Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2014_0427

Title: Advice of the Belgian Biosafety Advisory Council on application EFSA/GMO/UK/2009/76 from Monsanto under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/UK/2009/76 was submitted by Monsanto on 20 October 2009 within the framework of Regulation (EC) No. 1829/2003[1] for authorisation of genetically modified (GM) soybean MON 87769 for import and processing, and for food and feed uses.

Soybean MON 87769 was obtained through Agrobacterium-mediated transformation of conventional soybean. The introduction of two desaturase genes, Primula juliae Δ6 desaturase (Pj.D6D) and Neurospora crassa Δ15 desaturase (Nc.Fad3), results in the seedspecific production of the PjΔ6D and NcΔ15D proteins. These proteins desaturate certain endogenous fatty acids resulting in the production of SDA at approximately 20-30% of total fatty acids.

The application was officially acknowledged by EFSA on 15 February 2010. 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 GM organisms being part of the products).

Within the framework of this consultation, the Belgian Biosafety Advisory Council (BAC), under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier, chosen from the common list of experts drawn up by the BAC and the Biosafety and Biotechnology Unit (SBB). Eight experts answered positively to this request, and formulated a number of comments to the dossier, which were edited by the coordinator. See Annex I for an overview of all the comments and the list of comments actually placed on the EFSAnet on 12 May 2010.

The opinion of the EFSA GMO Panel was adopted on 9 April 2014 and published on 16 May 2014 (EFSA Journal 2014; 12(5):3644[2]). The responses from the Panel to comments submitted by the experts during the three-month consultation period were made available on 21 May 2014.

On 4 June 2014 the EFSA opinion and the responses from the EFSA GMO Panel were forwarded to the Belgian experts. They were invited to give comments and to react if needed to the answers given by the 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 EFSA opinion 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/fr/efsajournal/pub/3644.htm

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 Biosafety Advisory Council is of the opinion that the compositional analysis of GM soybean MON 87769 does not raise safety concerns. It shows that GM soybean MON 87769 differs from its conventional counterpart in its fatty acid composition in seeds, as expected, and that there are few additional significant differences that are well within the range of amounts of those substances present in conventional reference varieties.

The Biosafety Advisory Council would however like to raise the following comments on the compositional analysis with the aim to further improve future analyses:

1. It considers that even if the compositional analysis of the GM food/feed was performed according to the OECD consensus document, it lacks the analysis on dietary fibre. The majority of the Biosafety Advisory Council members recommend 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.

2. The applicant only provided data for vitamin E. It is generally recognised that soybean is an important source of vitamins in the human diet, in particular vitamin E and vitamin K. The Biosafety Advisory Council notes that in the revised version of the OECD Consensus Document on Compositional Considerations for New Varieties of soybean, Vitamin K is also listed as suggested constituent to be analysed related to food use.

3.2. Assessment of toxicity

With respect to toxicity, the Biosafety Advisory Council did not identify any safety concerns.

3.3. Assessment of allergenicity

The Biosafety Advisory Council is of the opinion that there are no indications that GM soybean MON 87769 would be more allergenic than its conventional counterpart.

[3] Since this application does not imply a cultivation of the GM crop in the EU, a full environmental assessment is not required in EFSA procedure and was not achieved.

3.4. Nutritional value

The Biosafety Advisory Council is of the opinion that the information provided is sufficient and does not raise any concerns.

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 EFSA opinion, the answers of the EFSA GMO Panel to the questions raised by the Belgian experts, the answers of the applicant to the questions of the EFSA GMO Panel and considering the data presently available, the Biosafety Advisory Council is of the opinion that in the context of its intended uses, GM soybean MON 87769 is unlikely to pose any risk to human and animal health.

Given the scope of the application of this GM soybean (no cultivation in EU) and the fact that the establishment of volunteer plants would be unlikely (soybean cannot survive without human assistance and is not capable of surviving as a weed in Europe), the potential environmental release of soybean MON 87769 is unlikely to pose any threat to the European environment.

