Bioveiligheidsraad
Conseil de Biosécurité

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Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2012_0850

Title: Advice of the Belgian Biosafety Advisory Council on the application EFSA/GMO/NL/2005/24 from Monsanto under Regulation (EC) No. 1829/2003

Context

The application EFSA/GMO/NL/2005/24 was submitted by Monsanto on 4 November 2005 for cultivation of the genetically modified (GM) soybean 40-3-2 within the framework of Regulation (EC) No. 1829/2003[1]. Soybean 40-3-2 expresses the gene of the CP4 EPSPS protein conferring tolerance to glyphosate-based herbicides. This GM soybean is since 1996 authorized by the European Commission for food and feed uses and the authorization was renewed on 10 February 2012[2].

The application was officially acknowledged by EFSA on 29 September 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 genetically modified organisms (GMOs) 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). Five 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 4 January 2007.

The opinion of the EFSA Scientific Panel on GMOs was adopted on 31 May 2012 (EFSA Journal 2012; 10(6):2753[3], and published together with the responses from the EFSA GMO Panel to comments submitted by the experts during the three-month consultation period. In delivering its Scientific Opinion, the EFSA GMO Panel considered notably the environmental risk assessment report of the German Competent Authority delivered on 3 October 2008 in line with Articles 6.3(c) and 18.3(c) of Regulation(EC) No 1829/2003.

On 25 June 2012 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

[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]GMO register : http://ec.europa.eu/food/dyna/gm_register/gm_register_auth.cfm?pr_id=53
[3] See http://www.efsa.europa.eu/en/efsajournal/pub/2753.htm

together with the opinion of EFSA including the answers of the EFSA GMO Panel form the basis of the advice of the BAC given below.

In addition, in August 2011 the food and feed uses of this GM soybean have been positively evaluated by the BAC in the frame of the application for renewal of authorization (EFSA/GMO/RX-40-3-2)[4]. Taking this into account the present advice deals only with the evaluation of the environmental risks of the cultivation of soybean 40-3-2 in the European Union (EU).

Environmental risk assessment and risk management strategies

1. Persistence and invasiveness

Soybean is a highly domesticated crop and its ability to survive as volunteers, or to establish feral populations under European environmental conditions is very low. The BAC agrees with EFSA that it is "*very unlikely that the establishment, spread and survival of soybean 40-3-2 would be increased due to the herbicide tolerance trait*". The risk for 40-3-2 soybean to be invasive or to persist without human intervention is considered negligible.

2. Selective advantage and disadvantage

The herbicide tolerance trait will only provide a selective advantage when glyphosate is applied. In the rare case volunteers would emerge (see section 1), they will be controlled by standard cultivation practices.

3. Potential for gene transfer

Gene transfer via the dispersal of pollen is very unlikely: soybean is a self-pollinating crop and in the EU there are no compatible wild relatives. Cross-pollination with other cultivated conventional soybean varieties cannot fully be excluded. We agree with EFSA that the consequences of cross-pollination should not be considered an environmental risk, but rather an agricultural management and coexistence issue.
Gene transfer via seeds is very unlikely: the seeds show no dormancy phase and will not survive cold climatic conditions. Survival of soybean outside of cultivation is very rare.
Horizontal gene transfer to bacteria is not likely to occur under natural conditions (EFSA, 2010[5]). However, in the unlikely event that the *cp4-epsps* gene is transferred and expressed, no environmental harm on microbial communities, environment or human/animal health is expected as various *epsps* variants, tolerant and non-tolerant to glyphosate, naturally occur in soil bacteria. The BAC therefore supports the conclusion of EFSA and of the German Competent Authority that "*the possibility of gene transfer from the genetically modified soybean 40-3-2 to microorganisms and its consequences is not regarded as a safety concern*".

4. Interactions of the GM plant with target organisms

The CP4 EPSPS protein conferring tolerance to glyphosate-based herbicides does not interact with any specific target organism.

[4] Document reference: BAC_2011_0745;
[5] Guidance on the environmental risk assessment of genetically modified plants. EFSA Journal 2010;8(11):1879

5. Interactions of the GM plant with non-target organisms

There are no indications that the CP4 EPSPS protein or the GM plant would have any adverse impacts on non-target organisms.

