acetolactate synthase (ALS). Expression of the GM-HRA protein in 305423 soybean confers tolerance to ALS-inhibiting herbicides.

Mean concentrations of GM-HRA protein measured in 305423 soybean.

Growth stage/ Tissue	ng/mg Tissue Dry Weight		Standard deviation
	Mean	Range	
V2/Leaf	3.1	0.99 – 9.0	1.9
V5/Leaf	2.7	0 – 7.7	1.9
R3/Leaf	4.0	1.2 – 6.3	1.8
R3/Forage	5.7	0.78 – 51	12
R3/Root	0.18	0 – 0.63	0.22
R8/Grain	2.5	0 – 4.9	1.1

Comment 2

Overall safety is based on:

- history of safe consumption
- absence of toxicity of the GM-HRA protein and equivalence of microbial-derived GM-HRA protein and 305423 soybean expressed GM-HRA.
- Lack of homology of GM-HRA to known toxins

Absence of adverse effects in a fast growing broiler study.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment1

The only newly expressed protein in 305423 soybean is the GM-HRA protein.

a) Degradation of the GM-HRA protein in simulated gastric fluid (author).

No data are provided. How fast and to what extend is the GM-HRA protein broken down in SGF. What kind of fragments are formed?

b) Degradation of the GM-HRA protein in simulated intestinal fluid (author).

No data are provided. How fast and to what extend is the GM-HRA protein being digested in SIF. What kind of fragments are formed?

c) Equivalency assessment of the GM-HRA protein derived from a microbial expression system with the GM-HRA protein derived from 305423 soybeans (Buffington, 2006).

Since a microbially-derived GM-HRA protein was used for acute toxicity testing in mice, equivalence between the microbially and soybean derived proteins must be demonstrated.
The results of this study indicate that the GM-HRA protein derived from a microbial expression system was equivalent to the GM-HRA protein derived from the 305423 soybean leaf tissue.
Therefore, GM-HRA protein derived from a microbial expression system is appropriate for utilization in safety assessment studies as a proxy for the GM-HRA protein contained in soybean plants.

d) GM-HRA: Acute Oral Toxicity Study in Mice (Finlay, 2006).

A single dose of GM-HRA test substance in water was administered by oral gavage to groups of 5 fasted male and 5 fasted female Crl:CD(ICR) mice at a dose of 2000 mg/kg. This corresponded to a per-animal exposure of at least 436, but less than 582, mg/kg recombinant GM-HRA protein. Two control groups, each consisting of 5 fasted male and 5 fasted female mice, were administered bovine serum albumin at a dose of 2000 mg/kg in water, or vehicle (water) alone, once by oral gavage. The mice were observed for mortality, body weight effects, and clinical signs for 14 days after dosing. The mice were sacrificed and given a complete gross pathology examination to detect grossly observable evidence of organ or tissue damage or dysfunction.
All mice survived until the scheduled sacrifice on Day 14. No clinical signs of systemic toxicity or test substance-related body weight losses were observed in any mice. No gross lesions were present in the mice at necropsy.
Under the conditions of this study, administration of recombinant GM-HRA test substance to male and female mice at a dose of 2000 mg/kg produced no test substance-related clinical signs of toxicity, body weight losses, gross lesions, or mortality.

Comment 2

Toxicological feeding studies are done with microbially derived GM-HRA. It is claimed that this test substance is equivalent with GM-HRA expressed in 305423 soy. Yet, MALDI-TOF MS analysis shows that there are peptide fragments in the plant-derived GM-HRA which are lacking in microbially derived GM-HRA and vice-versa (Part I, page 186, Table 18). **Q: Is this finding not an indication that both proteins are not identical? If so, how convincing is it that the safety results obtained with this protein are a good substitute to evaluate the safety of the novel expressed protein in 305423 soy?**
It is mentioned several times in Part I (e.g. page 63) that the only newly expressed protein in 305423 soybean is the GM-HRA protein.
Yet, evidence is presented that there are 27 new open reading frames in soy 305423 which are translationally competent (Annex 3, page 13). This may possibly result in novel proteins. The safety of such putative proteins was evaluated by sequence comparison with toxic and allergen data sets. Although he results indicate that allergic and toxicological problems are very unlikely, this was not substantiated by acute oral toxicity tests. Of course, such tests are superfluous when these putative novel proteins are not expressed. **Q: Are there data to show that these putative proteins are not expressed?**
It is mentioned several times (e.g. Part I, page 84) that the *gm-fad2-1* gene fragment does not code for a functional protein**. Q: does this inserted gene fragment code for a non-functional protein? If so,**

is this protein, which is a fragment of a larger natural protein, to be considered as a novel protein?