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

Annex I: Compilation of comments of experts in charge of evaluating application EFSA/GMO/UK/2009/76 and comments submitted on the EFSAnet (ref. BAC_2010_0397)

Bioveiligheidsraad
Conseil de Biosécurité

12-05-2010

Secretariaat
Secrétariat

N./réf. : WIV-ISP/41/BAC_2010_0397
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Compilation of comments of experts in charge of evaluating the application EFSA/GMO/UK/2009/76
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 9 March 2010
Coordinator: René Custers
Experts: Armand Christophe (UGent), Johan Claes (KH Kempen), Leo Fiems (ILVO), Lieve Gheysen (UGent), Peter Smet (Consultant), Wim Stevens (UA), Frank Van Breusegem (VIB), Johan Van Waes (ILVO)
Domains of expertise of experts involved: Molecular characterisation, genome analysis, genetic engineering, transgene expression, DNA/RNA/protein analysis, human & animal nutrition, substantial equivalence of alimentary products, analysis food/feed, industrial processing, fats and oils, Immunology, alimentary allergology, toxicology, agronomy, ecology, breeding techniques, biosafety, post-release monitoring, soybean
Secretariat (SBB): Didier Breyer, Adinda De Schrijver, Martine Goossens, Philippe Herman, Katia Pauwels

INTRODUCTION

Dossier **EFSA/GMO/NL/2009/76** concerns an application of the company **Monsanto** for the marketing of the genetically modified **Soybean MON87769** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 15 February 2010.
The scope of the application is:
☒ GM plants for food use
☒ Food containing or consisting of GM plants
☒ Food produced from GM plants or containing ingredients produced from GM plants
☒ GM plants for feed use
☒ Feed produced from GM plants
☒ Import and processing (Part C of Directive 2001/18/EC)
☐ Seeds and plant propagating material for cultivation in European Union (Part C of Directive 2001/18/EC)

Depending on their expertise, the experts were asked to evaluate the genetically modified plant considered in the application on its 1) molecular, 2) environmental, 3) allergenicity, 4) toxicity and/or 5) food and feed aspects. It was expected that the expert should evaluate if the information provided in the application is sufficient in order to state that the marketing of the genetically modified plant for its intended uses, will not raise any problems for the environment or human or animal health. If information is lacking, the expert was asked to indicate which information should be provided and what the scientifically reasoning is behind this demand.

The comments are structured as in the "Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed" (EFSA Journal (2004), 99, 1-94). Items are left blank when no comments have been received either because the expert(s) focused on other related aspects, or because for this dossier the panel of experts who accepted to evaluate the dossier didn't have the needed expertise to review this part of the dossier.
It should be noted that all the comments received from the experts are considered in the evaluation of this dossier and in formulating the final advice of the Biosafety Advisory Council. Comments placed on the EFSAnet are indicated in grey.

List of comments received from the experts

GENERAL COMMENTS

Comments/Questions of the expert(s)

Comment 1

According to the dossier the scope of application does not include the authorization for the cultivation of MON87769 soybean products in the EU. It can however be valuable to give some remarks on the different topics, dealing with cultivation and survivability of seeds, in the case that the applicant should ask in the near future for an extension for the scope of cultivation, especially for cultivation in some southern European countries.
So as agronomical expert I will also give some comments in this questionnaire, related to cultivation and the environmental aspect.
Remark SBB, agreed by the coordinator
Comments related to cultivation of this GMO could indeed be useful for information purpose, but should not be communicated to EFSA as they are out of scope for the risk assessment of the current application.

Comment 2

Minor comment: The reason given why alpha-linolenic acid (ALA) is an essential fatty acid (Part I, page 38) is not correct.
Additional comment from expert
It concerns the exact definition of essential fatty acid but has no further importance.
Comment SBB
This comment has nevertheless been sent to EFSA, as "nice to know" information.

Comment 3

The dossier is, in general, well-established and supports the approval of soybean MON87769 for use in human food and animal feed. Some comments can be made on specific topics. These are discussed below.

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

No questions nor comments

Comment 3

Information adequate: no comments

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

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Under "3. Survivability – ability to form structures for survival or dormancy" it is mentioned that it is not likely that soybean seed would overwinter and germinate the following spring. My question is : are there data available of overwintering of seed of soybean for example in Southern Europe and in that case how were the volunteers be destroyed?
Remark SBB, agreed by coordinator
Suggestion not to forward this comment to EFSA, because it is not relevant in the context of the current application (no cultivation). Same comment in a previous soybean dossier (2009/73) has not been sent to EFSA. Moreover, it should be noted that, for commercial reasons, MON87769 will be processed in dedicated facilities and therefore it is not expected that significant quantities of this GM soybean will commingle with general soybean supply in the EU.

