SCIENTIFIC OPINION

Scientific Opinion on applications (EFSA-GMO-RX-40-3-2$_{[8\text{-}1a/20\text{-}1a]}$, EFSA-GMO-RX-40-3-2$_{[8\text{-}1b/20\text{-}1b]}$) for renewal of authorisation for the continued marketing of (1) food containing, consisting of, or produced from genetically modified soybean 40-3-2; (2) feed containing, consisting of, or produced from soybean 40-3-2; (3) other products containing or consisting of soybean 40-3-2 with the exception of cultivation, all under Regulation (EC) No 1829/2003 from Monsanto[1]

EFSA Panel on Genetically Modified Organisms (GMO)[2, 3]

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ABSTRACT

This scientific opinion is an evaluation of a risk assessment for the renewal of authorisations for continued marketing of the genetically modified herbicide tolerant soybean 40-3-2 (Unique Identifier MON-Ø4Ø32-6) for (1) food containing, consisting of, or produced from genetically modified (GM) soybean 40-3-2; (2) feed containing, consisting of, or produced from soybean 40-3-2; and (3) of other products containing or consisting of soybean 40-3-2 with the exception of cultivation. Soybean 40-3-2 has been developed for tolerance to glyphosate herbicides by the introduction, via particle gun acceleration technology, of a gene coding for 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4 (CP4 EPSPS). Molecular analyses indicated that soybean 40-3-2 contains one functional insert expressing CP4 EPSPS and a non-functional insert consisting of a fragment of the CP4 EPSPS coding sequence. Updated bioinformatic analyses of the flanking sequences and the open reading frames spanning the insert-plant DNA junctions and the levels of the newly expressed protein in soybean 40-3-2 did not raise any safety concern. The stability of the inserted DNA was confirmed over several generations. Available compositional and agronomic data show that soybean 40-3-2 is compositionally and agronomically equivalent to its conventional counterpart and to other commercial soybean varieties, except for expressing the CP4 EPSPS protein.

[1] On request from the European Commission on applications (EFSA-GMO-RX-40-3-2$_{[8\text{-}1a/20\text{-}1a]}$ and EFSA-GMO-RX-40-3-2$_{[8\text{-}1b/20\text{-}1b]}$) submitted by Monsanto, Questions No EFSA-Q-2007-142, EFSA-Q-2007-141 adopted on 10 November 2010.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Molecular Characterisation, Food and Feed and Environment for the preparatory work on this scientific opinion, Boet Glandorf, Niels Hendriksen as external experts and EFSA's staff members Zoltán Divéki (MC), Karine Lheureux (ENV) and Claudia Paoletti (FF) for the support provided to this EFSA scientific opinion.

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It is estimated that the European consumers have been exposed to soybean 40-3-2 mainly via soybean oil Processed meal of soybean 40-3-2 has been given to farm animals in the EU at an estimated maximum dietary inclusion levels around 21% for broiler chickens, 18% for pigs, and 12% for dairy cattle. No adverse effects have been linked to these exposures. The safety assessment of the CP4 EPSPS protein expressed in soybean 40-3-2 and the whole soybean plant identified no concerns regarding potential toxicity and allergenicity of soybean 40-3-2. Considering the intended uses of soybean 40-3-2, which excludes cultivation within the European Union, no scientific assessment of potential environmental effects associated with cultivation of GM soybean 40-3-2 was required. In case of accidental release of viable grains produced by soybean 40-3-2 into the environment during transportation and processing, there are no indications of an increased likelihood of establishment and spread of feral soybean plants, except in the presence of the glyphosate herbicides. The EFSA GMO Panel considers unlikely that the recombinant DNA in soybean 40-3-2 transfers to bacteria and other micro-organisms and that the risk caused by a rare but theoretically possible transfer of the recombinant epsps gene from soybean 40-3-2 to environmental microorganisms is regarded to be negligible due to the lack of a selective advantage in the context of its intended use that would be conferred. Taking into account the scope of the application, the rare occurrence of feral soybean plants and the low levels of exposure through other routes, indicate that the risk to non-target organisms is extremely low. The scope of the monitoring plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean 40-3-2 since cultivation is excluded.
In conclusion, on the basis of the information considered in the original application, updated studies in the present applications, and other peer-reviewed scientific data on soybean 40-3-2, the EFSA GMO Panel confirms that soybean 40-3-2 is as safe and nutritious as the conventional counterpart and other commercial soybean varieties.

KEY WORDS

GMO, soybean, 40-3-2, glyphosate tolerance, risk assessment, food and feed safety, environment, import and processing, Regulation (EC) No 1829/2003, renewal.

SUMMARY

This document provides a scientific opinion of the Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) on two applications (References EFSA-GMO-RX-40-3-2$_{[8\text{-}1a/20\text{-}1a]}$ and EFSA-GMO-RX-40-3-2$_{[8\text{-}1b/20\text{-}1b]}$) submitted by Monsanto under Regulation (EC) No 1829/2003 for renewal of the authorisation for continued marketing of (1) food containing, consisting of, or produced from genetically modified (GM) soybean 40-3-2 (Unique Identifier MON-Ø4Ø32-6); (2) feed containing, consisting of, or produced from soybean 40-3-2; and (3) other products containing or consisting of soybean 40-3-2 with the exception of cultivation.

The scopes of the two renewal applications cover the continued marketing of:

- existing food containing, consisting of, or produced from soybean 40-3-2 (including food additives) (Reference EFSA-GMO-RX-40-3-2$_{[8\text{-}1a/20\text{-}1a]}$) that have been placed on the market in accordance with Part C to the Directive 90/220/EC before the entry into force of Regulation (EC) No 258/97 and under Directive 89/107/EEC (Commission Decision 96/281/EC);

- existing feed containing, consisting of, or produced from soybean 40-3-2 (Reference EFSA-GMO-RX-40-3-2$_{[8\text{-}1b/20\text{-}1b]}$) that have been placed on the market in accordance with Part C to the Directive 90/220/EEC (Commission Decision 96/281/EC) and as feed materials and feed additives subject to Directive 70/524/EEC;

- other products containing or consisting of soybean 40-3-2 with the exception of cultivation (Commission Decision 96/281/EC).

After the date of entry into force of the Regulation (EC) No 1829/2003, the products mentioned above were notified to the European Commission according to Articles 8 or 20 of this Regulation and subsequently included in the Community Register of GM food and feed.

Soybean 40-3-2 has been developed for tolerance to glyphosate herbicides by the introduction, via particle gun acceleration technology, of a gene coding for 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium tumefaciens* (renamed *Rhizobium radiobacter*) strain CP4 (CP4 EPSPS). In delivering its scientific opinion, the EFSA GMO Panel considered the renewal applications (EFSA-GMO-RX-40-3-2$_{[8\text{-}1a/20\text{-}1a]}$, EFSA-GMO-RX-40-3-2$_{[8\text{-}1b/20\text{-}1b]}$); a consolidated application on the cultivation of soybean 40-3-2 (application EFSA-GMO-NL-2005-24); additional information submitted by the applicant on request of the EFSA GMO Panel; the scientific comments submitted by Member States; and relevant scientific publications. In accordance with the Guidance Document for renewal of authorisations of existing GMO products, the EFSA GMO Panel has taken into account the new information, experience and data on soybean 40-3-2, which have become available during the authorisation period.

The EFSA GMO Panel assessed soybean 40-3-2 with reference to the intended uses and appropriate principles described in the Guidance Documents of the EFSA GMO Panel for the Risk Assessment of Genetically Modified Organisms and Derived Food and Feed and the Guidance Document of the Scientific Panel on Genetically Modified Organisms for renewal of authorisations of existing GMO products lawfully placed on the market. The scientific assessment included molecular characterisation of the inserted DNA and expression of the target proteins. A comparative analysis of agronomic traits and composition was undertaken and the safety of the new protein and the whole food/feed were evaluated with respect to potential toxicity, allergenicity and nutritional quality. An assessment of environmental impacts and the post-market environmental monitoring plans were undertaken.

The molecular characterisation data establish that the genetically modified soybean 40-3-2 contains one functional insert expressing CP4 EPSPS and a non-functional insert consisting of a 72 bp

fragment of the CP4 EPSPS coding sequence. No other parts of the plasmid used for transformation are present in the transformed plant. Updated bioinformatic analyses of the flanking sequences and the open reading frames spanning the insert-plant DNA junctions and the levels of the newly expressed protein in soybean 40-3-2 did not raise any safety concern. The stability of the inserted DNA was confirmed over several generations and a Mendelian inheritance pattern was demonstrated.

The EFSA GMO Panel compared the composition and agronomic characteristics of soybean 40-3-2 and its conventional counterpart, assessed all statistical differences identified, and came to the conclusion that soybean 40-3-2 is compositionally and agronomically equivalent to its conventional counterpart and other commercial soybean varieties, except for the expressing the glyphosate tolerance trait. The risk assessment of the newly expressed protein and the whole crop included an analysis of data from analytical studies, bioinformatics, and *in vitro* and *in vivo* studies. The EFSA GMO Panel concluded that the soybean 40-3-2 is as safe as its conventional counterpart and that the overall allergenicity of the whole plant is not changed.

According to the information provided by the applicant, food and feed products produced from soybean 40-3-2 have been consumed without reports of adverse effects since they were approved in the EU in 1996. Scientific publications which have become available since the previous evaluation of soybean 40-3-2 by the Advisory Committee of the Competent Authority of the United Kingdom (UK-ACNFP, 1995) did not raise safety issues. In addition, bioinformatics studies comparing the amino acid sequences of the newly expressed CP4 EPSPS protein in soybean 40-3-2 with amino acid sequences in updated databases of toxic or allergenic proteins confirmed the results of the older studies which identified no relevant similarities to known toxic or allergenic proteins.

The applications EFSA-GMO-RX-40-3-2 concern food containing, consisting of, or produced from genetically modified (GM) soybean 40-3-2 (Unique Identifier MON-Ø4Ø32-6); feed containing, consisting of, or produced from soybean 40-3-2; and other products containing or consisting of soybean 40-3-2 with the exception of cultivation. Therefore, there is no requirement for scientific assessment of possible environmental effects associated with the cultivation of soybean 40-3-2. There are no indications of an increased likelihood of establishment and spread of feral soybean plants in case of accidental release into the environment of viable grains produced by soybean 40-3-2 during transportation and processing, except in the presence of glyphosate herbicides. Taking into account the scope of the applications, the rare occurrence of feral soybean plants and the low levels of exposure through other routes, the risk to non-target organisms is extremely low. The EFSA GMO Panel considers unlikely that the recombinant DNA in soybean 40-3-2 transfers to bacteria and other microorganisms and that the risk caused by a rare but theoretically possible transfer of the recombinant epsps gene from soybean 40-3-2 to environmental microorganisms is regarded to be negligible due to the lack of a selective advantage in the context of its intended use that would be conferred. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan. The EFSA GMO Panel recommends that appropriate management systems should be in place to restrict seeds of soybean 40-3-2 entering cultivation as the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

In conclusion, the EFSA GMO Panel considers that the information available for soybean 40-3-2 addresses the scientific comments raised by the Member States and that the soybean 40-3-2, as described in these applications, is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment in the context of its intended uses. The EFSA GMO Panel concludes that soybean event 40-3-2 is unlikely to have any adverse effect on human and animal health and the environment, in the context of its intended uses.