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

However, more changes occur than just the ones mentioned here, at least if these are accepted as changes (see discussion under D 7.3). Although I think these are of no concern perhaps for the use of 305423 soybean as natural food and feed constituents, but they have to be considered and taken into account.

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment1

42-day poultry feeding study (Delaney and Smith, 2007)

Diets produced with soybean fractions from non-transgenic near-isoline (control), 305423 (test), and non-transgenic commercial reference varieties (93B86, 93B15, and 93M40) were fed to Ross x Cobb broilers (n = 120/group, 50% male and 50% female) for a period of 42 days.
No statistically significant differences were observed in mortality, weight gain, mortality adjusted feed efficiency, and carcass yields between broilers consuming diets produced with 305423 soybean fractions and those consuming diets produced with near isoline control soybean fractions.
Based on the results from this study, it was concluded that 305423 soybean was nutritionally equivalent to non-transgenic control soybean with a comparable genetic background.

Question
All samples of the 305423 test Starter and Finisher diets were negative for the MON-Ø4Ø32-6 event. However, six of the 12 PCR reactions from the 305423 test Grower diet were weakly positive for event MON-Ø4Ø32-6, indicating a very low level of the MON-Ø4Ø32-6 event in this diet. **Where does this contamination come from? Why is only the Grower diet being contaminated?**

Comment 2

Although no differences in performances or carcass characteristics in the broiler study were found, composition of the broiler fat, as well as softness of the fat would have been worthwhile information. It is well known that whole soybean included in poultry diets, depending on the level, can affect poultry fat composition ("oily birds syndrome") and since the soybean fat composition has been drastically altered in 305423 soybean grain, it could affect poultry fat composition and texture.

Comment 3

Sufficient

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Assessment of allergenicity of the introduced traits.
The reviewer agrees with the conclusion of the applicant when it is said that, according to current knowledge, GM-HRA is not likely to be allergenic.

Assessment of allergenicity of the whole GM plant.
For this evaluation, the number of soybean-sensitive sera that have been used is too limited. Given the high prevalence of soy allergy, additional sera can easily be found. It is recommended that at least 20 sera be used, in order to get a broader range of reactivity patterns. In addition, the sera must not be pooled, as some information (for example the visualisation of the emergence of an allergen for some patients) might be diluted and lost in a pool. Therefore, one cannot conclude, at the moment, whether the allergenicity of 305423 soybean is similar to that of control Jack soybean.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

Soy lecithin is one of the secondary products of processed soybean. No data are given on the fatty acid composition of lecithin derived from 305423 soybeans which is possibly (and probably) also affected. If so, this may affect the possibility of soy phospholipids to improve the lipid profile favorably (Evans et al., 2007)**. Q: Are data available on the fatty acid composition of phospholipids derived from 305423 soybeans?**

Comment 2

From summary: " A study was conducted to evaluate the changes in dietary fatty acids provided in the diet when commercial soybean frying oils as they exist in the marketplace today (*i.e.* baseline scenario) are substituted with oil from 305423 soybean (*i.e.* high oleic scenario) and to consider the nutritional implications of these changes.
Based on this assessment it was concluded that no changes are made in the overall quality of the fatty acid composition of the diet that are nutritionally or biologically meaningful when using oil from 305423 soybean".

Not very right, this oil will be better for some uses, so might be more used then soybean oil is today in these frying oils.

Comment 3

1) See earlier remarks on compositional differences as discussed under D.7.3

2) estimated changes for mean fatty acid intake for baseline and 305423 soybean in perspective to soybean oil dietary fatty acid intake is a lot when expressed as a % for C17:1, C17:0 or C18:2. What it means as a % change to the total dietary fatty acid intake for these fatty acids cannot be derived from this study. Therefore, the conclusion on p. 76 of part I that the greatest change in nutrient intake for all populations was that for which the modification in 305423 soybean oil was made: oleic acid, is correct in absolute amounts yes, but not necessarily as a % change. The question is what is most important here; and also whether it is significant from a nutritional point of view (even doubtful that it means a lot, but it has to be questioned). Also, nothing is mentioned anymore for the other fatty acid differences, C22:0, C24:0; this is also not mentioned anymore in fatty acid profile of table 39. Therefore, the conclusion on p. 77 of part I is a bit premature to me.

Comment 4

Sufficient

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

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Sufficient

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

Comments/Questions of the expert(s)

Comment 1

Not applicable

Comment 2

Not applicable

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)

Comment 1

However and regardless of these routes of dissemination, soybean seeds cannot survive without human assistance in non-agricultural environments in the EU.