Comment 3

Information adequate: no comments

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)io

Comment 1

No comments

Comment 2

Information adequate: 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

Information adequate: no comments

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

Comments/Questions of the expert(s)

Comment 1

The southern blot analyses have been done in detail and give clear results. I only have some minor comments. Page 54 and table 5 mention “expected size” of fragments such as the 4.2 kb one. However, according to figure 9, the *Psh*AI recognition site is outside the T-DNA, in the flanking soybean DNA. Unless the sequence of the flanking DNA was already available, this recognition site cannot be predicted.
It is nowhere mentioned how the flanking DNA was obtained and identified. On page 68, it is described that it was amplified using primers of the flanking soybean DNA, but first the sequence of these primers needs to be known.
On page 55, the higher than expected size is explained by higher salt concentration, but part of the discrepancy is obviously also due to the ‘reverse smiling’ of the gel.
Page 64: “Although a band at 2.0 kb that represents the Right Border sequence in the insert was also expected, this band was not visible in the Figure 18. This band was not observed in the reported exposure. The low hybridisation signal observed is expected”. This sentence refers to the observation that a 43 bp homology is not sufficient to give a signal that is strong enough to be observed with the exposure shown. I wonder if a longer exposure does reveal this fragment, and if these longer exposures then do not show any additional fragments. I realize that a longer exposure will also show higher background and result in a not so nice picture, but it would be useful to mention the results of this longer exposure. This to avoid possible later problems with discovery of other construct fragments inserted as has been the case with the first Roundup-Ready soybean event that was deregulated (Windels et al., 2001 and http://www.food.gov.uk/multimedia/pdfs/monsantosumcharater.pdf). As this transformation was done with Agrobacterium, in contrast to the RR soybean, it is less likely that additional small pieces of the vector are integrated in the soybean genome, but it cannot be excluded.

Comment 2

Information adequate: no comments

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 2

Information adequate: no comments

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

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Information adequate: no comments

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

Comments/Questions of the expert(s)

Comment 1

No comments

Comment 2

Information adequate: no comments

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

Comments/Questions of the expert(s)

Comment 1

No comments

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

D.7.1 Comparative assessment

Comments/Questions of the expert(s)

Comment 1

Through the introduction of two desaturase enzymes, the fatty acid composition of MON87769 has been intentionally altered to produce the omega-3 fatty acid, SDA. Given this shift in fatty acid metabolism, the fatty acid profile in MON87769 seed was expected to differ from conventional soybean. The compositional analyses confirmed that MON87769 harvested seed had the expected change in fatty acid composition, while the other components analysed in MON87769 seed were compositionally equivalent to conventional soybean seed.

Analysis of forage showed no significant differences between MON87769 and conventional control.

Comment 2

1) Although vitamin E is increased in processed soybean oil it is possible, based on the considerable differences in degree of unsaturation, that the oxidative stability of the GMO soybean oil may be lower than that of regular soybean oil. It would be of interest if data on oxidative stability were given.

2) It is likely that more than one trans-isomer will be formed from ALA and stearidonic acid (SDA) on processing. This is especially true when an oil is bleached (Loï C et al, 1998; Martin et al.; 2006) or heated (Chardigny et al.; 1996) but not reported for such an oil derived from MON87769 (Part I, Table 30). Yet the confidential report MSL 00221116 mentions the presence of other trans-isomers of alpha-linolenic acid (ALA) (MSL 00221116 page 70; page 159).
The isomer composition is not without of importance. For instance, it has been shown that the retina incorporates trans-isomers of docosaenoic acid acid (DHA) after consuming trans-isomers of ALA (Acar N. et al.; 2006); different trans-isomers of eicosapentaenoic acid, which may be formed from different trans-isomers of ALA and SDA, have different pathophysiological effects (Loï C et al, 1998) and so on.