The BAC is of the opinion that a study looking at the behaviour of honeybees on the GM soybean 40-3-2, as recommended by EFSA, is not necessary as there are no changes in the composition or the phenology of the plant.

6. Effects on human and animal health

In its advice of August 2011 the BAC concluded that the GM soybean 40-3-2 is unlikely to have adverse effects on human and animal health.

7. Interactions with biogeochemical processes and the abiotic environment

The BAC agrees with EFSA that "*the expression of the newly introduced trait is not expected to alter the natural interactions of soybean with the abiotic environment".*

8. Impacts of the specific cultivation, management and harvesting techniques

The BAC agrees with EFSA that the cultivation of this GM soybean will allow the use of glyphosate 'over the top of the crop', which might lead to a change in the cultivation and crop management practices compared with conventional soybean cultivation.

It cannot be excluded that the 'over the top' use of glyphosate-based herbicides has an effect on soil microorganisms harbouring forms of the EPSPS protein that are sensitive to glyphosate. It is known however that bacterial communities are flexible and dynamic. The EFSA GMO Panel considers that the use of glyphosate-based herbicides at recommended field application rates on soybean 40-3-2 is unlikely to cause adverse effects to the majority of soil microbial communities. However, bacterial symbionts of soybean that harbour sensitive forms of the target enzyme EPSPS (such as *Bradyrhizobium*) could be affected, which may have an effect on the nitrogen-fixation in the soybean. A wide array of studies under different environmental conditions however has shown that glyphosate application does not have an impact on performance and yield, suggesting that soybean has the ability to recover from glyphosate stress. In addition, in Europe due to the lack of spontaneously occurring bacterial symbionts for soybean, organic or inorganic nitrogen is generally supplied in soybean fields. And where the soybeans are inoculated with bacterial symbionts one can choose to do this with selected strains of *Bradyrhizobium* that are not affected by glyphosate.

The use of glyphosate-based herbicides may also have an effect on the biodiversity in the field due to shifts in and reduction of weeds. However, the magnitude of these potential adverse effects will depend on factors such as herbicide use management, crop rotation and receiving environment. The environmental impact of the cultivation of this GM soybean can therefore be lower or higher than the impact of non-GM soybean. When no effective resistance management strategy is implemented the repeated use of glyphosate-based herbicides may lead to glyphosate resistance in weed species, thereby reintroducing the need for use of additional (selective) herbicides.

9. Risk management strategies and post-market environmental monitoring

The BAC agrees with EFSA that "*current management practices are sufficient to cope with potential adverse effects on symbiotic nitrogen fixation arising from the use of glyphosate on soybean 40-3-2 » ;* The BAC also agrees with the recommendations of EFSA to put in place measures "*to manage potential herbicide effects, in order to ensure that glyphosate is used*

on soybean 40-3-2 in ways that result in similar or reduced adverse effects on farmland biodiversity compared with conventional soybean cultivation".
The Belgian experts insisted on the need for monitoring the effect of cultivation of GMHT soybean, including the use of glyphosate, on biodiversity.

As recommended by EFSA *(1) impartial and standardised interviews should be carried out by independent parties and effective quality and auditing procedures should be considered; and (2) relevant data as from other sources of information (e.g., official statistics on crop management practices) should/could be considered for validity check of the questionnaires (e.g., consistency, representativeness).*

The BAC is of the opinion that glyphosate resistance evolution in weeds should also be monitored, but recognizes that this issue is directly linked to the use of the herbicide which falls under the framework of Regulation 1107/2009 on plant protection products.

Conclusion

Based on the scientific assessment of the dossier done by the Belgian experts, taking into account the environmental risk assessment report of the German Competent Authority, 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, its advice of August 2011 concerning the food and feed safety of soybean 40-3-2 and considering the data presently available, the Belgian Biosafety Advisory Council is of the opinion that the genetically modified soybean 40-3-2 does not provoke health and environmental problems.