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BACKGROUND

On 29 June 2007, the European Food Safety Authority (EFSA) received from the European Commission two applications submitted under Regulation (EC) No 1829/2003 for renewal of the authorisation of (1) food containing, consisting of, or produced from genetically modified (GM) soybean 40-3-2 (Unique Identifier MON-Ø4Ø32-6); (2) feed containing, consisting of, or produced from soybean 40-3-2; and (3) other products containing or consisting of soybean 40-3-2 with the exception of cultivation, developed by Monsanto to provide tolerance to glyphosate herbicides.

The scopes of the two renewal applications cover the continued marketing of:

- existing food containing, consisting of, or produced from soybean 40-3-2 (including food additives) (Reference EFSA-GMO-RX-40-3-2$_{[8\text{-}1a/20\text{-}1a]}$) that have been placed on the market in accordance with Part C to the Directive 90/220/EC before the entry into force of Regulation (EC) No 258/97 and under Directive 89/107/EEC (Commission Decision 96/281/EC);

- existing feed containing, consisting of, or produced from soybean 40-3-2 (Reference EFSA-GMO-RX-40-3-2$_{[8\text{-}1b/20\text{-}1b]}$) that have been placed on the market in accordance with Part C to the Directive 90/220/EEC (Commission Decision 96/281/EC) and as feed materials and feed additives subject to Directive 70/524/EEC;

- other products containing or consisting of soybean 40-3-2 with the exception of cultivation (Commission Decision 96/281/EC).

After the date of entry into force of the Regulation (EC) No 1829/2003, the products mentioned above were notified to the European Commission according to Articles 8 or 20 of this Regulation and subsequently included in the Community Register of GM food and feed.

Soybean 40-3-2 was the subject of an earlier safety assessment (UK-ACNFP, 1995) and has been authorised (EC, 1996) under Directive 90/220/EEC. In addition, national approvals for the food and feed use of soybean 40-3-2 and its derivatives were received from the United Kingdom, The Netherlands and Denmark prior to the entry into force of Regulation (EC) No 258/97.

After receiving the renewal applications (EFSA-GMO-RX-40-3-2$_{[8\text{-}1a/20\text{-}1a]}$ and EFSA-GMO-RX-40-3-2$_{[8\text{-}1b/20\text{-}1b]}$) and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed Member States as well as the European Commission and made the summary of these applications publicly available on the EFSA website[4]. EFSA initiated a formal review of the renewal applications to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 03 March 2008, EFSA received additional information requested under completeness check (requested on 14 January 2008) and on 12 March 2008, EFSA declared the applications as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid applications available to Member States and the European Commission, and consulted nominated risk assessment bodies of Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC (EC, 2001) following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. The Member State bodies had 3 months after the date of receipt of the valid applications (until 13 June 2008) within which to make their opinion known.

[4] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2007-141 and http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2007-142

The EFSA GMO Panel carried out the safety evaluation of the renewal applications of the soybean 40-3-2 in accordance with the appropriate principles described in the EFSA GMO Panel Guidance Documents for the risk assessment of GM plants and derived food and fed (EFSA, 2006b) and the Guidance Document of the Scientific Panel on Genetically Modified Organisms for renewal of authorisations of existing GMO products lawfully placed on the market (EFSA, 2006a). In addition, the scientific comments of Member States, the additional information provided by the applicant; the information provided in the context of application EFSA-GMO-NL-2005-24 and relevant scientific publications were taken into consideration.

The EFSA GMO Panel requested additional information from the applicant on (1) 15 July 2008, 12 September 2008, 11 December 2008 and 16 March 2010 for application EFSA-GMO-RX-40-3-2$_{[8\text{-}1a/20\text{-}1a]}$; (2) 12 September 2008, 11 December 2008 and 16 March 2010 for application EFSA-GMO-RX-40-3-2$_{[8\text{-}1b/20\text{-}1b]}$. The applicant provided the requested information on (1) 01 December 2008, 23 December 2008, 20 August 2009 and 15 July 2010 for application EFSA-EFSA-GMO-RX-40-3-2$_{[8\text{-}1a/20\text{-}1a]}$; (2) 23 December 2008, 20 August 2009 and 15 July 2010 for application EFSA-GMO-RX-40-3-2$_{[8\text{-}1b/20\text{-}1b]}$. Moreover, the EFSA GMO Panel considered the application and additional information submitted in the context of application EFSA-GMO-NL-2005-24 (soybean 40-3-2 for cultivation).

In giving its scientific opinion on soybean 40-3-2 to the European Commission, the Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of six months from the acknowledgement of the valid applications. As additional information was requested by the EFSA GMO Panel, the time-limit of six months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

According to Regulation (EC) No 1829/2003, this scientific opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation and thus will be part of the overall opinions in accordance with Articles 6(5) and 18(5).

TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific assessment of soybean 40-3-2 for the renewal of authorisation of (1) existing food containing, consisting of, or produced from soybean 40-3-2 (including food additives) (Reference EFSA-GMO-RX-40-3-2$_{[8\text{-}1a/20\text{-}1a]}$) that have been placed on the market in accordance with Part C to the Directive 90/220/EC before the entry into force of Regulation (EC) No 258/97 and under Directive 89/107/EEC (Commission Decision 96/281/EC); (2) feed containing, consisting of, or produced from soybean 40-3-2 (Reference EFSA-GMO-RX-40-3-2$_{[8\text{-}1b/20\text{-}1b]}$) that have been placed on the market in accordance with Part C to the Directive 90/220/EEC (Commission Decision 96/281/EC) and as feed materials and feed additives subject to Directive 70/524/EEC; and (3) other products containing or consisting of soybean 40-3-2 with the exception of cultivation (Commission Decision 96/281/EC). Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of GMOs or food/feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environments and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)e of Regulation (EC) No 1829/2003.

The EFSA GMO Panel was not requested to give a scientific opinion on information required under Annex II of the Cartagena Protocol. Furthermore, the EFSA GMO Panel did not consider proposals for labelling and methods of detection (including sampling and the identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to risk management.

ASSESSMENT

1. Introduction

The initial safety assessment of soybean 40-3-2 (Unique Identifier MON-Ø4Ø32-6) was conducted according to Directive 90/220/EEC. During this process, the Advisory Committee on Releases to the Environment (ACRE), acting as the scientific authority of the UK Competent Authority, its sister organisations within the UK, as well as the Competent Authorities of the other Member States, concluded that this product did not pose safety concerns and that no risk management measures such as specific monitoring were required. This led to the Commission giving consent under Directive 90/220/EEC in 1996 (EC, 1996). In addition, national approvals for the food and feed use of soybean 40-3-2 and its derivatives were received from the United Kingdom, The Netherlands and Denmark prior to the entry into force of Regulation (EC) No 258/97. Switzerland also granted approval for import and use in 1996.

In addition to the renewal applications EFSA-GMO-RX-40-3-2$_{[8\text{-}1a/20\text{-}1a]}$ and EFSA-GMO-RX-40-3-2$_{[8\text{-}1b/20\text{-}1b]}$, the applicant submitted to EFSA an application under Regulation EC No 1829/2003 (EFSA-GMO-NL-2005-24) for cultivation of soybean 40-3-2, which gather all the data supporting the safety of soybean 40-3-2 and complement the renewal applications. The scientific assessment in the cultivation application included the transformation process, the vectors used and the transgenic constructs in the GM plants. A comparative analysis of agronomic traits and composition was undertaken and the safety of the new proteins and the whole food/feed was evaluated with respect to toxicology and allergenicity. Although it also contained an extensive environmental risk assessment, this information was not necessary for the renewals concerned in the present opinion.

The assessment presented here is based on the information provided by the applicant in the renewal applications EFSA-GMO-RX-40-3-2$_{[8\text{-}1a/20\text{-}1a]}$ and EFSA-GMO-RX-40-3-2$_{[8\text{-}1b/20\text{-}1b]}$ for continued marketing of food containing, consisting of, or produced from genetically modified (GM) soybean 40-3-2; feed containing, consisting of, or produced from soybean 40-3-2; and other products containing or consisting of soybean 40-3-2 with the exception of cultivation, appropriate sections of the application EFSA-GMO-NL-2005-24 for cultivation of soybean 40-3-2, additional information submitted by the applicant in response to questions requested from the EFSA GMO Panel, as well as comments from Member States and relevant scientific publications. The assessment has taken into account the appropriate principles described 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, 2006b), and the Guidance Document of the Scientific Panel on Genetically Modified Organisms for renewal of authorisations of existing GMO products lawfully placed on the market, notified according to Articles 8 and 20 of Regulation (EC) No 1829/2003 (EFSA, 2006a).

Information in the applications include 1) updated information on the comparative compositional analysis; 2) an estimation of the human and live-stock exposure in Europe to soybean 40-3-2; 3) an update on peer-reviewed scientific data on soybean 40-3-2, and 4) updated information on potential for allergenicity and toxicity, including updated homology searches between the newly expressed proteins and known toxic and allergenic proteins.

2. Issues raised by the Member States

The comments raised by the Member States are addressed in Annex G of the EFSA overall opinions[5] and have been considered in this scientific opinion.

3. Molecular characterisation

3.1. Evaluation of relevant scientific data

3.1.1. Transformation process and vector constructs[6]

Soybean tissue, derived from cultivar A5403 was transformed with plasmid PV-GMGT04 using particle acceleration. The plasmid PV-GMGT04 contains two CP4 *epsps* expression cassettes conferring resistance to glyphosate herbicides, the marker gene *uidA* coding for β-D-glucuronidase (GUS) derived from *Escherichia coli* and the neomycin phosphotransferase (*npt*II) gene conferring resistance to kanamycin and neomycin for selection in *E. coli* and the *E. coli* origin of replication ColE1.

The first CP4 epsps expression cassette consists of the following elements: an enhanced 35S promoter derived from *Cauliflower mosaic virus*, the CTP4 N-terminal chloroplast transit peptide sequence from the *epsps* gene of *Petunia hybrida*, the coding sequence of CP4 *epsps* from *Agrobacterium tumefaciens* (renamed *Rhizobium radiobacter*) and the 3' *nos* terminator from *A. tumefaciens*. The second CP4 *epsps* expression cassette contains the same elements as the first cassette except for the *fmv* promoter from the *Figwort mosaic virus* which replaces the 35S promoter. The *uidA* gene is under control of the mannopine synthase (*mas*) promoter from *A. tumefaciens* and the 3' terminator from soybean 7S globulin gene.

3.1.2. Transgene constructs in the genetically modified plant[7]

Southern analysis of genomic DNA isolated from leaves of soybean 40-3-2 digested with three different restriction enzymes was performed using the complete vector PV-GMGT04 as a probe. This analysis demonstrated the presence of two inserts: a functional and a non-functional one. Southern analysis also demonstrated the absence of the *fmv* promoter and the *uidA* gene in soybean 40-3-2. The ColE1 origin of replication and the *npt*II gene were not detected by PCR analysis.

Sequencing of the functional insert in soybean 40-3-2 demonstrated that in the 5' region of the insert the first 354 bp of the 35S promoter are absent, thereby removing a duplicate portion of the 35S enhancer region. An additional 250 bp of CP4 *epsps* was found adjacent to the 3' *nos* terminator. With these exceptions, the nucleotide sequence of the insert is identical to the corresponding sequence of PV-GMGT04.

Sequencing demonstrated that the non-functional insert consists of 72 bp of the CP4 *epsps* coding sequence.

[5] http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2007-141 and http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2007-142

[6] Technical Dossier / Section C1

[7] Technical Dossier / Section D2

Nucleotide sequences of the flanking regions have been determined. This includes 186 bp (at the 5') and 534 bp (at the 3') flanking the functional insert and 505 bp (at the 5') and 502 bp (at the 3') flanking the non-functional insert. The 3' flanking sequence of the functional insert has been shown to be rearranged soybean genomic DNA. The results of BLASTn and BLASTx analyses of the flanking sequences of both inserts do not indicate the disruption of known coding or regulatory sequences in 40-3-2 of soybean.