3) The fatty acid composition of phospholipids is not reported. From the data given on “crude lecithin” it is clear that the preparation must contain a lot of triglycerides. Indeed, the fatty acid composition of soybean phospholipids is quite different from that of soybean oil (Dasgupta et al., 2009). This was not found in “crude lecithin” of either the control or the GMO oil (report MSL 00221116, page 93 - 101).
The fatty acid composition of soy lecithin is of importance as the latter is used in infant formula and sold as supplements (e.g. Jorissen et al., 2002) and because soy phospholipids containing polyene fatty acids as probably those of MON87769 (no data given; the introduced genes code for desaturases which use phosphatidylcholine linked fatty acids as substrates; MLS-19874 report, page 9) have been described as having special nutritional effects (e.g. Ristic et al., 2006). Phospholipids enriched in SDA, if they occur as expected in MON87796 “crude” lecithin (no data given), may result in increased levels of such phospholipids in blood and tissues when consumed. Such phospholipids may be prone to oxidation. The biological activities of oxidized phospholipids have been reviewed (Bochkov et al., 2010). Recently, soy lecithin has also been used to improve the productive and

reproductive performance of hens, (Attia et al., 2009), and when fed to cattle to change the fatty acid composition of milk (Gaby, 2009).

Questions

1) Are there data on the identity of the different 18:3 omega3 trans-isomers in RBD oil derived from MON87769 soybean?
2) Has it been considered that the differences between the control and MON87769 soybean in amino-acids (differentially increased) and protein levels (increased) **considered together** may point to differences in protein composition?
3) Are there data on the oxidative stability of oil derived from MON87769 soybean?

Comment 3

For the comparative assessment, five sites from 2006 and five from 2007 are selected. Although 10 sites are sufficient for the comparative assessment, it is not clear from the dossier, how these sites are selected out of eight sites from each year. The dossier would be stronger if the selection of the sites is discussed as well.

In many tables that discuss the comparative assessment (Tables 19-22, Tables 25-28), only the statistical differences are shown. Showing all data (and not only for the components that are different) would allow an easier evaluation.

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

No comments

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

1) The selection of the material and the compounds for analysis is logical.

2) As pointed out in previous evaluation reports, it is suggested that saponins would be included in the compositional analysis of soybean. Indeed, saponins are present in soy in relatively high quantities (Berhow et al., 2003) 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). Soya

saponins (Ali et al., 2009; Zhang et al., 2010) and sapogenols, obtained by hydrolysis of saponins, clearly have important biological effects (e.g. Zhang et al., 2008).
Remark SBB, agreed by coordinator
For consistency with previous dossiers we suggest to send the comment concerning saponins preceded with the following sentence :
"Although the OECD consensus document on "Compositional considerations for new varieties of soybean: key food and feed nutrients and anti-nutrients" does not prescribe the analysis of saponins, one expert has suggested to include saponins in the compositional analysis."

3) As phytosterols and phytosterol glycosides are bioactive in humans (and because soy phytosterols are used in food applications; Jones et al.; 1997) it has been suggested that they should be analysed (Lin et al. , 2009)

Comment 2

No comments

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

It is mentioned that from field trials MON87769 soybean is equivalent to the traditional soybean, except for the introduced trait.
From this information we can conclude that there is no significant difference in the agronomical value between the MON 87769 soybean and the traditional type. Is this conclusion correct?
Remark SBB, agreed by coordinator
In this context, equivalence relates to the composition of the GMO, not to its agronomical value. Suggestion not to send this comment to EFSA.

Comment 2

No comments

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 2

No questions.

Comment 3

No comments

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

Minor comment: as hydrogenation will not be applied to SDA soybean oil (part I, page 173) some of the applications of soybean oil products mentioned on the same page (such as the production of shortening) may not apply.

Comment 2

No comments

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

It is stated that the oil derived from MON87769 may be used amongst others as a replacement for fish oil (Part I, page 175). This is not completely true as SDA oil is a poor precursor of DHA which is also present in fish oil.

Comment 2

See comment about the margin of exposure in 7.8.1.

D.7.8 Toxicology

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

Both proteins were detected in immature seed, mature seed, and at low levels in forage because this tissue usually contains a small amount of immature seed.