The (repeated) application of glyphosate-based herbicides may however, depending on the way they are being used, have small and temporary effects on soil micro-organisms harbouring sensitive forms of the EPSPS protein, on the biodiversity in the field, and may lead to the emergence of glyphosate resistance in weed species. These effects can be countered using the mitigation measures as recommended by EFSA. The BAC supports the use of these mitigation measures and recommends:

- To promote the application of an effective resistance management to prevent the emergence of glyphosate tolerant weeds.
- To pay attention to floristic and faunistic biodiversity in monitoring plans.

Additionally the BAC wishes the competent authority to take into account that the large scale cultivation of any glyphosate tolerant crop (as this soybean) may increase environmental problems due to herbicide residues in soil and water, as is the case with any herbicide that is applied on large surfaces.

For information the BAC would like to add that Belgium has no experience in cultivation of soybean. Until now the cultivation of this subtropical crop is restricted to the Southern regions of Europe. In a near future it is unlikely that soybean 40-3-2 will be cultivated in Belgium. However currently research is being done in countries beyond the Southern part of the EU (e.g.,Belgium, The Netherlands) to develop new soybean varieties, less susceptible to frost.

f.o. M.Th. Herman

Prof. D. Reheul
President of the Belgian Biosafety Advisory Council

Annex I: Full comments of experts in charge of evaluating application EFSA/GMO/NL/2009/73 and *comments submitted on the EFSAnet (ref. BAC_2010_0211)*

Bioveiligheidsraad
Conseil de Biosécurité

4 January 2007

Secretariaat
Secrétariat

N./réf. : WIV-ISP/BAC/2007/PT/431
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Comments of experts in charge of evaluating the application EFSA/GMO/NL/2005/24
and
Comments submitted on the EFSAnet on mandate of the Biosafety Council

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 23 October 2006
Coordinator: Prof. Thierry Hance
Experts: Armand Christophe, Eddy Decuypere, Jean-Pierre Hernalsteens, André Huyghebaert, Jean-Marie Saint-Rémy
Domains of expertise of experts involved: Genetic engineering, human nutrition, animal nutrition, biochemistry of food/feed, toxicology, immunology, alimentary allergology, risk analysis, industrial processing, traceability of alimentary chain, soybean.
Secretariat: Adinda De Schrijver, Martine Goossens

INTRODUCTION

Dossier **EFSA/GMO/NL/2005/24** concerns an application of the company **Monsanto Europe S.A.** for the marketing of the genetically modified **40-3-2 Soybean** for food and feed applications under Regulation (EC) 1829/2003.
The application has been officially acknowledged by EFSA on 29 September 2006.
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 – including the impact of its cultivation in Europe, 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. Comments placed on the EFSAnet are indicated in grey.

List of comments received from the experts

A. GENERAL INFORMATION

Comments/Questions of the expert(s)

Comment 1

40-3-2 Roundup Ready Soybean (40-3-2 soybean) is tolerant to glyphosate, the active component in Roundup.
The phosphonomethyl-glycine blocks the activity of 5-enolpyruvylshikimate-3-phosphate synthase or EPSPS, which is a key enzyme in the shikimic pathway leading to the formation of aromatic amino acids (tyrosine, phenylalanine and tryptophane) in plants, bacteria and fungi, but not in animals. Why the term "low toxicity" in animals is used for glyphosphate in the Oxford dictionary of Biology ? Since there is no shikimic pathway in animals, has the enzyme other functions ?
In this part a remark or question can be made about part IV "Labelling and Unique Identifier".
It is stated in the text that inserts in 40-3-2 do not contain human or animal genes consistent with Monsanto's commitment not to use animal or human genes in GM plants for food or feed use, and that therefore 40-3-2 is not considered to give rise to ethical or religious concerns.
This is a rather strange reasoning as ethical concerns may be related to a certain view on nature and what we can do or how we can interfere; ethical concerns may therefore arise but not be based on any safety concerns, but simply on subjective reasons originating from a certain view on mankind, nature and world.
Therefore, freedom of choice as a consumer to accept or reject products from GM-plants, seems to be important and requires labelling anyway.