Comment 2

No comments

Comment 3

Sufficient
Comments : Soybean is a non-invasive plant in Belgium (and non frost tolerant).

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Sufficient

Comments : release of 305423 soybean will be confined to unintended grain spillage during import, storage and processing. The specific advantages introduced by the genetic modification do not confer any selective advantage to the plants in the natural Belgian environment.

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Sufficient

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Not applicable

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

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Not applicable

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1

An argument given for the production of high oleic acid soy oil is that it can replace trans fats for certain applications. Trans fats have indeed nutritionally less desirable properties. However, this is

only part of the truth. High oleic acid soy oil is lower than regular soy oil in alpha-linolenic acid which has beneficial properties and the consumption of which is lower in Belgium and in many European countries than recommended. Thus, from a nutritional point of view, the use of soybean oil from 305423 soy may have advantages when used for certain applications but general use would probably be contra-indicated.

Comment 2

No comments , but see also remark under D.7.3.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

No comments

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

Comment 1

No comments

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

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Sufficient
Comments: Soybean is not cultivated in Belgium.

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

Comment 1

No comments

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Sufficient

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

Comment 1

Sufficient

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

Comment 1

Sufficient

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

Comments/Questions of the expert(s)

Comment 1

Sufficient

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

Comment 1

Sufficient

References

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Bioveiligheidsraad
Conseil de Biosécurité

21-01-2008

Secretariaat
Secrétariat

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Application EFSA/GMO/NL/2007/45
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 10 December 2007
Coordinator: René Custers
Experts: Pascal Cadot (Consultant), Armand Christophe (UGent), Eddy Decuypere (KUL), Peter Smet (Consultant), Nancy Terryn (UGent), Michel Van Koninckxloo (HEPHO)
Domains of expertise of experts involved: Genetic engineering, genome analysis, transgene expression, toxicology, immunology, alimentary allergology, human nutrition, biochemistry and analysis of food/feed, animal nutrition, agronomy, crop protection management, agro-ecology, herbicide tolerance, soybean
Secretariat: Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman

INTRODUCTION

Dossier **EFSA/GMO/NL/2007/45** concerns an application of the company **Pioneer Hi-Bred International** for the marketing of the genetically modified **soybean 305423** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 19 October 2007.
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)

Comments posted on the EFSAnet

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). Below you will find the comments as they were forwarded to the EFSAnet, and in a separate document the compilation of all the comments that were given by the experts (including the references). Only those comments that raised a question or a concern were forwarded to the EFSAnet. The fact that comments that did not raise a question or a concern were not forwarded to the EFSAnet does not diminish the value of these comments. They are absolutely necessary for the complete analysis of the dossier, and will be used in formulating the final advice by the Biosafety Advisory Council.

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

As the genetic modification involves the introduction of a herbicide tolerant acetolactate synthase (ALS) which is involved in the synthesis of the branched amino-acids leucine, isoleucine and valine, particular attention has to be drawn on the composition of these branched amino acids .

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

The information provided in this part of the dossier is regarded adequate.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

The information provided in this part of the dossier is regarded adequate.

D. INFORMATION RELATING TO THE GM PLANT

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

The information provided in this part of the dossier is regarded adequate.

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

Comments/Questions of the expert(s)

There are four insertion sites that have a rather complex nature. These sites have been well characterized on the molecular level and have been followed for their stability. The four insertion

sites behave like one locus, which means that on the genomic level, they are quite close together. But they are not so close together that they can be found on one and the same cosmid clone. The remark has been made that more detailed information on the largest distance between the insertion loci could give a more precise estimation of the chances that the insertion loci would segregate.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

According to one of our experts it is not completely correct to state:
" In addition, comparable transcript levels of the endogenous and constitutively expressed *FAD2-2* and *FAD3* genes (Figures 24 and 25, respectively) were observed in leaves of 305423 and non-GM control soybean plants as expected". Like for figure 28 it should be stated that the expression of the endogenous *FAD2-2* gene is somewhat lower in 305423 than in non-GM control soybean plants.

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

The information given in this part of the dossier is regarded adequate.

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

See earlier comment under D.2

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

The information provided in this part of the dossier is regarded adequate.

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

The information provided in this part of the dossier is regarded adequate.

D.7.2 Production of material for comparative assessment

The information provided in this part of the dossier is regarded adequate.

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Although the OECD consensus document on "Compositional considerations for new varieties of soybean: key food and feed nutrients and antinutrients" does not prescribe the analysis of saponins, one expert has suggested to include saponins in the compositional analysis as they are present in soy in relatively high quantities (Berhow et al, 2006) and although poorly absorbed in humans (Hu et al., 2004), they can cause bloat in ruminants (Van Haver et al., 2003) and induce enteritis in salmon (Knudsen et al., 2007).