Updated bioinformatic analyses (2010)[8] of the DNA sequences of the functional and non-functional inserts and their flanks have been provided. The results indicate that in the unlikely event that any of the ORFs spanning the junctions were to be transcribed and translated, the translation products would not share significant similarity to known allergens, toxins, or other bioactive peptides.

3.1.3. Information on the expression of the insert[9]

Analysis of CP4 EPSPS protein levels was carried out by ELISA using seed and leaf samples from plants grown in 1992 and 1993 in the USA (at seven and four locations, respectively) and in 1998 at seven European locations in France and Italy. Mean protein levels in leaves of unsprayed plants in 1998 ranged from 0.32 to 0.62 µg/mg fresh weight (fw), and from 0.31-0.86 µg/mg fw in 1993. CP4 EPSPS protein levels were not determined in leaves in 1992. In seed samples of unsprayed plants mean protein levels were 0.09 to 0.27 µg/mg fw in 1998, 0.26 to 0.38 µg/mg fw in 1992 and 0.17 to 0.29 µg/mg fw in 1993. No significant differences in CP4 EPSPS protein levels were observed between glyphosate treated and non-treated samples in the European or USA studies. The levels of the newly expressed protein do not pose a safety concern (see also section 4.2.3. and 5.).

Northern analysis indicates that soybean 40-3-2 produces read-through transcripts initiated by the 35S promoter and which extend through the nos terminator into soybean genomic sequences flanking the 3' end of the functional insert (Rang et al., 2005). These transcripts are produced at very low levels (estimated to be 75 times lower than the intended transcript). However, no fusion proteins that might result from these read-through transcripts were detected by Western analysis (Rogan et al., 1999). If a fusion protein were to be produced at a level below the detection limit, bioinformatic analysis indicates that such a protein would not show similarity to known allergens or toxins.

3.1.4. Inheritance and stability of inserted DNA[10]

The inheritance of the introduced trait in soybean 40-3-2 follows a Mendelian pattern. Phenotypic stability was determined by application of glyphosate herbicides over multiple generations in two breeding lines. In addition, phenotypic stability was demonstrated in trials over four generations of soybean 40-3-2 in different genetic backgrounds at multiple geographical locations in the USA. Genetic stability of soybean 40-3-2 was demonstrated over four generations by Southern analysis.

3.2. Conclusion

The molecular characterisation data establish that the GM soybean 40-3-2 contains one functional insert expressing CP4 EPSPS and a non-functional insert consisting of a 72 bp fragment of the CP4 EPSPS coding sequence. No other parts of the plasmid used for transformation are present in the transformed plant. Updated bioinformatic analyses of the flanking sequences and the open reading

8 Additional information, July 2010
9 Technical Dossier / Section D3
10 Technical Dossier / Section D5

frames spanning the insert-plant DNA junctions and the levels of the newly expressed protein in soybean 40-3-2 did not raise any safety concern. The stability of the inserted DNA was confirmed over several generations and a Mendelian inheritance pattern was demonstrated. The EFSA GMO Panel considers that the molecular characterisation does not indicate a safety concern.

4. Comparative analysis

4.1. Comparative compositional and agronomic/phenotypic assessment

The original food safety assessment of soybean 40-3-2 within the European Union was performed by the Advisory Committee on Novel Foods and Processes in the UK(UK-ACNFP, 1995). Similarly, the Advisory Committee on Release to the Environment (ACRE) to the Secretary of State for the Environment, Transport and the Regions and Minister of Agriculture, Fisheries and Food of the UK advised on the importation storage and use of soybean 40-3-2 for processing to non-viable soybean fractions suitable for use in animal feeds, foods and any other products in which soybean fractions are used. On that occasion ACRE concluded that the risk of marketing this product would be no different from that of other soybeans marketed for the same purposes[11].

4.1.1. Choice of comparator and production of material for the compositional assessment

The original field trials giving comparative data on agronomic and phenotypic characteristics, and materials for investigation of the chemical composition on soybean 40-3-2 and an appropriate non-GM soybean conventional counterpart were performed in Puerto Rico (1991-1992) and the USA (1992 and 1993), and were subsequently extended with compositional data of seed material collected in field trials in France (1998) and Italy (1998). The design of these field trials with respect to choice of comparator, replication, herbicide spraying regime, materials collected for compositional analysis, and compounds analysed, varied considerably, and were not in accordance with the current EFSA Guidance document (EFSA, 2006b). Following a request for a comprehensive assessment of these field trial data during an ongoing assessment of an application to cultivate soybean 40-3-2 within the EU (EFSA-GMO-NL-2005-24), the applicant provided compositional data on soybean forage and seeds from an additional field trial in Romania in 2005. This field trial, which was designed essentially according to the EFSA Guidance document, compared the composition of soybean 40-3-2 with a conventional soybean variety having a comparable genetic background. The EFSA GMO Panel made a comprehensive comparative assessment of the compositional data in the application, but particularly focused on the data from the Romanian field trials.

In most compositional studies, the genetically modified (GM) soybean 40-3-2 was compared to the non-transgenic Asgrow variety A5403, which is the commercial soybean variety originally used when the soybean was transformed to establish transformation event 40-3-2. In cases where the GM event 40-3-2 had been bred into a soybean variety with another genetic background, the corresponding non-GM variety was used as conventional counterpart (Dekabig).

4.1.2. Compositional analysis

The Romanian field trials in 2005 were replicated and performed at five sites, and included soybean 40-3-2, the non-GM conventional counterpart (Dekabig), and a set of different conventional soybean varieties (Harrigan et al., 2007). The conventional soybeans were reference lines aimed to provide

11 http://tna.europarchive.org/20031224105948/http://www.defra.gov.uk/Environment/acre/annrep4/2.htm#2.4

data on the natural variation in composition of this food and feed plant. Whereas all varieties were treated with required conventional pesticides, soybean 40-3-2 was additionally treated with a glyphosate herbicide.

Soybean seeds were harvested and analysed for proximates (protein, fat, ash, and moisture), fibre fractions, amino acids, fatty acids, vitamin E, anti-nutrients (i.e. phytic acid, trypsin inhibitor, lectins, stachyose and raffinose) and other secondary metabolites (isoflavones). Forage was analysed for proximates, including fibre fractions. In total 63 different compounds were analysed in the materials from the Romanian field trials, fifty-six in seeds and seven in forage, essentially those recommended by OECD (2001). The early field studies were analysed for a lower number of constituents.

When the compositional data for forage and seed samples from the Romanian field trial were evaluated across sites, a statistically significant difference between soybean 40-3-2 and its conventional counterpart was found only for four of the 49 comparisons. These were acid detergent fibre in forage (31.93% vs. 30.26% dry weight (DW)), and isoleucine (1.69% vs. 1.73% DW), valine (1.80% vs. 1.84% DW), and genistein (1642 vs. 1717 μg/g DW) in seeds. However, when evaluated per site, the level of these constituents was significantly different at only one of the five field trial sites. Differences were small and levels fell within the normal variation of soybean constituents demonstrated by the reference soybeans included in the study and described in the ILSI (2006) and the USDA-ISO (2006) isoflavone databases. In addition to the differences mentioned above additional statistically significant differences were found for other constituents in the per site analysis. Twenty of these were found at one site only, and four at two of the five sites. Also in these cases differences were small, not consistent, and levels fell within the normal variation established by the reference lines.

The EFSA GMO Panel concludes that the data of the Romanian field trials confirmed the data from field trials in France and Italy in 1998, and the United States in 1992 and 1993. The studies from the United States have been published (Padgette et al., 1996; Taylor et al., 1999) and were considered by UK-ACNFP in their original safety assessment of soybean 40-3-2. Materials from the 1992 harvest were also used to analyse processed products. Defatted toasted meal was analysed for proximates, trypsin inhibitor, lectins, urease, isoflavones, staychose, raffinose and phytate; non-toasted meal for proximates, urease, trypsin inhibitor; protein isolate and protein concentrate for proximates; lecithin for phosphorylated compounds, and refined, bleached, deodorised soybean oil for fatty acids. It was concluded that the composition of processed products of soybean 40-3-2 were equivalent to those of the convention counterpart.

Further compositional comparisons (proximates, lectin, trypsin inhibitor, and isoflavones) between seeds of soybean event 40-3-2 crossed into soybeans of diverse genetic background and seeds of the corresponding conventional counterpart without the 40-3-2 event harvested in the 2000, 2001 and 2002 field seasons in the United States and Canada have been published by McCann et al. (2005). These additional studies found that the level of the measured analytes sometimes varied considerably across years but that the mean and range in soybean 40-3-2 is similar to the mean and range of conventional soybean varieties.

Since the original safety assessment of UK-ACNFP (1995), several investigators have confirmed the compositional equivalence of soybean 40-3-2 and commercial soybean varieties with regard to the content of isoflavone isomers, saponins, phospholipids, trypsin inhibitors, and lectins (List et al., 1999; Novak and Haslberger, 2000; Goda et al., 2002; Wei et al., 2004). One report claims soybean 40-3-2 to contain 12-14% less isoflavones (mainly genistin) than conventional non-GM soybeans (Lappé et al., 1998/1999). On the other hand, several investigators have reported that these compounds vary significantly in soybeans (Taylor et al., 1999; Wei et al., 2004). It has also been reported that various strategies for glyphosate herbicides applications to soybean 40-3-2 have no market influence on the isoflavone content (Duke et al., 2003). The EFSA GMO Panel concludes that

the data obtained since the original safety assessment of soybean 40-3-2 confirms that it is compositionally equivalent to its conventional counterpart and to other commercial soybean varieties.

4.1.3. Agronomic traits and GM phenotype, including ecological interaction

The applicant performed comparative assessments of the phenotypic and agronomic characteristics, and of the reproduction, dissemination, and survivability of soybean 40-3-2 and conventional soybeans based on field trials in the USA and Puerto Rico (1991-1994), Argentina (1993-1994), Canada (1993-1994), France (1994), and Italy (1994, 1996, and 1997). Parameters studied included date of emergence, % emergence, plant count, plant height, vigour and colour, morphological changes, date at 50% flowering, susceptibility to insects, nodes per plant, pods per plant, % lodging, % leaf drop, yield and % moisture, reproduction, dissemination and survivability. No meaningful difference between soybean 40-3-2 and its conventional counterpart were identified, except the expected difference in tolerance to glyphosate herbicides.

After commercial introduction of soybean 40-3-2 in North America, various research groups have published data on yield, height and glyphosate tolerance (Delannay et al., 1995; Elmore et al., 2001a, 2001b), as well as data on susceptibility of soybean 40-3-2 to insect pests (Morjan and Pedigo, 2002; McPherson et al., 2003), nematode damage (Koennig, 2002; Yang et al., 2002), and diseases, including resistance to fungal pathogens (Lee et al., 2000; Sanogo et al., 2000, 2001; Harikrishnan and Yang, 2002; Mueller et al., 2003; Njiti et al., 2003). These data contribute to the conclusion that the characteristics of soybean 40-3-2 do not differ from those of conventional soybean varieties, except for soybean 40-3-2 giving a slightly reduced yield (Elmore et al., 2001a), still within the range in yield of commercial soybean varieties, and being glyphosate tolerant as a consequence of the newly introduced trait. The EFSA GMO Panel accepted the applicants conclusion that soybean 40-3-2 is phenotypically and agronomically equivalent to traditional soybeans, except for the introduced glyphosate tolerance trait.