Comment 2

I followed the guidelines, written down in " The safety assessment of genetically modified crops for food and feed use" chapter VI Food nutrition evaluation.

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

Comments/Questions of the expert(s)

Comment 1

No questions

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

Comments/Questions of the expert(s)

Comment 1

What is the exact difference between the plant EPSPS and the EPSPS from Agrobacterium CP4 so that glyphosate does not block the latter but does so with the plants EPSPS ?
If most bacterial EPSPS are also not tolerant for glyphosate, is this also the case for the rhizobia that form a symbiosis with soybean plants and are responsible for N-fixation ? Are they also killed by glyphosate or not ?
I also wonder if the use of 40-3-2 soybean that will promote the use of Roundup will not affect in this way soil life (bacteria and/or fungi) that are sensitive to glyphosate ?

Comment 2

The insert in soybean 40-3-2 consists of only the CP4 EPSPS gene, responsible for the glyphosate tolerance, and its regulatory sequences. This gene can allow more efficient weed control: an herbicide with environmentally and toxicologically acceptable characteristics can be used on this crop and can be applied only when this is required. This is potentially advantageous, both for the agronomic use of the crop and for the environmental impact of its production.

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 questions

Comment 2

The absence of marker genes from the 40-3-2 plants is optimal for the safety of this line. The only expressed transgene is the *cp4 epsps* gene (full length and truncated non-functional insert), encoding the desired tolerance trait.

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

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

The presence of rearrangements at the borders of the insert (Windels *et al*., 2001) is a typical consequence of the transformation method and has no direct consequence on the safe use of the resulting transgenic plant. Its substantial equivalence to traditional soybean (except for the introduced glyphosate-tolerance trait) was previously established by the detailed analysis that was performed before the approval of this soybean for import and processing.

D.3. INFORMATION ON THE EXPRESSION OF THE INSERT

Comments/Questions of the expert(s)

Comment 1

No questions

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 differences found between traditional soybean and 40-3-2 soybean; but what is meant by "nodes per plant" ? Has this to do with the morphology of the plant or with the nodulation of the roots by rhizobia ?
Has the rhizobia nodulation been locked for after the application of Roundup ?

Comment 2

From the knowledge of the insert, no difference in these properties should be expected. This is confirmed by several years of experience with large scale cultures of this transgenic line.

D.5. GENETIC STABILITY OF THE INSERT AND PHENOTYPIC STABILITY OF THE GM PLANT

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

There are no reasons to anticipate instability.

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 questions

Comment 2

The probability of gene transfer by hybridisation from cultured soybean is very low. Cultured soybeans are the only possible recipient. No related plants that could receive genes from soybeans are present in the European environment.

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

No differences found.

Comment 2

1. In the proximate analysis the crude fibre method has been applied. This is the conventional approach for animal feed. For many years, the dietary fibre method is applied for foods. Is there material available to confirm the equivalence for an important group of constituents like dietary fibre?

2. In the same line of thinking carbohydrates were assessed by calculation. Nowadays there are however a range of methods available for the direct assessment of carbohydrates. These methods give more accurate information about the composition of the beans. Are further data available for individual carbohydrates?
3. Anti-nutrients are studied in depth. However data are rather poor for a group of nutrients like vitamins. I found only data for vitamin E. This is an important vitamin. However other vitamins are important as well, particularly when soy beans are used as a food. Is there any additional information about the vitamin content?

Comment 3

1. Considering the noted variation in moisture content of the seed samples and the fact that the oil is used as such in many applications, it is suggested that the fatty acids are expressed not only on a fresh weight basis (as is reported) but also on an oil basis to determine whether there is compositional equivalence or not. However, even if small differences in fatty acid composition would be found, it is expected that they would not negatively affect nutritional properties

   Question: What is the fatty acid composition of the oil (with total fatty acids = 100%). Are there differences between the 40-3-2 oil and oils from non-modified soybeans?