Margins of exposure between 270 and 980 were calculated based on daily soybean consumption, expression levels and NOAELs from the acute toxicity studies.

a) Degradation of the PjΔ6D protein in simulated gastric fluid (**From CBI:** Kapadia et al., 2008b).

The results of the study demonstrated that greater than 99% of the full-length PjΔ6D protein was digested within 30 s of incubation in SGF when analyzed using stained gel, and greater than 96% was digested when analyzed using western blot with an antibody raised against full-length PjΔ6D protein. Fragments were observed up till 60 min. Their origin could not be established.

b) Degradation of the PjΔ6D protein in simulated intestinal fluid (**From CBI:** Kapadia et al., 2008b).

After digestion of PjΔ6D protein in SGF for 2 min, the reaction was quenched and the mixture was exposed to further digestion in SIF. The data indicate that the stable fragments observed in SGF rapidly degrade upon short exposure to SIF (<5 min).

c) PjΔ6D: Acute Oral Toxicity Study inMice (**From CBI:** MSL21314, 2008).

No mortality occurred during the study, and no test article-related clinical findings were observed. There were no differences in body weights, body weight changes, or food consumption in Delta 6 Desaturase-treated or Delta 15 Desaturase-treated animals when compared to Bovine Serum Albumin-treated control animals. No test article-related gross necropsy findings were present.

Delta 6 Desaturase induced neither mortality nor other adverse effects when administered to mice by single oral gavage at a dose of **4.66 mg/kg (NOAEL of PjΔ6D)**, nor was there mortality or other adverse effects induced by Delta 15 Desaturase when administered to mice by single oral gavage at a dose of **37.3 mg/kg (NOAEL of NcΔ15D).**

d) PjΔ6D: Assessment of Amino Acid Sequence Homology with Known Toxins (**From CBI:** Tu and Silvanovich, 2009a).

The results of the bioinformatic analyses demonstrated that no structurally relevant similarity exists between the PjΔ6D protein and any known toxic or other biologically active proteins that would be harmful to human or animal health.

e) Degradation of the NcΔ15D protein in simulated gastric fluid (**From CBI:** Kapadia et al 2008a).

The results of the study demonstrated that greater than 96% of the full-length NcΔ15D protein was digested within 30 s of incubation in SGF when analyzed using either stained gel or western blot with a NcΔ15D specific antibody. Several fragments (~17 kDa, ~12 kDa, ~5 kDa and ~4 kDa) were observed at various time points during digestion in SGF when specimens were analyzed using a stained gel.

f) Degradation of the NcΔ15D protein in simulated intestinal fluid (**From CBI:** Kapadia et al 2008a).

Digestion of the NcΔ15D fragments observed in SGF was further evaluated in SIF, where they were rapidly degraded upon exposure to SIF when analyzed using stained gel (<5 min) and western blot (<30 s).
The results of the study also demonstrated that greater than 96% of the full-length NcΔ15D protein was digested in SIF within 5 min when analyzed using western blot. No proteolytic fragments were observed at any time points.

g) NcΔ15D: Acute Oral Toxicity Study inMice (**From CBI:** MSL21314, 2008).

Cfr. point c) of this section.

h) NcΔ15D: Assessment of Amino Acid Sequence Homology with Known Toxins (**From CBI:** Tu and Silvanovich, 2009a)

The results of the bio-informatic analyses demonstrated that no structurally relevant similarity exists between the NcΔ15D protein and any known toxic or other biologically active proteins that would be harmful to human or animal health.
Remark SBB, agreed by coordinator
We guess that the overall conclusion from the comments made by the expert is that the data provided are sufficient to demonstrate the safety of the newly expressed protein.
Suggestion not to forward these comments to EFSA.

Comment 2

I agree with the statement that there is a reasonable certainty that consumption of the newly expressed proteins from food derived from MON87769 will not affect human health.