4.1.4. Conclusion

The EFSA GMO Panel considered the total set of compositional and agronomical data that have become available since the safety assessment of soybean 40-3-2 by the UK-ACNFP was published in 1995. Any statistically significant differences identified between soybean 40-3-2 and its conventional counterpart were assessed in the light of the field trial design, the level of the studied compounds in relation to identified biological variation, and agronomic and phenotypic characteristics in conventional soybean varieties. The EFSA GMO Panel concludes that soybean 40-3-2 is compositionally and agronomically equivalent to its conventional counterpart, and other conventional soybean varieties, except for the expression of the CP4 EPSPS protein. Furthermore, no unintended effects have appeared as a result of the genetic modification.

4.2. Food and feed safety assessment

In originally assessing the food safety of soybean 40-3-2 and products derived from them, the UK-ACNFP (1995) used a comparative approach to determine whether these soybeans are nutritionally, and with regard to safety, similar to conventional soybeans and products derived from them. Issues related to feeds were considered by the UK Inter-Departmental Group on New Feed Developments. The advisory committee noted that soybeans are not consumed or used in food (and feed) in an unprocessed form because they naturally contain anti-nutrients such as trypsin inhibitors which may give adverse effects if not destroyed by heating. The Committee was satisfied that the genetic

modification procedure had proceeded as intended and that the only complete novel gene present in soybean 40-3-2 is the CP4 *epsps* gene. The enzyme expressed from this gene is found only at very low levels in the GM soybeans (<0.1%) and is not detectable in oil derived from the GM beans. Soybeans are known to be allergenic. However, the levels of known allergenic proteins found in the modified beans were also similar to those found in conventional beans. The UK-ACNFP concluded that the GM soybeans and products derived from them are comparable to and as safe for human consumption as conventional, unmodified soybeans and products derived from them (UK-ACNFP, 1995). The Committee, therefore, recommended clearance for use in food of soybeans from the genetically modified soybean 40-3-2 and other glyphosate tolerant lines derived from subsequent crosses of this line with other commercial soybean cultivars.

In addition to the information available in the original applications, taken into account by the UK-ACNFP when giving its opinion on the food safety of soybean 40-3-2 (UK-ACNFP, 1995), the present renewal applications contain a few updated studies (bioinformatics comparison of amino acid sequence similarity of the newly expressed protein to known toxic or allergenic proteins), and a commentary on peer-reviewed publications on food and feed issues related to soybean 40-3-2 published after the approval to market these products were given in 1996. Issues specifically addressed in the update included information on areas where soybean 40-3-2 have been cultivated and the quantity produced, amounts imported into the EU, and the known and estimated human and animal exposure to soybean 40-3-2.

4.2.1. History of exposure to soybean 40-3-2 in Europe

Soybean 40-3-2 was first cultivated in the U.S.A. and Argentina in 1996, and subsequently commercialised in Canada, Uruguay, South Africa, Brazil, Romania and Paraguay. Thus, in Romania the bean was commercially produced between 1999 and 2006, prior to the accession to the EU in 2007. Production of soybean 40-3-2 was rapidly adopted in many markets, but most notably in the U.S.A. and Argentina, where current adoption rates exceed 90% of total soybean production area. When soybean 40-3-2 production was discontinued in Romania in 2006 it was cultivated on 84% of the area devoted to soybean cultivation.

Based on data on import of soybean seed, soybean meal and soybean oil into the 27 countries of the European Community from five 40-3-2 soybean producing countries (Argentina, Brazil, Canada, Paraguay and the USA) during the years 2003-2006, the applicant calculated that around 55% of soybean seed, 61% of soybean meal and 54% of soybean oil used in the EU might be based on soybean 40-3-2. It should be noted, however, that the calculations of these figures are based on several assumptions. Because operators in the food and feed chain in some Member States of the European Community have made efforts to preferentially source non-GM soybean products, the actual consumption of products derived from soybean 40-3-2 in food and feed may vary between Member States.

Based on FAO Statistics from 1997 to 2001, the human soybean oil consumption in Europe was calculated at 6.3-7.0 g/person/day. Assuming that 54% of the soybean oil was derived from soybean 40-3-2, an estimated average exposure of the European consumer to products of soybean 40-3-2 would be in the range of 3.4-3.7 g/person/day.

Animal feed is the major end use of soybean meal. The applicant calculated, based on data from 2006, that the maximum inclusion levels (% of the diet) of soybean 40-3-2 meal in the EU would be 21% for broiler chickens, 18% for pigs and 12% for dairy cattle.

Although no post-market monitoring for food and feed safety of soybean 40-3-2 has formally been performed, there is no evidence of any adverse effects being associated with the consumption of soybean 40-3-2 as food or feed within the European community.

4.2.2. Effects of processing

In the initial risk assessment the UK-ACNFP noted that the only protein present in soybean 40-3-2 as a result of the newly introduced DNA is the CP4 EPSPS enzyme. The enzyme is responsible for the soybean becoming tolerant to herbicides containing the active principle glyphosate. Soybean 40-3-2 will be used for production and manufacturing of food and feed products, as any other commercial soybean variety. Taking into account the compositional analysis providing no indication of relevant compositional changes, the EFSA GMO Panel has no reason to assume that the characteristics of soybean 40-3-2 and derived processed products would be different from those of the respective products derived from conventional soybean varieties. Intermediate temperatures (55°C) will reduce the activity of the CP4 EPSPS enzyme, whereas higher temperatures (65° and 75°C) will completely inactivate the enzyme. The pH will have less influence on the activity, only slightly lowering it at the low end of the pH range 4-11. Studies by Kim et al. (2006b) showed that the CP4 EPSPS enzyme is degraded during preparation of foods such as tofu and soybean paste.

Similarly, other investigators processed glyphosate tolerant soybeans by grinding, cooking, blending, homogenisation, sterilisation and spray-drying in order to study the fate of the soybean DNA in foods such as bean curd, soy milk and soy powder (Chen et al., 2005). In these studies an endogenous gene (*lectin*) present in all soybean varieties was compared with the CP4 *epsps* gene specific for soybean 40-3-2. Although both genes were degraded to various extents by the different processing procedures, the endogenous *lectin* gene was more stable than the introduced CP4 *epsps* gene. Large DNA fragments were affected more by processing than small ones. Thus, in processed foods and feeds mainly fragments of the CP4 *epsps* gene can be expected, and the size of the fragment would be dependent on the type of processing applied (Chen et al., 2005). Bauer et al. (2003) confirm that pH and temperature are important factors for DNA degradation when preparing foods from soybean 40-3-2.

4.2.3. Toxicological assessment of expressed novel protein in soybean 40-3-2

Submitted data indicated that CP4 EPSPS is unlikely to constitute a hazard to health. Thus, in an acute toxicity study in mice (Harrison et al., 1996), the CP4 EPSPS protein resulted in no adverse effects up to the highest dose administered (572 mg/kg body weight). Furthermore, the original data demonstrated a low expression of the CP4 EPSPS protein in soybean 40-3-2 (<0.1%). The protein was not detectable in soybean oil and showed no meaningful amino acid sequence homology to known toxic proteins (UK-ACNFP, 1995). Since the original submission of the soybean 40-3-2 application in 1994, the databases used to compare newly expressed proteins with known toxins (TOXIN database) have been updated several times and been published. Bioinformatics-supported studies with the updated databases, revealed no biologically relevant structural similarities between CP4 EPSPS and known toxic proteins.

Degradation in the gastrointestinal tract

UK-ACNFP also assessed *in vitro* digestion studies using simulated gastric fluid, which demonstrated that CP4 EPSPS is rapidly degraded at conditions mimicking the stomach (Harrison et al., 1996). Rapid digestion of microbially produced CP4 EPSPS, as well as of CP4 EPSPS extracted from soybean 40-3-2, has later been confirmed in studies using pepsin and pancreatin digestion assays

(Okunuki et al., 2002; Chang et al., 2003; Kim et al., 2006b). Pre-heating of soybean extracts containing the enzyme increased digestibility. No stable degradation fragments were formed. In the original risk assessment UK-ACNFP (1995) considered the potential for genetic transfer of the CP4 *epsps* gene from soybean 40-3-2 and derived products to human consumers, or their gut microflora, and concluded that the risk was negligible since the soybeans would not be consumed in a viable form and the processes used to derive the soybean products would destroy the DNA and protein. Subsequently, the fate of dietary CP4 *epsps* DNA and CP4 EPSPS protein as compared to plant DNA and proteins in general, have been studied both in laboratory animals and farm animals. Data are available from rats, broiler chickens, pigs, cows, salmon, rainbow trout and rabbits. There are also data from *in vitro* studies using tissues. These studies show that the CP4 EPSPS protein is easily degraded as levels are below the limit of detection in eggs, liver and faeces (Ash et al., 2003) as well as in muscle tissue (Jennings et al., 2003; Zhu et al., 2004) of hens, pigs and rats fed soybean 40-3-2. Thus, digestion seems to result in levels where no detectable protein is absorbed in the tissues investigated.

Data have been published about the fate of the CP4 *epsps* gene during digestion of various raw and processed dietary products of soybean 40-3-2. The results from a study employing an *in vitro* systems in which DNA was incubated subsequently with pepsin and ileal digesta in order to simulate the human digestive system have shown that less than 5% of the CP4 *epsps* transgenic DNA survive for three hours. It was considered that the DNA that survived, may be so fragmented that it is of limited biological significance, and thus may represent no apparent health risk (Martin-Orue et al., 2002). With detection methods sensitive enough, fragments of the CP4 *epsps* gene can easily be detected early in the digestive tract of broilers, but less easily further down in the tract and in the faeces (Deaville and Maddison, 2005). Fragments of the CP4 *epsps* gene were not detected in animal tissues (Deaville and Maddison, 2005; Jennings et al., 2003; Tudisco et al., 2006), or in blood (Chainark et al., 2008; Deaville and Madditon, 2005; Tudisco et al., 2006). Whereas some investigators found no CP4 *epsps* fragments in milk (and other tissues) of dairy cows (Phipps et al., 2002; 2003) and in the liver, muscle, and brain tissue of Atlantic salmon (Sanden et al., 2004), others reported that foreign DNA can be taken up by Atlantic salmon intestinal tissue and rainbow trout leukocytes, head kidney and muscle (Chainark et al., 2006, 2008; Sanden et al., 2007). The detection of transgenic DNA in fish tissue seems to be transient as it is no longer detectable in rainbow trout organs a couple of days after the intake of the 40-3-2 soybean meal (Chainark et al., 2006, 2008). It should be noted, however, that the multi-copy *rubisco* gene, common in plants, has been detected in several tissues of tested animals, such as in the blood and the milk (Deaville and Maddison, 2005; Phipps et al., 2003). It has been reported that CP4 *epsps* DNA has been detected in milk from the Italian market (Agodi et al., 2006), although in this case it is not known whether the molecules have a dietary origin or have contaminated the milk via air, animal feed or faeces.

Taken together, the studies investigating the digestive fate of the CP4 EPSPS protein and the CP4 *epsps* gene, indicate that no CP4 EPSPS protein accumulate in tissues of tested organisms, and that only fragments of DNA can be detected. Comparative studies on the digestive fate of endogenous and transgenic plant genes, show that these genes behave in a similar way.

4.2.4. Toxicological assessment of the whole soybean 40-3-2 food/feed

Although the chemical analysis provided showed soybean 40-3-2 to be compositionally equivalent to conventional soybean varieties (except for the newly expressed CP4 EPSPS protein), the applicant referred to four rat feeding studies with the GM soybean. Two of these were over four weeks with processed and unprocessed soybean 40-3-2, respectively. The other two were over thirteen and fifteen weeks with processed and heat-treated soybean 40-3-2, respectively.