2. As I could not find the referenced article, nor its modification referred to (Thompson et al, 1989), it is not clear what is meant by vitamin E expressed in mg/g. In soybean oil, there are several vitamin E vitamers with different biological activities. One way of expressing them together is as alpha-tocopherol equivalents (Eggermont, 2006). In order to judge compositional equivalence (and to calculate alpha-tocopherol equivalents), the tocopherol composition should be known. This may be of importance as different tocopherols exhibit different (patho)physiological properties (Morris et al., 2005).

   Question: is the vitamer E composition and alpha-tocoferol equivalents similar in the genetic modified soybean compared to non modified controls?

3. Soy sterols/stanols can be used for incorporation in foods or in food supplements (Spilburg et al., 2003).

   Question: is the stanol/sterol composition of the genetically modified soybean similar compared to non modified controls?

Additional comment from the coordinator

The **40-3-2 Soybean** imply for its cultivation the use of glyphosate. What is the influence of glyphosate application on the plant protein-expression? Is it known? Is there any indication of glyphosate or metabolites presence in the soybean plants after glyphosate application and if yes what are their consequences on human health?

D.7.2 Production of material for comparative assessment

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No further comments or questions.

D.7.3 Selection of material and compounds for analysis

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No further comments or questions.

D.7.4 Agronomic traits

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No further comments or questions.

Comment 3

This is not within my expertise. Besides the herbicide tolerance, no modified traits can be logically expected.

D.7.5 Product specification

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No further comments or questions.

Comment 3

This is not within my expertise. No modified traits can be logically expected.

D.7.6 Effect of processing

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

I agree with the conclusion that there is no difference with the equivalent foods and feed originating from traditional soybeans as far as processing is concerned.

Comment 3

This is not within my expertise. No modified traits can be logically expected.

D.7.7 Anticipated intake/extent of use

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No further comments or questions.

D.7.8 Toxicology

Comments/Questions of the expert(s)

Comment 1

No indications for additional toxicity in 40-3-2 soybean; same level of antinutritional factors as in traditional non-GM soybean.

Comment 2

Based on the arguments, supported by experimental data, given by the company on 1) history of safe use of 40-3-20 soybean on a commercial scale since 1996 as source of foods and feeds*; 2)* the lack of acute oral toxicity of the CP4 EPSP protein in mice and of feeding the processed soybean in rats*;* 3) lack of allergenicity in groups of subjects who are sensitive to allergies, lack of structural homology to known toxins and protein allergens and the results of the in vitro digestion experiments and in vivo feeding experiments in several animal species, it appears to me that it would be very unlikely that 40-3-2 soybean would cause major food and feed problems. Nonetheless, there remain a few questions in my mind concerning the compositional analyses and potentially harmful effects in subgroups of the population.

Dangers to humans and domesticated animals (I am not qualified to comment on wild animals, invertebrates and environmental aspects) which may occur due to cultivation in the European Union of soy 40-3-2 seem very unlikely. Adverse occupational health effects associated with the storing and handling of 40-3-2 soy bean and its products are not expected to be different from these of traditional soy bean(products) as 1) no differences in the content of the known allergens in soy were found between the genetically and non-genetically modified organisms; 2) there is evidence that the newly expressed protein is not allergic nor toxic; 3) it is claimed that post-market experience in the US has shown no evidence of extra occupational hazard between 40-3-2 and traditional soybean.

Comment 3

This is not within my expertise. Toxicology was thoroughly evaluated before the approval of this soybean for import and processing. Use on a commercial scale for several years has to the best of my knowledge not shown any unexpected or undesirable properties.

D. 7.8.1 Safety assessment of newly expressed proteins

Comments/Questions of the expert(s)

Comment 1

Safety assessment of the newly expressed protein was based on:
- protein specificity
- no homology with known protein toxins

- very quickly digested in vitro and therefore very little chance that intestine would be exposed to feed allergen
- no acute toxicity by mouse acute gavage studies

Comment 2

1. Soybean protein isolate is the base of soy-based infant formula. The protein isolate of soy 40-3-2 is expected to contain the modified CP4 EPSPS protein.
   Question: Is there a history of safe use of 40-3-20 soybean protein isolate as a base of baby food?
2. From the stained gel showing the gastric simulated digestion of purified CP4 EPSPS protein (Technical dossier, part 1, page 89) it appears that fragments of still considerable length (MW< 2.5 dalton) would not be detected. If such fragments would survive peptic digestion and enter the small intestine, it can not be excluded that they could exhibit physiological effects.
   Question: Has the size range of the peptic digestion products been determined and its physio(pathological) implications considered? (Zaloga et al, 2004)

Comment 3

Only a single new protein is expressed, the CP4 EPSPS. Use on a commercial scale for several years has to the best of my knowledge not shown any unexpected or undesirable properties of this protein.