Comment 3

Throughout the document (and especially in Table 37), the margin of exposure is used to evaluate the acute dietary intake. It is not always agreed among scientists that this is a good measure to evaluate the risk (see, e.g., Constable and Barlow, 2009). Therefore, a statement like on page 221 *"This dose was several orders of magnitude higher than conservative estimates for human exposure to the PjΔ6D from consumption of MON 87769 (Table 37)."* might be subject to misinterpretation and should be avoided.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

Based on the molecular species found in soy phosphatidylcholine (Le Grandois et al., 2009) it is likely that phospholipids derived from MON87769 could have "unusual" **combinations** of sn-1 and sn-2 acyl groups (different molecular species, e.g. SDA/SDA; SDA/GLA…). These may have to be considered as new constituents, especially as the biological activity of such "essential" phospholipids may exceed that of the constituent fatty acids (Jayaraman et al., 2008; Hiratsuka et al., 2008; Okiyama et al., 2009).

Comment 2

No comments

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

Detailed information is given on the nutritional impact of fatty acids whose concentration changes in the oil derived from MON87769. However, the effects of uptake of GLA in combination with SDA (both are present in the oil) has not been addressed. Although GLA and SDA are present together in some foods, the consumption of the latter is usually small. Moreover usually the level of these fatty acids is relatively low. Echium oil contains both fatty acids in relatively high and about the same concentration. This oil has been approved as novel food but I do not know whether the combined presence of both fatty acids in relatively high concentration has been considered.
When the levels of GLA and SDA in the diet are low, as is usually the case, delta-6 desaturase is the limiting enzyme in the fatty acid cascade in humans. By feeding GLA and SDA, delta-5 desaturase may become limiting and their elongation products (respectively 20:3n-6 and 20:4n-3) may accumulate. It has been shown that GLA and SDA interact resulting for instance in the accumulation of dihomogamma-linolenic acid (20:3n-6) in tissues (e.g. Miles et al., 2004). Increased tissue levels of 20:3n-6 results in increased formation of prostaglandin E_1 (Levin et al., 2002). 20:4n-3 seems not to accumulate to any significant extend in tissue lipids. Yet it can not be excluded a priory that temporarily increased levels of this fatty acid, in case it would occur, may compete with other polyunsaturated fatty acids with 20 carbon atoms, affecting eicosanoid metabolism. If so, this may have profound effects (Lagarde et al., 2010). This does not mean that the combination of SDA with GLA is not safe or even beneficial. It just means that there is uncertainty about possible unexpected effects. Some slight differences between rats fed the MON87769 derived oil or control soybean oil were noted in the 90-day rat one generation study but they were considered not to be of toxicological significance.

Minor comment: some of the arguments given to conclude that the SDA oil is safe are not very convincing (e.g. the estimation that the use of the SDA oil, considering the fraction that is converted into EPA, would result in an intake of EPA plus DHA not exceeding the maximum guidance level (Part I, page227) (for reasons see above).

Comment 2

The occurrence of trans-fatty acids in MON87769 is, to my opinion, not properly discussed. In recent years, there is an increased concern about negative health effects of trans-fatty acids (EU Commission, 2005).

In this dossier, it is advisable to discuss this issue in more detail, and based on relevant recent literature. In addition, the consequences for labelling of the trans-fatty acids in foodstuffs must be considered. Examples of recent literature include, among many others Micha and Mozaffarian (2008), Mensink *et al.* (2003), Chardigny *et al.* (2008), WHO (2003).

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

a) 42-day feeding study in broiler chickens (**From CBI:** MSL21498, 2008).
There were no biologically relevant differences in broiler performance, carcass yield or meat composition between broilers fed diets containing MON87769 and those fed diets containing conventional control soybean meal.

b) 90-Day rat feeding study (**From CBI:** MSL0021746, 2008).

MACROSCOPIC EXAMINATION
Gross observation of red stomach content in two female rats of the 15% test diet group was unconfirmed in the absence of correlating histologic findings. The same event was seen in 2 male rats of the 5% test diet group. No such event occurred in the control group. Although there is no real dose-response, can these observations be seen as coincidence?

CONCLUSIONS
There were no test substance-related clinical observations. There were no test substance-related adverse effects on body weights, food consumption or organ weights.
There were no test substance-related adverse effects on clinical pathology parameters. There were no adverse test substance-related macroscopic or microscopic findings.
In conclusion, administration of meal from MON87769 to rats for at least 90 consecutive days at concentrations up to 15% (w/w) in the diet (equivalent to 10,915 mg/kg body weight/day for males and 12,597 mg/kg body weight/day for females) had no adverse effects on the growth or health of Sprague-Dawley (Crl:CD[SD]) rats.