In the first of the two 28-day studies, Charles River CD rats of both sexes (10 animals/sex) were fed *ad libitum* a diet with 24.8% processed (dehulled, defatted and toasted) soybean material from either event 40-3-2 or a conventional counterpart. An additional group of animals were fed a commercial rat diet containing dehulled soybean meal. Test animals survived and appeared healthy. The diet neither influenced feed consumption and body weights of the rats, nor had any significant influence on organ weights (only liver, testes, and kidneys measured). The few findings in the histopathological examinations at necropsy were randomly distributed among treatment groups and were commonly observed in control animals of this rat strain in the testing laboratory.

The second 28-day study had an experimental design very similar to the first study and also used CD rats of both sexes, but instead of feeding the animals processed soybean meal unprocessed meal was applied at inclusion rates of 5% and 10% of the diet. Such low inclusion rates might have been required as monogastric animals usually are not fed unprocessed soybeans due to the presence of anti-nutritive factors in the raw bean. Ruminants tolerate the raw material as the anti-nutrients are degraded by the rumen microflora. In this study test animals appeared healthy, and the diet neither influenced feed consumption, body weight and cumulative body weight gain, nor had any significant influence on absolute and relative organ weights (only liver, testes, and kidneys measured) in relation to the conventional counterpart. When soybean 40-3-2 fed rats were compared with rats fed the commercial rat feed, a slightly increased relative kidney weight was observed at a dose of 5% soybean 40-3-2 but not at the higher dose. As the influence on kidney weight was not dose-related, the finding was not considered relevant to the treatment. Animals that received the higher dose unprocessed soybean frequently showed darker livers, possibly related to the inclusion rate of unprocessed soybean and not to the genetic modification. The few findings in the histopathological examinations at necropsy were randomly distributed among all groups as in the first experiment. Since unprocessed soybean meal contains trypsin inhibitors that can cause hypertrophy of the pancreas when soybeans are the sole protein source (Liener and Kakade, 1980), this organ was examined histologically for all animals in the study. No pathological lesions, but minimal to mild microscopic changes were observed in the pancreas of animals of all groups. Thus, this characteristic was not related to the treatment with soybean 40-3-2.

The third study was a 90-day feeding study in Sprague-Dawley rats fed *ad libitum* diets with processed soybean 40-3-2 meal or meal from a conventional soybean (Zhu et al., 2004). The test diets contained 30%, 60% or 90% processed soybean 40-3-2 meal or 60% traditional/commercial soybean meal (conventional counterpart). The only deviation in feed intake and body weight was observed during the first week in rats of both sexes fed 90% soybean 40-3-2 meal, apparently due to the exposure to high protein levels and not to the exposure to soybean 40-3-2. Later on in the study, there was no influence on feed intake and body weight gain. No treatment-related adverse effects were observed in the study. There were also no meaningful differences in gross necropsy findings, haematology or urinalysis parameters between rats fed processed 40-3-2 and conventional soybean meal.

The final study was a 15-week rat feeding study with heat-treated soybean meal in female Brown Norway rats and female B10A mice, aiming to study potential effects on the immune system (Teshima et al., 2000). The heat-treated soybean meal was incorporated at a level of 30% in the rat and mice feed produced from soybean 40-3-2 in the test group and produced from a closely related conventional non-GM soybean in the control group. No treatment-related changes in growth, food consumption, liver and spleen weight between rats and mice fed 40-3-2 and animals fed the control soybean meal were observed. Based on the level of soybean-specific IgG and IgE in rodent sera and histological examination of immune-related organs, it was concluded that soybean 40-3-2 was not more antigenic or immunogenic than traditional soybeans.

A few additional rodent feeding studies with diet containing soybean 40-3-2 is available in the peer-reviewed literature. In two long-term studies over 52 and 104 weeks, respectively, Japanese

investigators fed F344 DuCrj rats diets that contained 30% either of a powder of processed soybean 40-3-2 or of the non-GM soybean conventional counterpart having a similar genetic background to soybean 40-3-2, or a basal diet (CE-2) (Sakamoto et al., 2007, 2008). When the three groups were compared, some statistically significant differences in animal growth, food intake, serum biochemical parameters and histological findings were noted, in particular between rats fed the two types of soybean diet (with GM and non-GM soybean) and the rats fed the basal diet. However, body weight and food intake were similar for the rats fed soybean 40-3-2 and conventional soybean. Gross necropsy findings, haematological and serum biochemical parameters, organ weights, and microscopic findings were comparable between rats fed soybean 40-3-2 and conventional soybean. In the 2-year study, the histopathological investigations did not reveal an increase in the incidence, nor in any specific type of non-neoplastic or neoplastic lesions in the GM soybean-exposed group of both sexes. The investigators concluded that the long-term effects of soybean 40-3-2 are not different than the long-term effects of non-GM soybeans.

Brake and Evenson (2004) fed pregnant C57Bl/6J mice transgenic (40-3-2) or non-transgenic soybean meal as 21.35% of the diet through gestation and lactation, and followed up by maintaining weanling young male mice on the respective diets until an age of 87 days. After different length of treatment, mice were killed, the testes surgically removed, and the cell populations measured by flow cytometry techniques. Multi-generational studies were conducted in a similar manner. The studies showed that soybean 40-3-2 had no different effect on macromolecular synthesis or cell growth and differentiation (as evidenced by no differences in the percentages of testicular cell populations) than conventional non-GM soybean. Furthermore, the different treatments resulted in no difference in litter size and body weights of mice. The investigators concluded that diets containing soybean 40-3-2 had no negative effect on foetal, postnatal, pubertal or adult testicular development.

Malatesta and co-workers in a series of publications summarised their result of studies in which histo-cytochemistry was performed on cells of specific organs, such as liver, pancreas, and testis, of progeny of Swiss mice fed during pregnancy and/or for 1, 2, 5, 8 or 24 months after weaning diets containing 14% soybean 40-3-2 or wild type soybean (Malatesta et al., 2002a, 2002b, 2003, 2005, 2008; Vecchio et al., 2004). In most studies only female mice were used. Although growth was comparable in animals receiving the two types of diets, and no macroscopic alterations or pathological lesions were found, the investigators reported to have identified differences in transcriptional activity, revealed as alterations in staining characteristics of chromatin-associated elements in cell nuclei. The investigators concluded from three animals per treatment only, and that no information was available on the natural variability in the specific histocytochemical endpoints analysed. The authors claimed that the altered staining characteristics indicate that feeding diets containing GM soybean may be associated with reversible changes in nucleic transcriptional activity, possibly as a consequence of exposure to residues of glyphosate, differences in phytoestrogen content between the diets, the genetic modification in soybean 40-3-2, or a combination of these. However, the experimental designs of the studies and their evaluation can be criticised. In particular the studies do not provide detailed account of the origin and characteristics of the control soybeans used, or whether the soybeans were processed or not. The levels of soybean bioactive constituents in the two diets were not stated. In addition, it is noted that in these studies particular biological phenomena were examined but not those parameters which are normally regarded as indicative for specific organ toxicity. Also the statistical evaluation of the data has been criticised. Therefore, the toxicological relevance of the findings, if any, is not clear.

More recently, the same research team reported on preliminary observations indicating that a diet containing soybean 40-3-2 neither affects fertility of female mice raised since weaning on a diet containing 14% GM soybean, nor parturition time or litter health (Cisterna et al., 2008). From a limited dataset, they concluded that a transient depression in pre-mRNA transcription and processing take place at the 2- to 8-cell stage of embryos, but that this transient episode does not affect foetal development. Also this study is weakened by a non-appropriate experimental design.

A transient mild histological alteration in the pancreas and a fast recovery has been reported in rats fed up to 30 days with a diet containing 18% soybean protein (Magaña-Gómez et al., 2008). Unfortunately, also in this study it is unclear whether the control diets used was based on soybean isogenic to soybean 40-3-2 or another type of commercially available non-GM soybean. It is also unclear whether the soybean products used have been appropriately processed before being included in the diet. Thus, it cannot be excluded that the transient alterations reported could have been the result of non-controlled levels of anti-nutrients in the diet.

The EFSA GMO Panel concludes that the feeding studies with laboratory animals to investigate potential toxicity demonstrate that soybean 40-3-2 and its derived products are as safe as conventional soybean varieties and their products.

4.2.5. Allergenicity

Assessment of allergenicity of the newly expressed protein

Theoretical assessment of the allergenic potential of the CP4 EPSPS protein by UK-ACNFP (1995) showed that it is unlikely to be an allergen since i) the CP4 *epsps* gene was taken from a source not known to be allergenic, and ii) the molecular weight of the protein and its glycosylation characteristics and acid lability are not indicative of an increased risk of allergenicity. In addition, a bioinformatics-supported comparison of the amino acid sequence of the CP4 EPSPS protein with the sequences of known allergens, gliadins, and glutenins (which included an updated analysis with published databases), identified no similarities which would cause concern.

European and Asian patients allergic to soybean and/or other foods do not express IgE that specifically bind the purified CP4 EPSPS protein (Chang et al., 2003; Batista et al., 2005; Kim et al., 2006a, 2006b; Hoff et al., 2007). The purified CP4 EPSPS enzyme also did not result in pronounced change in histamine release or cytokine production in sensitised peritoneal mast cells or unsensitised but antisera-labelled mast cells cultivated *in vitro* (Chang et al., 2003). The EFSA GMO Panel considers that these studies further confirm that the newly expressed CP4 EPSPS protein is unlikely to be allergenic.

Assessment of allergenicity of the whole GM plant

UK-ACNFP (1995) noted that soybeans are known to be allergenic for certain individuals. However, studies supplied in the original notification under Directive 90/220/EEC (Burks and Fuchs, 1995), allowed to conclude that the levels of known allergenic proteins in soybean 40-3-2 does not differ from the levels in non-GM soybeans. The results of these initial pre-marketing studies have recently been confirmed after the product has been on the market for some time. Using two-dimensional gel electrophoresis followed by peptide tandem mass spectrometry to identify soybean proteins, and Western analysis to evaluate the IgE response of soybean allergic individuals, Batista et al. (2007) were able to show that none of the five soybean-allergic individuals tested reacted differently to soybean 40-3-2 and its appropriate conventional counterpart. Similarly, several other investigations based on blood/sera of soybean allergic patients (from Denmark, Korea, Portugal) or on skin prick tests have found no difference in allergenic potential of extracts of soybean 40-3-2 and extracts of non-GM soybeans (Park et al., 2001; Sten et al., 2004; Batista et al., 2005; Kim et al., 2006a, 2006b; Hoff et al., 2007). Furthermore, another study (Hoff et al. 2007) did not observe cross-reactivity between CP4 EPSPS and known allergens including the mite allergen Der f 2 using sera of patients allergic to certain foods and mites.

Further support for unaltered allergenic potential for soybean 40-3-2 was presented by Gizzarelli et al. (2006), who developed and characterised a murine model (Balb/c mice) of IgE-mediated soybean

sensitisation induced by intragastric immunisation (in the presence of Cholera Toxin) with soybean extracts. Extracts of soybean 40-3-2 induced an immunological response that was comparable with that induced by non-GM soybean extracts. In other sensitisation studies, the purified CP4 EPSPS protein, homogenates of soybean 40-3-2 and control soybean were subcutaneously injected for three weeks (3 times/week) at various doses into male Sprague Dawley rats (Chang et al., 2001, 2003). A week after the last sensitisation antisera were recovered from individual animals and injected intradermally into unsensitised rats followed by a challenge with soybean homogenate. There were no signs of passive cutaneous anaphylaxis. Furthermore, sera of rats treated with both types of soybean homogenate resulted in comparable histamine release in cultured peritoneal mast cells. In addition, as already mentioned above, Teshima et al. (2000) were unable to identify effects on biomarkers for immunotoxicity and allergenicity in rodents fed a diet with 30% heat-treated soybean meal for 15 weeks, the test group receiving meal from soybean 40-3-2, the control group meal from a closely related conventional non-GM soybean.