D.7.8.2 Testing of new constituents other than proteins

Comments/Questions of the expert(s)

Comment 1

Not applicable

Comment 2

I agree with the conclusion.

Comment 3

This is not within my expertise. However, by the nature of the genetic modification no differences can reasonably be anticipated.

D.7.8.3 Information on natural food and feed constituents

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

The dossier contains an in depth study of known anti-nutritional factors. I agree with the conclusion that there is equivalence in terms of content and inactivation during processing.

D.7.8.4 Testing of the whole GM food/feed

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No further questions. I agree with the conclusion.

D.7.9 Allergenicity

Comments/Questions of the expert(s)

Comment 1

Assessing potential allergenicity is not an easy task, as there are no single characteristics, which would qualify a protein as an allergen. Indirect methods include or take into account sequence homology with known allergens for B cell epitopes, resistance to digestibility under acidic conditions and resistance to heat denaturation. All these methods are unsatisfactory and have severe limitations. The applicant is requested to take this into account and to be less affirmative in his statements, perhaps by providing a short introduction on such limitations. In particular:
The search for sequence homology is carried out only for linear sequences. It is well known that B cell epitopes are primarily conformational, in particular or IgE antibodies thought to be key players in allergic reactions, which would escape detection by the methods referred to here. Besides, one would question the reason as to why stretches of 8 contiguous aminoacids, and not 6 as commonly used, though a minimal epitope for antibody can be constituted of only 3 aminoacids. Likewise, a search for T cell epitope homology is commendable. Algorithms are freely available to predict main T cell epitopes and should be used in any evaluation of allergenicity.
Assays for assessing digestibility in so-called simulated gastric fluid are highly variable in terms of pH values, concentration of pepsin, etc. The recommendation is to use at least two complementary

experimental conditions. However, many allergens are not degraded by digestion, so whatever the results, digestibility remains a weak argument.
Heat resistance if not mentioned in the present application.

Comment 2

Same remarks as for 7.8

Comment 3

It has been shown in gastric simulated digestion that matrix effects are not important for proteins that are slowly digested (Ashwood et al, 1996 prot). Is the same true for proteins that are degraded very fast in pure form? If not, polypeptides of sufficient length considered to be potentially allergenic (Thomas et al,2004) could be formed.
Question: Is it certain that the CP4 EPSPS protein in a soy-based food matrix would be digested to the same extent as the purified protein? (Teuber, 2002)

Question: Has the potential allergenicity (and toxicity) been considered in subgroups of the population such as patients with pancreatic insufficiency in whom postprandial gastric function is disturbed? (Reagen et al, 1979)

D.7.10 Nutritional assessment of GM food/feed

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No further comments or questions.

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

Comments/Questions of the expert(s)

Comment 1

No questions

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

Comments/Questions of the expert(s)

Comment 1

No questions

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

No risk for 40-3-2 soybean to be invasive or to persist without human intervention.
But again, the question remains what happens with the soybean-rhizobium interaction with or after the use of Roundup ? Can it affect other microbial-plant interactions ?

Comment 2

When no glyphosate is applied, the genetic modification will not affect the properties of the plant. This is confirmed by several years of experience with large scale cultures of this transgenic line outside the European Union.

Additional comment from the coordinator

What are the consequences of Glyphosate application on soil microbiological life and on invertebrate? Previous studies have showed that wide spectrum herbicides uses have also negative consequences on invertebrate fauna, particularly Carabids.

D.9.2 Selective advantage or disadvantage

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

Idem D.9.1.

D.9.3 Potential for gene transfer

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

The unlikely transfer of the *cp4 epsps* gene to other cultivated soybeans would have no predictable negative consequences, as cultivated soybeans have no potential to become weedy.