Comment 2

No questions.

Comment 3

No comments

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

PjΔ6D and NcΔ15D proteins are not known hitherto as allergenic proteins. In contrast the soy product (Glycine max) is known as an allergenic plant: Gly m1 (hydrophobic protein from soybean) and Gly m2 (defensin) act as inhalant allergens, Gly m3 is a profilin, common to many other plants, Gly m4 is PR-10 protein. Gly m5 (conglycinin) and Gly m6 (glycinin) are storage proteins that can be responsible for food allergy especially in children; there is no reason to suppose that the GM plant MON 87769 will be devoid of these proteins, which means that MON87769 will be as allergenic but not more than the natural soy plant.
http://www.allergen.org/Allergen.aspx (14.03.2010)

Comment 2

Data are presented to expect that the newly expressed proteins are unlikely to be allergenic and that MON87769 soybean has no greater allergenic potential than other soybean varieties on the market.

Comment 3

The rapid digestibility of PjΔ6D and NcΔ15D proteins in simulated digestive fluids is not a guarantee for safety. There are indications that the use of the SGF technique to predict the allergenic status of the proteins remains uncertain. Bannon et al. (2003), Meredith (2005), Herman et al. (2006) and Spök et al (2005) have shown that digestibility studies can not be considered as suitable tools to address the allergenic potential of a protein. However, based on the weight-of-evidence approach (Goodman et al., 2008) in this dossier:

- the history of safe use
- the lack of structural similarities of PjΔ6D and NcΔ15D proteins with known protein toxins and allergens
- the rapid digestibility of PjΔ6D and NcΔ15D proteins
- MON 87769 soybean shows no IgE binding to sera from non-allergic patients

we may assume that MON87769 does not pose a serious allergenic risk, and that it is comparable with conventional soybean with regard to allergenicity.

Comment 4

No comments

Remark SBB
The allergenicity of the whole GM plant has been assessed (i) by determining binding levels of human IgE antibody collected from clinically documented, soybean allergic patients to protein extracts prepared from MON87769 and various controls, and (ii) by performing IgE western blots using one-dimensional (1D) and two-dimensional (2D) gel electrophoresis.
Remark Coordinator
I conclude that the experts are of the opinion that the MON87769 soybean does not have an altered allergenic profile. So we don't need to forward any comments on this issue.

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

1) The intended level of SDA in a food serving has been based amongst others on the percent conversion of this fatty acid into EPA and on recommendations for EPA **plus** DHA (there are no recommendations for intake of EPA and DHA separately). Firstly, it has to be realized that some of the effects of EPA and DHA are different (e.g. Anderson et al, 2009). Moreover, SDA may have effects of its own (note in this respect that maximal estimated intake of SDA from all the proposed uses is about 100 times higher than estimated baseline intake; 2.5g/day vs 200 mg/day; part I, page 258). For instance, it has been shown that gene expression of fatty acid elongase and delta-5 desaturase is more increased by feeding diets with SDA than with fish oil (Miller et al., 2008). For ALA, it has been shown that, on a weight basis, its effects on hemostatic factors are similar to those of marine long chain fatty acids (Freeze et al., 1997), though its conversion into EPA is very low. Thus the effect on bleeding time for instance of SDA might be comparable to that of EPA (theoretically, no experimental data available) and not just 17-33% as effective as assumed in the text.

2) If it is assumed that a good guidance for EPA intake would be half of that for EPA plus DHA (as done for some calculations in the application; there are good reasons for this assumption, given on page 257), potential exposure of EPA at the 97.5th percentile (1.0-1.8 g/person/day) may come close or possibly even exceed half of the FDA guidance level for EPA plus DHA (3 g/person/day /2=1.5 g/person/day) especially when Echium oil is also consumed. On page 259 potential exposure of EPA is compared to the FDA guidance level for EPA plus DHA.

3) It may be of importance to mention that MON87769 feed products might be used in the future to increase SDA and/or EPA content of milk (O'Donnal et al, 2010), farmed fish (Diaz-Lopez et al., 2009), meat (Kitessa et al., 2009), eggs a.s.o.