The EFSA GMO Panel concludes that the information presented confirms that the overall allergenicity of the whole soybean 40-3-2 plant is not changed compared with that of its conventional counterpart.

4.2.6. Nutritional assessment of soybean 40-3-2

To substantiate that soybean 40-3-2 has equivalent nutritional quality to conventional soybeans, as indicated by equivalent chemical composition, the applicant originally supplied short-term feeding studies with soybean 40-3-2 on the target animals broiler chicken, quail, swine, dairy cow and catfish. The EFSA GMO Panel considered the feeding studies on broiler chickens, swine and catfish for the nutritional assessment of soybean 40-3-2 as compared to its conventional counterpart. The study with dairy cattle was not considered by the EFSA GMO Panel because the study had a short duration (3 weeks only) and additional weaknesses in experimental design (Flachowsky and Aulrich, 1999). The feeding study in quails was not considered due to its short duration, five days only.

Broiler chickens were fed starter diets containing 32.9% processed (dehulled, defatted and toasted) soybean meal (soybean 40-3-2 or an appropriate non-GM soybean) from day 0 to 21, and grower/finisher diets containing 26.6% soybean meal from day 22 to 42, when the study was terminated (Hammond et al., 1996). In these 42 days the broilers reached a market weight of approximately 2 kg. The experimental diets had no influence on feed intake, weight gain, feed conversion, and liveability (percent live birds; survival rate). There were also no significant difference in the performance parameters investigated (breast muscle weight and abdominal fat pad weight; in both cases total weight and percent of body weight) between broilers fed diets with soybean 40-3-2 and broilers fed its conventional counterpart. Additional information on broiler chickens is available from a small feeding study in which the birds were given a diet with 24-25% soybean meal (Deaville and Maddison, 2005). The broilers fed soybean 40-3-2 had as high feed intake, growth and feed conversion ratio as broilers fed control soybean.

One hundred cross-bred pigs of both sexes were fed for about 100 days with soybean meal diets containing about 14-24% (depending on age of animals) of dehulled soybean meal derived from either the GM event 40-3-2 or its conventional counterpart (Cromwell et al., 2002). During the feeding period the pigs grow in weight from about 24 kg to 111 kg. No difference between treatment groups were observed for feed intake, efficiency of feed utilisation and body weight gain, scanned backfat and longissimus area, and calculated carcass lean percentage. The sensory characteristics of the longissimus muscles were not influenced by treatment. The differences observed were not between pigs given the different feeds but those expected between sexes.

The fish feeding study was performed on 300 fingerling channel catfish (*Ictalurus punctatus*) of mixed sex. The study duration was over 10 weeks with diets containing processed meal (45-47% w/w) (Hammond et al., 1996). There was no statistically significant difference in survival, feed conversion ratio, and percentage weight gain between the groups receiving diets based on control soybean meal and glyphosate tolerant soybean meal. Although fish receiving the diet with soybean 40-3-2 meal consumed slightly less feed (2.85% of their body weight) than fish fed a diet with the control soybean meal (3.63%), this did not influence body composition data. There were no differences in moisture, protein, fat or ash among fish regardless of dietary treatment.

Feeding studies to investigate the nutritional wholesomeness of soybean 40-3-2 have also been performed. Norwegian investigators in a series of publications presented data on the nutritional adequacy of soybean 40-3-2 for the Atlantic salmon, *Salmo salar*, and concomitantly studied selected parameters of fish health. In one set of studies post-smolt salmon (average weight 104 g) were fed for 3 months with diets containing 17.2% soybean meal prepared either from genetically modified (GM) soybean event 40-3-2 or a non-appropriate non-GM soybean (Bakke-McKellep et al., 2007; Hemre et al., 2005; Sanden et al., 2004), and in another set of studies salmon parr (average weight 0.2 g) were fed for 8 months a diet in which 12.5% were soybean full-fat meal either from GM event 40-3-2 or from a non-appropriate non-GM soybean (Bakke-McKellep et al., 2008; Sanden et al., 2005, 2006). As the control materials in these studies were not suitable to assess the influence of the specific genetic modification in soybean 40-3-2, they were not used in the assessment of the nutritional wholesomeness of soybean 40-3-2.

Two later studies performed by the same research team, however, used an appropriate control material, and the studies give a valuable contribution to the assessment of the nutritional quality of soybean 40-3-2 as compared to a non-GM soybean with a comparable genetic background. In the first of these studies, farmed Atlantic salmon (weighing around 700g) were fed for four weeks a diet with 15% or 30% full-fat meal of soybean 40-3-2 or non-GM conventional soybean counterpart (Frøystad et al., 2008; Sagstad et al., 2008). Meal of soybean 40-3-2 neither affected growth, feed utilisation and proximate composition, nor organ weights and haematology. Spleen somatic index was higher in fish fed soybean 40-3-2 than in fish given non-GM soybean, while the plasma triacylglycerol (TAG) level was lower. The investigators subsequently concluded that this observation is unlikely to be related to the genetic modification per se (Sissener et al., 2009a). The investigators considered the possibility of whether the altered spleen somatic index could indicate a possible immune response (Sagstad et al., 2008), but experimental support for this speculation was not provided. In the same experiment, Frøystad et al. (2008) investigated gene expression in the distal intestine. Whereas most genes studied were equally expressed in fish fed diets with soybean 40-3-2 and fish fed diets with the non-GM soybean, expression of a lectine gene was down-regulated in salmon fed diets containing soybean 40-3-2. The investigators hypothesised, without supporting data, that this could have relevance for the local immunity in the distal gastrointestinal tract. In the second experiment, Sissener et al. (2009a) conducted a seven month feeding trial on Atlantic salmon (initial weight 40 g) going through the parr-smolt transformation and fed a full fat soybean meal derived from soybean 40-3-2 or its conventional counterpart at an inclusion rate of 25% of the diet. The two diets were compositionally similar in all analysed macro- and micro-nutrients. The parr-smolt transformation stage is a particular sensitive stage of Atlantic salmon as it enables the fish to migrate from freshwater to seawater, a process comprising a range of preparatory physiological adaptations that are dependent on nutritional status and energy turnover above a certain level. The performance and health of the fish were assessed by growth, body composition, organ development, haematological parameters, clinical plasma chemistry and lysozyme levels, with fish samples collected both in the freshwater and seawater stages. At the last sampling the average fish weight was around 190 g. In all parameters studied no diet-related differences were observed. The investigators concluded that soybean 40-3-2 can be used as an equivalent and safe substitute for conventional soybean varieties in feeds for Atlantic salmon. The wholesomeness of soybean 40-3-2 was further supported by histo-morphological analysis of these fish (Sissener, 2009), and proteomic profiling of their livers (Sissener et al., 2009b).

Similarly, Chainark et al. (2006) reported no difference in growth and feed performance of rainbow trout fed a fish diet with soybean 40-3-2 or non-GM soybean meal.

Tudisco et al. (2006) reported a 40 day feeding study in New Zealand rabbits given a diet with 20% soybean meal derived from soybean 40-3-2 or non-GM soybean. There was no differential influence of the two treatments on body weight, fresh organ weights, and serum and tissue enzyme levels in both males and females, with exception of a slight increase in lactic acid dehydrogenase 1 in the kidneys and heart of animals fed soybean 40-3-2. No difference was observed in the muscle.

Some additional studies have investigated the influence of diets containing soybean 40-3-2 as compared to diets with non-GM soybeans on the feed intake, growth rate, serum biochemistry, muscle composition and organ weights of the studied animals (Soares et al., 2005, de Silva Faria et al., 2009; Brasil et al., 2009), but as the diets have not been appropriately defined or chosen, these studies only marginally contribute to the safety assessment of soybean 40-3-2.

In conclusion, feeding studies with several target animal species (swine, broiler chickens, rabbits, catfish and salmon) have shown that soybean 40-3-2 is nutritionally equivalent to a non-GM soybean with a comparable genetic background. The risk assessment concluded that no data have emerged to indicate that soybean 40-3-2 is any less safe than its non-GM comparator. In addition, soybean 40-3-2 is, from a nutritional point of view, equivalent to conventional soybean. Thus, the EFSA GMO Panel, like previously the UK-ACNFP (1995), concludes that soybean 40-3-2 is nutritionally equivalent to the conventional counterpart and to other commercial soybean varieties, and in line with its Guidance document (EFSA, 2006b), the EFSA GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

4.2.7. Conclusion

The exposure assessment indicated an average exposure of the European consumer to products of soybean 40-3-2 (mainly soybean oil) in the region 3.4-3.7 g/person/day, and a maximum dietary inclusion levels of soybean 40-3-2 meal (% of diet) for farm animals in the EU being around 21% for broiler chickens, 18% for pigs, and 12% for dairy cattle. No adverse reactions have been reported upon exposure of humans and animals to products of soybean 40-3-2. Recombinant DNA and the CP4 EPSPS protein is to a large extent degraded during processing of food and feed. Furthermore, the CP4 EPSPS is quickly degraded in simulated gastric fluid. Bioinformatic studies demonstrated that the CP4 EPSPS protein shows no similarities to known toxic and allergenic proteins. The CP4 EPSPS protein induced no toxicity when administered orally to mice in an acute toxicity study. A number of feeding studies of various duration on laboratory rodents given processed and unprocessed soybean 40-3-2 in the diet indicated no toxicity related to the genetic modification. Whole-product testing with sera from soybean-allergic patients showed that the overall allergenicity of soybean 40-3-2 is not different from that of the conventional counterpart. Feeding studies on broiler chickens, rabbits, swine, catfish and salmon show that soybean 40-3-2 is nutritionally equivalent to the conventional counterpart. The EFSA GMO Panel is of the opinion that soybean 40-3-2 is as safe as the conventional counterpart and commercial varieties, and considers that no additional animal safety or nutritional wholesomeness studies are needed.

In conclusion, on the basis of the original information considered in the original application, updated studies in the present applications, and peer-reviewed scientific data on soybean 40-3-2, the EFSA GMO Panel confirms that soybean 40-3-2 is as safe and nutritious as the conventional counterpart and other commercial soybean varieties.

5. Environmental risk assessment and monitoring plan

5.1. Environmental risk assessment

The scope of applications EFSA-GMO-RX-40-3-2$_{[8\text{-}1a/20\text{-}1a]}$ and EFSA-GMO-RX-40-3-2$_{[8\text{-}1b/20\text{-}1b]}$) is for renewal of the authorisation of (1) food containing, consisting of, or produced from genetically modified (GM) soybean 40-3-2 (Unique Identifier MON-Ø4Ø32-6); (2) feed containing, consisting of, or produced from soybean 40-3-2; and (3) other products containing or consisting of soybean 40-3-2 with the exception of cultivation. Considering the intended uses of soybean 40-3-2, the environmental risk assessment is concerned with the exposure through manure and faeces from animals fed grain produced by soybean 40-3-2 and with the accidental release into the environment of viable grains produced by soybean 40-3-2 during transportation and processing.