D.9.4 Interactions between the GM plant and target organism

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

No effect of the *cp4 epsps* transgene on other organisms can be anticipated.

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

Comments/Questions of the expert(s)

Comment 1

No questions

Comment 2

Idem as D.9.4.

D.9.6 Effects on human health

Comments/Questions of the expert(s)

Comment 1

No effects on human health of 40-3-2 soybean nor of its specific CP4-EPSPS protein are likely.

Comment 2

Not anticipated by the nature of the genetic modification. To the best of my knowledge there is sufficient experience of large scale safe use.

D.9.7 Effects on animal health

Comments/Questions of the expert(s)

Comment 1

No effects on animal health of 40-3-2.

Comment 2

Not anticipated by the nature of the genetic modification. To the best of my knowledge there is sufficient experience of large scale safe use.

D.9.8 Effects on biogeochemical processes

Comments/Questions of the expert(s)

Comment 1

No evidence that this GM-soybean would be any different from traditional soybean regarding its direct influence on nutrient levels in the soil: the N-fixating capability is presumably the same. Also there is no, or at least very unlikely, interaction between CP4-EPSPS-producing soybean and decomposers or detritivores in the receiving environment, but what with the effect of glyphosate on those ?
And the production of CP4-EPSPS soybean is just making the use of glyphosate possible !!!

Comment 2

No effect should be anticipated by the nature of the genetic modification.

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

Comments/Questions of the expert(s)

Comment 1

It is stated that there is a negligible potential for adverse environmental effects from the recommended use of glyphosate in 40-3-2 soybean.
But is this also for other organisms ? Or only for the 40-3-2 soybean itself ? How has the term "environmental" in this sentence to be understood ? As no effect on other micro-organisms or fungi ? No effect on the N-fixating capability ? It is stated that this is minimal and transient but this should be more documented.

D.10. POTENTIAL INTERACTIONS WITH THE ABIOTIC ENVIRONMENT

Comments/Questions of the expert(s)

Comment 1

As for the potential interactions with the abiotic environment, again the question does not only seem to be how innocuous CP4-EPSPS protein in 40-3-2 soybean is, but what the effect of glyphosate on the environment really means.
The widespread use of glyphosate will just be made possible by the use of genetically manipulated plants, e.g. 40-3-2, with CP4-EPSPS.

D.11. ENVIRONMENTAL MONITORING PLAN

D.11.1 General

Comments/Questions of the expert(s)

Comment 1

- Same remarks as D10
- Additionally, since the key metabolic pathway in the biosynthesis of the aromatic amino acids occurs in plants, bacteria and fungi, but not in animals, the glyphosate blocks the synthesis of aromatic amino acids by interfering with the shikimic pathway in all of them except in some species when the EPSPS is not sensitive for glyphosate.
- Therefore, the generalized use of Roundup may not only affect weeds but also micro-organisms and fungi in the soil, hence soil life, unless persistence in soil is very low, or very low quantities of glyphosate reach the soil when applied on leafs. These aspects should be more explicited in the application.
- If there is however no negligible potential for any direct or indirect, nor immediate or delayed adverse environmental effects from the recommended use of glyphosate, as stated in the text and

as derived from earlier work (Directive 91/414/EEC), but not further explicited in the application, then previous remarks under D10 and D11 may not be relevant anymore.

Additional comment from the coordinator

There is a strong need of the evaluation of the effect on biodiversity of the new agricultural practices required with the uses of 40-3-2 Soybean**, particularly glyphosate application at a large scale,** as required in the Directive 2001/18/EC.

D.11.2 Interplay between environmental risk assessment and monitoring

Comments/Questions of the expert(s)

D.11.3 Case-specific GM plant monitoring

Comments/Questions of the expert(s)

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

Comments/Questions of the expert(s)

D.11.5 Reporting the results of monitoring

Comments/Questions of the expert(s)

Additional comment from the coordinator

Farmers who shall use 40-3-2 Soybean should address their observation concerning any side effect to an independent authority and not directly to Monsanto.

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