4) The expected small changes in intake of fatty acids other than SDA when SDA soybean oil would be used (Table 47, page 264) are not of nutritional importance.

Overall conclusion.
To my knowledge there are no adverse effects reported when oils rich in SDA, GLA or containing both fatty acids are fed to humans. Moreover it is probable that intake of such oils would have health benefits for the general population (e.g. Whelan, 2009) and for certain patient groups (e.g. Chilton et al., 2008). Yet, as these fatty acids and their elongation products interact with each other, possibly influencing eicosanoid metabolism and levels of the different eicosanoids which are physiologically very active, there is a remote possibility that in some circumstances or some individuals the use of MON87769 derived products may have negative effects. It is suggested that some clinical experiments are done in human volunteers using SDA oil (e.g. determination of hemostatic factors).

Question
In the EU authorisation of Echium oil, supplementation levels of SDA in foods were authorized from 75 to 750 mg **per 100 g** of food. The intended level of SDA in a **food serving** is 375 mg. How much would that be /100g for the different types of servings mentioned?

Comment 2

Why is only the presence of vitamin E presented and discussed in the Technical Dossier ?

Comment 3

No comments

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

Comments/Questions of the expert(s)

Comment 1

To my knowledge there are no adverse effects reported when oils rich in SDA, GLA or containing both fatty acids are fed to humans. Yet, as these fatty acids are physiologically active and interact with each other (see above), it is my opinion that post-market monitoring would be wise.

Remark SBB
The possibility of adverse effects on human health linked to increased or high consumption of oils or other products enriched in omega-3 fatty acids is still under question. Post-market monitoring could be an option to ensure a follow-up. We do not think that request for this type of monitoring falls within the scope of the evaluation of this GMO application. Shouldn't we nevertheless send a general comment to EFSA ?
Additional comment from coordinator
I don't see why a request for post-market monitoring would not be possibe for this type of GMO application. Post-market monitoring can be triggered by either environmental concerns or human health concerns.
I have more doubts about whether there is a true rationale for asking for post-market monitoring in this particular case. As it will be difficult if not impossible to distinguish between the effects of omega-3 fatty acids from the GM soybeans, and the effects of the consumption of other forms of foods rich in omega-3 fatty acids. In other words: case-specific monitoring is almost impossible. And on top of that I also think there is no post-market monitoring for the use of echium oil as such.

Comment 2

No comments

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

Comments/Questions of the expert(s)

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)

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment 2

During the last decades, interest in the health effects of trans fatty acids has focused largely on the potential adverse effects for cardiovascular disease. Dietary trans fatty acids come mostly from the industrial hydrogenation of unsaturated vegetable oils; they are also formed in the rumen of ruminants

(Motard-Bélanger et al., 2008). As MON87769 soybean oil contains trans-stearidonic and trans-alpha-linolenic acid, attention may be paid to the presence of these trans fatty acids. However, the importance should not be exaggerated as their presence is limited to <0.3 and <0.5 % of total fatty acids, respectively, and total trans fatty acid content (0.6% of total fatty acids) is of the same magnitude as in commercial soybean oil.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

No questions.

Comment

The percentages of the PjΔ6D and NcΔ15D proteins consumed as part of the daily protein intake are low, so that there is little risk to animals that are fed diets containing MON87769.

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

Comment 1

In this paragraph it is mentioned again that the scope of application does not include cultivation of soybean plants in the EU. Nevertheless I give some remarks in the case that the applicant should ask in the near future for an extension for the scope of cultivation. In the framework of the EU- regulation 2002/53 a new variety have to be submitted to DUS (Distinctness, Uniformity, Stability) and VCU (Value for Cultivation and Use) tests before the variety can be commercialised. The new variety has to be compared with the best existing standard varieties. So my question here is : can the GM- soybean be incorporated in normal VCU trials, without adapting the field conditions for the specific trait?
Remark SBB, agreed by coordinator
Suggestion not to forward this comment to EFSA, because it is not relevant in the context of the current application (no cultivation). Similar comment in a previous soybean dossier (2009/73) has not been sent to EFSA.

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

Comment 1

The proposed environmental monitoring plan is OK.

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

Comment 1

Based on the scope of application (no cultivation) I can agree with the remark of this chapter.

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

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