Comparisons are made with non-GM control soybean with comparable genetic background, across different locations, but also comparisons are made with 4 commercial soybean varieties as well as publicly available literature reference data. Attention could also be drawn a bit more on similarities or differences of the comparisons between GM and non-GM soybean in US/Canada on the one hand and Chili/Argentina on the other hand.

In US/Canada comparisons:

- in grain, crude fat, ash and calcium were significantly lower in 305423 soybean compared in non-GM soybean, but within the combined literature range. Oleic acid was much higher and linoleic and linolenic acid much lower in 305423 soybean grain as expected due to variation of the gm-fad2-1 gene fragment; but levels of myristic acid (14:0), palmitic acid (16:0) were lower in 305423 soybean (compared to non-GM soybean as well as to the literature range for these fatty acids), while heptadecanoic acid (17:0), heptadecenoic acid (17:1), arachidic acid (20:0), eicosenoic acid (20:1) and lignoceric acid (24:0) were higher in 305423 soybean compared to non GM. No possible explanation or hypothesis is given why this could be the case; it is just stated that it causes no problem for the consumption of 305423 grain.
- No differences were found for amino acids.
- Vit B1 was significantly lower in 305423 soybean compared to non-GM as well as compared to the literature range of 4 commercial breeds. Also trypsin inhibitor was lower.

In Chili/Argentina comparisons:

- same observations as for fatty acid differences between 305423 soybean and non-GM soybean as found in US/Canada trials, and here also Behenic acid (22:0) was higher in 305423 soybean.
- Same observations also for total fat, ash, calcium and Vit B1 as for the US/Canada trials.

However, here valine, leucine, isoleucine were significantly lower in 305423 soybean compared to non-GM, and these are exactly the branched amino acids that could have been influenced by gm-hra gene. However, no effect of sprayed versus non-sprayed with ALS-inhibiting herbicides was found and levels of amino-acid remained within the literature range.
This striking difference between US/Canada and Chili/Argentina trials may question if possible genotype-environment interactions could occur for the introduced gm-hra gen ???
For the Chili/Argentina trials, the least square mean for crude protein in forage of sprayed 305423 soybean is not significantly different (table 25), the range is between 19.3-25.8 as % dry weight comparable to all other values of non-sprayed 305423 and non-GM soybean, but the mean is 39.9 which is almost double as non-sprayed 305423 or non-GM soybean: I think this must be an error: is it not the mean of grain that is erroneously typed here ??

- Genistin, malonylgenistin, daidzin, malonyl-daidzin, glycitin, malonylglycitin, are outside literature ranges both in US/Canada as in Chili/Argentina trials ? Although no differences

between GM and non-GM soybean were found here, the question remains why such values for these isoflavones ? (table 22 & 31). Is this due to this type of soybean used in the trials ? Or is this due to the methodology of measurements used ? Anyway, no remarks are given on such observations. It is stated that they are within their respective tolerance intervals and/or literature ranges, but is this not a bit misleading ? The tolerance intervals are defined as the intervals for 4 commercial soybean varieties containing 99 % of observed values for each analysis; with a broad variance and without any data of skewness or kurtosis of the distribution, almost anything can then lay within such tolerance intervals. Moreover, the sentence "and/or" is in this sense misleading since a lot of these insoflavones do not lay within the literature ranges given.

Therefore, even if the overall conclusion may remain that it causes no problem for the consumption of 305423 grain, a number of points as were raised here could have been described and questioned in a bit more scientific and critical way, e.g. as for isoflavone levels, or e.g. to what happened to the equilibria in the fatty acid synthesis pathways by changing one of the pathways ?

D.7.4 Agronomic traits

The information provided in this part of the dossier is regarded adequate.

D.7.5 Product specification

The information provided in this part of the dossier is regarded adequate.

D.7.6 Effect of processing

The information provided in this part of the dossier is regarded adequate.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

It is argued that high oleic soy oil can replace trans fats for certain applications. Trans fats have indeed nutritionally less desirable properties. However, this is only part of the truth. High oleic acid soy oil is lower than regular soy oil in alpha-linolenic acid which has beneficial properties and the consumption of which is lower in Belgium and in many European countries than recommended. Thus, from a nutritional point of view, the use of soybean oil from 305423 soy may have advantages when used for certain applications but general use would probably be contra-indicated. The applicant however seeks approval for all applications, most probably because it cannot exclude the use of the soy oil in applications other than frying and spray treatments. If indeed the 305423 soy oil will also be used as any other soy oil, what would then be the impact on the dietary intake of alpha-linoleic acid in European populations?