As the scope of the present applications excludes cultivation, environmental concerns related to the use of glyphosate herbicides on soybean 40-3-2 apply only to imported and processed soybean products that may have been treated with those herbicides in countries of origin. The EFSA GMO Panel is aware that the risk assessment of active substances falls within the scope of Directive 91/414/EEC concerning the placing of plant protection products on the market.

5.1.1. Unintended effects on plant fitness due to the genetic modification[12]

Cultivated soybean (*Glycine max* (L.) *Merr.*) is a species in the subgenus *Soja* of the genus *Glycine*. The species originated from eastern Asia and is a highly domesticated crop (Liu and Agresti, 2005). The major worldwide soybean producers are the United States (USA), Brazil, Argentina, China, North Korea and South Korea. In the European Union, soybean is mainly cultivated in Italy, France and Romania (Dorokhov et al., 2004).

Cultivated soybean seeds rarely display any dormancy characteristics and only under certain environmental conditions grow as volunteers in the year following cultivation (OECD, 2000). In soybean fields, seeds usually do not survive during the winter due to predation, rotting, germination resulting in death, or due to management practices prior to planting the subsequent crop (Owen, 2005).

Applicant's field trials have been conducted at several locations in USA, Puerto Rico, Argentina, Canada, France and Italy. Information on phenotypic and agronomic characteristics was provided to assess the agronomic performance of soybean 40-3-2 in comparison with its conventional counterpart. These field trial data did not show changes in plant characteristics that indicate altered fitness and invasiveness of GM soybean 40-3-2 compared to its conventional counterpart, except in the presence of glyphosate herbicides (according to field studies carried out in United States, Puerto Rico (1991-1994), Argentina (1993-1994), Canada (1993 and 1994) and field trials carried out in Europe in France and Italy (1994) and Italy (1996, 1997). In addition to the data presented by the applicant, the EFSA GMO Panel is not aware of any scientific report of increased spread and establishment of existing GM soybeans and any change in survival capacity, including overwintering (Dorokhov et al., 2004, Owen, 2005, Bagavathiannan and Van Acker, 2008, Lee et al., 2009).

Furthermore there is no evidence that the glyphosate tolerant trait introduced by genetic modification results in increased invasiveness of any crop species, except when glyphosate herbicides are applied. Thus, the accidental release of GM soybean 40-3-2 seeds would not result in the establishment of plants exhibiting dissemination capabilities different from existing conventional soybean varieties and would not create additional agronomic or environmental impacts. The GM soybean plants will only be

[12] Technical Dossier / section D9.1

fitter in the presence of glyphosate herbicides which are not currently used in most areas where the GM soybean might be spilled.

Survival of soybean plant outside cultivation or other areas is mainly limited by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens and cold climate conditions. Since these general characteristics are unchanged in soybean 40-3-2, it can be considered that soybean 40-3-2 has no altered survival, multiplication or dissemination characteristics, except when glyphosate herbicides are applied. Therefore, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects of the soybean 40-3-2 in Europe will not be different from that of conventional soybean varieties.

5.1.2. Gene transfer[13]

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer of DNA, or vertical gene flow via seed dispersal and cross-pollination.

a) Plant to bacteria gene transfer

Genomic DNA is a component of many food and feed products derived from soybean. It is well documented that DNA present in food and feed becomes substantially degraded during digestion in the human or animal gastrointestinal tract. However, a low level of exposure of fragments of ingested DNA, including the recombinant fraction of such DNA, to micro-organisms in the digestive tract of humans, domesticated animals, and other animals feeding on soybean 40-3-2 is expected (see section 4 of the scientific opinion).

Current scientific knowledge of recombination processes in bacteria indicates that horizontal transfer of non-mobile, chromosomally-located DNA fragments between unrelated organisms (such as plants to microorganisms) is not expected to occur at detectable frequencies under natural conditions (see EFSA, 2009c for further details).

A successful horizontal transfer would require stable insertion of the transgene sequences into a bacterial genome enabling it to multiple at a higher rate than non-transformed cells. The only known mechanism that facilitates horizontal transfer of non-mobile, chromosomal DNA fragments into bacterial genomes is homologous recombination (HR). HR depends on the presence of stretches of similar DNA sequences between the recombining DNA molecules. In addition to substitutive recombination events, HR can also facilitate the insertion of non-homologous DNA sequences into bacterial genomes (additive recombination) if the flanking regions share sequence similarity.

The CP4 *epsps* gene originates from a bacterium and therefore the recombinant DNA contains sufficient sequence similarity for homologous recombination to take place in related bacterial species. However, such a hypothesised horizontal gene transfer event is not likely to be maintained in bacterial populations due constraints to efficient expression and a limited selective advantage for gene transfer recipients in the case of CP4 *epsps* expression. In addition to homology-based recombination processes, illegitimate recombination that does not require the presence of DNA similarity between the recombining DNA molecules is also theoretically possible. However, the transformation rates for illegitimate recombination were considered to be 10^{10}-fold lower than for homologous recombination (EFSA 2009c, Hülter and Wackernagel, 2008). Illegitimate recombination events have not been detected in studies that have exposed bacteria to high concentrations of GM-plant DNA (see EFSA 2009c). For these reasons, illegitimate recombination is not further considered here.

[13] Technical Dossier / section D9.2

The exposure of bacterial communities to the recombinant genes in soybean 40-3-2 must be seen in the context of the natural occurrence and level of exposure to alternative sources of similar genes to which bacterial communities are continually exposed. The protein encoded by CP4 EPSPS is an enzyme involved in the biosynthesis of chorismate, the common precursor of numerous aromatic compounds in bacteria, fungi and plants. Thus, it can be expected that both sequence-similar and divergent *epsps* genes are widely distributed in gut inhabiting and other environmental microorganisms.

In the context of its intended use as food and feed, there is no direct exposure of microorganisms to the herbicidal compound glyphosate. The selective advantage of glyphosate resistance in bacteria is therefore predicted to be limited. The hypothetical rare acquisition of the CP4 *epsps* from recombinant DNA plants is therefore not considered to confer a selective advantage to microorganisms that would allow them to enhance their viability or to alter their habitat range.

The EFSA GMO Panel concludes that the recombinant DNA in soybean 40-3-2 does not represent an environmental risk in relation to its potential for horizontal transfers to bacteria and other microorganisms.

(b) Plant to plant gene transfer

Considering the intended uses of soybean 40-3-2 and physical characteristics of soybean seeds, a possible pathway of gene dispersal is from seed spillage and pollen of occasional feral GM soybean plants originating from accidental seed spillage during transportation and/or processing.

The genus *Glycine* is divided into two distinct subgenera: *Glycine* and *Soja*. Soybean is in the subgenus *Soja*. The subgenus *Glycine* contains 16 perennial wild species, whilst the cultivated soybean, *Glycine max*, and its wild and semi-wild annual relatives, *Glycine soja* and *Glycine gracilis*, are classified in the subgenus *Soja* (OECD, 2000). Due to the low level of genomic similarity among species of the genus *Glycine*, *Glycine max* can only cross with other members of *Glycine* subgenus *Soja* (Hymowitz et al., 1998, Lu, 2005). Hence, the three species of *Soja* are capable of cross-pollination and the hybrid seed that is produced can germinate normally and produce plants with fertile pollen and seed (Abe et al., 1999, Nakayama and Yamaguchi, 2002). However, since *Glycine soja* and *Glycine gracilis* are indigenous to China, Taiwan, Korea, Japan, the Far East Region of Russia, Australia, the Philippines and South Pacific, and since they have not been reported in other parts of the world, where the cultivated soybean is grown (Dorokhov et al., 2004, Lu, 2005), the plant to plant gene transfer from soybean is restricted to cultivated areas and the occasional soybean plants resulting from seed spillage in the EU.

Soybean (*Glycine max*) is an annual almost completely self-pollinating crop in the field, which has a percentage of cross-pollination usually lower than 1% (Weber and Hanson, 1961, Caviness, 1966, Ray et al., 2003, Lu, 2005, Yoshimura et al., 2006, Abud et al., 2007). Soybean pollen dispersal is limited because the anthers mature in the bud and directly pollinate the stigma of the same flower (OECD, 2000). However, cross-pollination rates as high as 6.3% have been reported for closely spaced plants (Ray et al., 2003), suggesting the potential of some within-crop gene flow. These results indicate that natural cross-pollination rates can fluctuate significantly among different soybean varieties under particular environmental conditions, such as favourable climate for pollination and abundance of pollinators (Gumisiriza and Rubaihayo, 1978, Ahrent and Caviness, 1994, Ray et al., 2003, Lu, 2005).

Plant to plant gene transfer could therefore occur under the following scenario: imports of soybean 40-3-2 grains (while most soybean 40-3-2 grains will be processed in countries of production), processing outside of importing ports, transportation in regions of soybean production in Europe, spillage of GM grains during transportation, germination and development of spilled grains within

soybean fields or in very close vicinity of cultivated soybean fields, overlap of flowering periods and environmental conditions favouring cross-pollination. The overall likelihood of cross-pollination between GM soybean plants and cultivated soybean is therefore extremely low. Apart from seed production areas, GM plants and derived from out-crossing with this GM soybean will not persist overtime. Dispersal of soybean seeds by animals is not expected due to the characteristics of the seed, but accidental release into the environment of seeds may occur during transportation and processing for food, feed and industrial uses. However, cultivated soybean seeds rarely display any dormancy characteristics and only under certain environmental conditions grow as volunteers in the year following cultivation (OECD, 2000). Even in soybean fields, seeds usually do not survive during the winter due to predation, rotting, germination resulting in death, or due to management practices prior to planting the subsequent crop (Owen, 2005).

The EFSA GMO Panel takes into account that these applications do not include cultivation of the soybean within the EU so that the likelihood of cross-pollination between cultivated soybean and occasional soybean plants resulting from grain spillage is considered extremely low. However, in countries cultivating this GM soybean and producing seed for export, there is a potential for admixture in seed production and thus the introduction of GM seeds through this route. Hence, it is important that appropriate management systems are in place to restrict seeds of soybean 40-3-2 entering cultivation as this would require specific approval under Directive 2001/18/EC or Regulation (EC) 1829/2003.

In conclusion, since soybean 40-3-2 has no altered survival, multiplication or dissemination characteristics, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from soybean 40-3-2 in Europe will not differ from that of conventional soybean varieties.

5.1.3. Interactions of the GM plant with target organisms

Due to the intended uses of soybean 40-3-2, which exclude cultivation and due to the low level of exposure to the environment, potential interactions of the GM plant with target organisms were not considered an issue by the EFSA GMO Panel.

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

Due to the intended uses of soybean 40-3-2, which exclude cultivation and due to the low level of exposure to the environment, potential interactions of the GM plant with non-target organisms were not considered an issue by the EFSA GMO Panel.

5.1.5. Interactions with the abiotic environment and biochemical cycles

Due to the intended uses of soybean 40-3-2, which exclude cultivation and due to the low level of exposure to the environment, potential interactions of the GM plant with abiotic environment and biochemical cycles were not considered an issue by the EFSA GMO Panel.

5.2. Post-market environmental monitoring[14]

The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC are to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment are correct and to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment which were not anticipated in the environmental risk assessment.

Monitoring is related to risk management, and thus a final adoption of the monitoring plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gives its opinion on the scientific quality of the monitoring plan provided by the applicant (EFSA, 2006). The potential exposure to the environment of soybean 40-3-2 would be through manure and faeces from animals fed with GM soybean or through accidental release into the environment of GM soybean grains during transportation and processing. The EFSA GMO Panel is aware that, due to physical characteristics of soybean seed and methods of transportation, accidental spillage cannot be excluded. Therefore, the EFSA GMO Panel recommends that appropriate management systems are introduced to actively monitor the occurrence of feral soybean plants in areas where soybean spillage and plant establishment are likely to occur as proposed in the EFSA Guidance Document (EFSA, 2006a) and the scientific opinion of the EFSA GMO Panel on post-market environmental monitoring (EFSA, 2006b).