D.7.8 Toxicology

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

It is remarked that in Part I of the dossier it is mentioned several times (e.g. page 63) that the only newly expressed protein in 305423 soybean is the GM-HRA protein. We find this a rather blunt statement, as it has not been checked whether one or more of the 27 new putative open reading frames caused by the genetic modification produce any peptide/protein. We do realize that according to the EFSA guidelines in this case it is not a requirement to check for protein production from these open reading frames, as there is nothing to suggest that any of the possible peptides/proteins would have any toxic or allergenic effect.

D.7.8.2 Testing of new constituents other than proteins

The information provided in this part of the dossier was regarded adequate.

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

It is remarked that more changes occur than just the ones mentioned in this part of the dossier, at least if these are accepted as changes (see discussion under D 7.3). Although these other changes may be of no concern for the use of 305423 soybean as natural food and feed constituents, they have to be considered and taken into account.

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Although no differences in performances or carcass characteristics in the broiler study were found, composition of the broiler fat, as well as softness of the fat would have been worthwhile information. It is well known that whole soybean included in poultry diets, depending on the level, can affect poultry fat composition ("oily birds syndrome") and since the soybean fat composition has been drastically altered in 305423 soybean grain, it could affect poultry fat composition and texture.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Assessment of allergenicity of the whole GM plant.
For this evaluation, the number of soybean-sensitive sera that have been used is too limited. Given the high prevalence of soy allergy, additional sera can easily be found. It is recommended that at least 20 sera be used, in order to get a broader range of reactivity patterns. In addition, the sera must not be pooled, as some information (for example the visualisation of the emergence of an allergen for some patients) might be diluted and lost in a pool. Therefore, one cannot conclude, at the moment, whether the allergenicity of 305423 soybean is similar to that of control Jack soybean.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Soy lecithin is one of the secondary products of processed soybean. No data are given on the fatty acid composition of lecithin derived from 305423 soybeans which is possibly (and probably) also affected. If so, this may affect the possibility of soy phospholipids to improve the lipid profile favorably (Evans et al., 2007)**.** Are data available on the fatty acid composition of phospholipids derived from 305423 soybeans?

See earlier remarks on compositional differences as discussed under D.7.3

It is remarked that the estimated changes for mean fatty acid intake for baseline and 305423 soybean in perspective to soybean oil dietary fatty acid intake is a lot when expressed as a % for C17:1, C17:0 or C18:2. What it means as a % change to the total dietary fatty acid intake for these fatty acids cannot be derived from this study. Therefore, the conclusion on p. 76 of part I that the greatest change in nutrient intake for all populations was that for which the modification in 305423 soybean oil was made: oleic acid, is correct in absolute amounts yes, but not necessarily as a % change. The question is what is most important here; and also whether it is significant from a nutritional point of view (even doubtful that it means a lot, but it has to be questioned). Also, nothing is mentioned anymore for the other fatty acid differences, C22:0, C24:0; this is also not mentioned anymore in fatty acid profile of table 39. Therefore, the conclusion on p. 77 of part I may be a bit premature.

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

The information provided in this part of the dossier is regarded adequate.

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

Not applicable

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

The information provided in this part of the dossier is regarded adequate.

D.9.2 Selective advantage or disadvantage

The information provided in this part of the dossier is regarded adequate.

D.9.3 Potential for gene transfer

The information provided in this part of the dossier is regarded adequate.

D.9.4 Interactions between the GM plant and target organism

The information provided in this part of the dossier is regarded adequate.

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

The information provided in this part of the dossier is regarded adequate.

D.9.6 Effects on human health

Comments/Questions of the expert(s)

See earlier comments under D.7.3. and D.7.7.

D.9.7 Effects on animal health

The information provided in this part of the dossier is regarded adequate.

D.9.8 Effects on biogeochemical processes

The information provided in this part of the dossier is regarded adequate.

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

The information provided in this part of the dossier is regarded adequate.

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

The information provided in this part of the dossier is regarded adequate.

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

The information provided in this part of the dossier is regarded adequate.

D.11.2 Interplay between environmental risk assessment and monitoring

The information provided in this part of the dossier is regarded adequate.

D.11.3 Case-specific GM plant monitoring

The information provided in this part of the dossier is regarded adequate.

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

The information provided in this part of the dossier is regarded adequate.

D.11.5 Reporting the results of monitoring

The information provided in this part of the dossier is regarded adequate.

References
see document BAC_2008_PT_633 in annex