The scope of the monitoring plan provided by the applicant is in line with the intended uses. Since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects, no case-specific monitoring is necessary.

The general surveillance plans proposed by the applicant includes (1) the description of an approach involving operators (federations involved in soybean import and processing) reporting to the applicants via a centralised system any observed adverse effect(s) of GMOs on human health and the environment, (2) a coordinating system established by EuropaBio for the collection of information recorded by the various operators (Lecoq et al., 2007, Windels et al., 2008), (3) the use of networks of existing surveillance systems. The applicant proposes to submit a general surveillance report on an annual basis and a final report at the end of the consent[15].

Issues relating to the practical implementation of general surveillance and the evaluation of monitoring results are currently outside the remit of the EFSA GMO Panel. Details of the specific plans and methods of monitoring in each country should be developed by the applicant after the applications have been accepted (EFSA 2006).

The EFSA GMO Panel is of the opinion that the scope of the monitoring plans proposed by the applicant are in line with the intended uses of soybean 40-3-2 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

5.3. Conclusion

The scope of the applications EFSA-GMO-RX-40-3-2$_{[8\text{-}1a/20\text{-}1a]}$ and EFSA-GMO-RX-40-3-2$_{[8\text{-}1b/20\text{-}1b]}$) is for renewal of the authorisation of (1) food containing, consisting of, or produced from genetically modified (GM) soybean 40-3-2 (Unique Identifier MON-Ø4Ø32-6); (2) feed containing, consisting of, or produced from soybean 40-3-2; and (3) other products containing or consisting of soybean 40-3-2 with the exception of cultivation. Considering the intended uses, the environmental risk

[14] Additional information / December 2008
[15] Technical Dossier / section D11

assessment is concerned with indirect exposure mainly through manure and faeces from animals fed grains produced by soybean 40-3-2 and with the accidental release into the environment of viable grains by soybean 40-3-2 during transportation and processing.

In case of accidental release into the environment of viable grains of soybean 40-3-2 during transportation and processing, there are no indications of an increased likelihood of establishment and spread of feral soybean 40-3-2 plants, except in the presence of glyphosate herbicides. In addition, the low levels of environmental exposure of these GM soybean plants and the newly expressed protein through other routes indicate that the risk to non-target organisms is extremely low. The EFSA GMO Panel considers that it is unlikely that the recombinant DNA in soybean 40-3-2 transfers to bacteria and other microorganisms and that the risk caused by a rare but theoretically possible transfer of the recombinant epsps gene from soybean 40-3-2 to environmental microorganisms is regarded to be negligible due to the lack of a selective advantage in the context of its intended use that would be conferred. The scope of the post-market environmental monitoring plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean 40-3-2.

The EFSA GMO Panel is aware that, due to physical characteristics of soybean seed and methods of transportation, accidental spillage cannot be excluded. Therefore, the EFSA GMO Panel recommends that, within general surveillance, appropriate management systems are introduced to actively monitor the occurrence of feral soybean plants in areas where spillage and soybean plant establishment are likely to occur as proposed in the EFSA Guidance Document (EFSA, 2006a) and the scientific opinion of the EFSA GMO Panel on post-market environmental monitoring (EFSA, 2006b).

The EFSA GMO Panel also recommends that appropriate management systems should be in place to restrict seeds of soybean 40-3-2 entering cultivation as this would require specific approval under Directive 2001/18/EC or Regulation (EC) 1829/2003.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was requested to deliver a scientific opinion for renewal of the authorisation for continued marketing of existing products from GM soybean 40-3-2 (references EFSA-GMO-RX-40-3-2$_{[8\text{-}1a/20\text{-}1a]}$ and EFSA-GMO-RX-40-3-2$_{[8\text{-}1b/20\text{-}1b]}$) under Regulation (EC) No 1829/2003. The scope of these applications cover the continued marketing of (1) existing food containing, consisting of, or produced from soybean 40-3-2 (including food additives) (Reference EFSA-GMO-RX-40-3-2$_{[8\text{-}1a/20\text{-}1a]}$); (2) existing feed containing, consisting of, or produced from soybean 40-3-2 (Reference EFSA-GMO-RX-40-3-2$_{[8\text{-}1b/20\text{-}1b]}$); (3) other products containing or consisting of soybean 40-3-2 with the exception of cultivation (Commission Decision 96/281/EC) which were lawfully placed on the market in the Community before the date of entry into force of Regulation (EC) No 1829/2003 and included in the Community Register of genetically modified food and feed.

In delivering its scientific opinion, the EFSA GMO Panel considered the renewal applications (EFSA-GMO-RX-40-3-2$_{[8\text{-}1a/20\text{-}1a]}$, EFSA-GMO-RX-40-3-2$_{[8\text{-}1b/20\text{-}1b]}$); a consolidated application on the cultivation of soybean 40-3-2 (application EFSA-GMO-2005-NK-24); additional information submitted by the applicant on request of the EFSA GMO Panel; the scientific comments submitted by Member States; and relevant scientific publications. In accordance with the Guidance Document for renewal of authorisations of existing GMO products, the EFSA GMO Panel has taken into account the new information, experience and data on soybean 40-3-2, which have become available during the authorisation period.

The EFSA GMO Panel is of the opinion that the molecular characterisation data provided for soybean 40-3-2 are sufficient. The results of the bioinformatic analyses of the inserted DNA and the flanking

regions do not raise safety concern. The levels of CP4 EPSPS in soybean 40-3-2 have been sufficiently analysed and the stability of the genetic modification has been demonstrated. The EFSA GMO Panel considers that the molecular characterisation does not indicate a safety concern.

The new data from field trials confirms that soybean 40-3-2 is compositionally, agronomically and phenotypically equivalent to the conventional counterpart and to other commercial soybean varieties, except for being tolerant to glyphosate herbicides. The updated bioinformatics analysis of the newly expressed protein provided by the applicant and the safety assessment of the whole soybean plant identified no concerns regarding potential toxicity and allergenicity of soybean 40-3-2. Feeding studies on laboratory animals and several farm animals and fish confirmed the nutritional equivalence of soybean 40-3-2 to its conventional non-GM counterpart. New information available in peer-reviewed scientific literature and supplementary studies supplied by the applicant confirms that soybean 40-3-2 is as safe and as nutritious as the conventional counterpart and to other commercial soybean varieties. The European consumers have been exposed to soybean 40-3-2 mainly via soybean oil at levels around 3.4-3.7 g/person/day. Processed meal of soybean 40-3-2 has been given to farm animals within the EU at maximum dietary inclusion levels around 21% for broiler chickens, 18% for pigs, and 12% for dairy cattle. No adverse effects have been reported.

Considering the intended uses of soybean 40-3-2, which exclude cultivation, there is no requirement for scientific assessment on possible environmental effects associated with the cultivation of soybean 40-3-2. In case of accidental release into the environment of viable grains of soybean 40-3-2 during transportation and processing, there are no indications of an increased likelihood of establishment and spread of feral soybean 40-3-2 plants, except in the presence of glyphosate herbicides. In addition, the low levels of environmental exposure of these GM soybean plants and the newly expressed protein through other routes indicate that the risk to non-target organisms is extremely low. The EFSA GMO Panel considers that it is unlikely that the recombinant DNA in soybean 40-3-2 transfers to bacteria and other microorganisms and that the risk caused by a rare but theoretically possible transfer of the recombinant epsps gene from soybean 40-3-2 to environmental microorganisms is regarded to be negligible due to the lack of a selective advantage in the context of its intended use that would be conferred. The scope of the post-market environmental monitoring plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean 40-3-2. The EFSA GMO Panel is aware that, due to physical characteristics of soybean seed and methods of transportation, accidental spillage cannot be excluded. Therefore, the EFSA GMO Panel recommends that, within general surveillance, appropriate management systems are introduced to actively monitor the occurrence of feral soybean plants in areas where soybean spillage and plant establishment are likely to occur.

The EFSA GMO Panel recommends that appropriate management systems should be in place to restrict seeds of soybean 40-3-2 entering cultivation as the latter requires specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

In conclusion, the EFSA GMO Panel considers that the information available for soybean 40-3-2 addresses the scientific comments raised by the Member States and that the soybean 40-3-2 assessed in these applications is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment in the context of its intended uses. The EFSA GMO Panel concludes that soybean event 40-3-2 is unlikely to have any adverse effects on human and animal health and the environment, in the context of its intended uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the MS, received 15 May 2007, concerning a request for placing on the market of 40-3-2 (8-1a_20-1a and 8-1b_20-1b) Soybean by Monsanto in accordance with Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 20 July 2007, from EFSA to the Competent Authority of the MS.

3. Letter from EFSA to applicant, dated 14 January 2008, requesting additional information under completeness check (Ref. SR/KL/shv (2008) 2619864).

4. Letter from applicant to EFSA, received 3 March 2008 providing additional information under completeness check.

5. Letter from EFSA to applicant, dated 12 March 2008, delivering the 'Statement of Validity' for applications EFSA-GMO-RX-40-3-2 (8-1a_20-1a and 8-1b_20-1b) Soybean submitted by Monsanto under Regulation(EC) No 1829/2003 (Ref. SR/KL/md (2008) 2768971).

6. Letter from EFSA to applicant, dated 15 July 2007, requesting additional information and stopping the clock for application EFSA-GMO-RX-40-3-2 (8-1a_20-1a). (Ref. PB/KL/md (2008) 3172306).

7. Letter from EFSA to applicant, dated 12 September 2008, requesting additional information and maintaining the clock stopped for applications EFSA-GMO-RX-40-3-2 (8-1a_20-1a and 8-1b_20-1b).(Ref.PB/KL/md(2008) 3288577).

8. Letter from applicant to EFSA, received 1 December 2008 providing additional information for applications EFSA-GMO-RX-40-3-2 (8-1a_20-1a and 8-1b_20-1b).

9. Letter from EFSA to applicant, dated 11 December 2008, requesting additional information and maintaining the clock stopped for applications EFSA-GMO-RX-40-3-2 (8-1a_20-1a and 8-1b_20-1b).(Ref. PB/KL/md(2008) 3522843).

10. Letter from applicant to EFSA, received 23 December 2008 providing additional information for applications EFSA-GMO-RX-40-3-2 (8-1a_20-1a and 8-1b_20-1b).

11. Letter from applicant to EFSA, received 26 May 2009 providing additional information for applications EFSA-GMO-RX-40-3-2 (8-1a_20-1a and 8-1b_20-1b).

12. Letter from applicant to EFSA, received 20 August 2009 providing additional information for applications EFSA-GMO-RX-40-3-2 (8-1a_20-1a and 8-1b_20-1b).

13. Letter from EFSA to applicant, received 16 March 2010, requesting additional information and maintaining the clock stopped for applications EFSA-GMO-RX-40-3-2 (8-1a_20-1a and 8-1b_20-1b) (Ref.PB/KL/ZD/shv (2010) 4722621).

14. Letter from applicant to EFSA, received 15 July 2010, providing additional information for applications EFSA-GMO-RX-40-3-2 (8-1a_20-1a and 8-1b_20-1b).

15. Letter from EFSA to applicant, dated 17 July 2010 restarting the clock (Ref.PB/KL/lg (2010) 5143985).

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