



VKM Report 2015: 13

Final health and environmental risk assessment of genetically modified soybean 356043

**Scientific opinion on herbicide tolerant, genetically modified soybean 356043
from Pioneer Hi-Bred for food and feed uses, import and processing under
Regulation (EC) No 1829/2003 (Application EFSA/GMO/UK/2007/43)**

**Opinion of the Panel on Genetically Modified Organisms of the Norwegian
Scientific Committee for Food Safety**

Report from the Norwegian Scientific Committee for Food Safety (VKM) 2015: 13
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Committee for Food Safety
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Assessed and approved

The opinion has been assessed and approved by the Panel on Genetically Modified Organisms. Members of the panel are: Åshild Andreassen (chair), Per Brandtzæg, Knut Helkås Dahl, Knut Tomas Dalen, Hilde-Gunn Hoen-Sorteberg, Olavi Junttila, Richard Meadow, Kåre M. Nielsen, Monica Sanden, and Rose Vikse.

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Competence of VKM experts

Experts working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

Table of Contents

| | |
|---|-----------|
| Abstract | 6 |
| Summary | 7 |
| Sammendrag på norsk | 11 |
| Abbreviations and glossary | 14 |
| Background | 16 |
| Terms of reference | 17 |
| Assessment | 19 |
| 1 Introduction | 19 |
| 2 Molecular characterisation | 21 |
| 2.1 Conclusions | 21 |
| 3 Comparative assessments | 22 |
| 3.1 Production of material for comparative assessment..... | 22 |
| 3.2 Compositional analysis | 22 |
| 3.3 Agronomic traits and GM phenotype | 24 |
| 3.4 Conclusion..... | 24 |
| 4 Food and feed safety assessment | 25 |
| 4.1 Previous evaluations by the VKM GMO panel and EFSA | 25 |
| 4.2 Product description and intended uses..... | 25 |
| 4.3 Effects of processing | 26 |
| 4.4 Toxicological assessment of soybean 356043 | 27 |
| 4.4.1 Toxicological assessment of the expressed novel proteins..... | 27 |
| 4.4.1.1 Acute toxicity testing | 27 |
| 4.4.1.2 Repeated-dose toxicity testing | 28 |
| 4.4.1.3 Toxicological assessment of new constituents other than proteins..... | 28 |
| 4.4.2 Toxicological assessment of the whole GM food/feed..... | 29 |
| 4.4.3 Allergenicity | 30 |
| 4.4.3.1 Assessment of allergenicity of the newly expressed proteins | 30 |
| 4.4.3.2 Assessment of allergenicity of the whole GM plant..... | 30 |
| 4.4.3.3 Assessment of allergenicity of proteins derived from the GM plant | 30 |
| 4.4.4 Assessment of adjuvanticity | 30 |
| 4.5 Nutritional assessment of GM food and feed..... | 31 |
| 4.5.1 Intake information/exposure assessment..... | 31 |

| | | |
|---------------------|---|------------|
| 4.5.2 | Nutritional assessment of feed derived from the GM plant..... | 34 |
| 4.6 | Conclusion..... | 35 |
| 5 | Environmental risk assessment..... | 37 |
| 5.1 | Unintended effects on plant fitness due to the genetic modification..... | 37 |
| 5.2 | Potential for gene transfer | 38 |
| 5.2.1 | Plant to micro-organisms gene transfer | 38 |
| 5.2.2 | Plant to plant gene flow | 39 |
| 5.3 | Interactions between the GM plant and target organisms | 39 |
| 5.4 | Potential interactions between the GM plant and non-target organisms (NTOs) | 39 |
| 5.5 | Potential interactions with the abiotic environment and biochemical cycles | 40 |
| 5.6 | Conclusion..... | 40 |
| 6 | Post-market environmental monitoring..... | 41 |
| 7 | Conclusions | 42 |
| 8 | Data gaps | 44 |
| 9 | References | 45 |
| Appendix I | | 49 |
| Appendix II | | 70 |
| Appendix III | | 111 |

Abstract

Soybean 356043 expresses both the *gat* gene from the soil bacterium *Bacillus licheniformis* and the *gm-hra* gene, an optimised form of the endogenous acetolactate synthase (*als*) coding sequence from soybean (*Glycine max*; *gm*). The encoded GAT4601 protein, glyphosate acetyltransferase, confers the ability to inactivate the active herbicidal substances glyphosate and glyphosate-ammonium to N-acetyl glyphosate, which does not have herbicidal activity. The encoded GM-HRA protein confers increased tolerance to the active, ALS-inhibiting, herbicidal substances chlorimuron, thifensulfuron and sulfonyleureas. Bioinformatics analyses of the inserted DNA and flanking sequences in soybean 356043 have not indicated a potential production of putative harmful proteins or polypeptides caused by the genetic modification. Genomic stability of the functional insert and consistent expression of the *gat* gene, have been shown over several generations of soybean 356043. Data from several field trials performed in USA, Canada, Chile and Argentina during 2005-2006 show that soybean 356043 contains higher levels of especially the acetylated amino acid N-acetyl aspartate, but also N-acetyl glutamate and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acids, in addition to expression of the newly expressed proteins. Otherwise the soybean 356043 is compositionally, morphologically and agronomically equivalent to its conventional counterpart and other commercial soybean cultivars. The acetylated amino acids and odd-chain fatty acids are normal constituents of plant and animal-derived foods and feeds, and an in-depth toxicity and intake assessment did not reveal safety concerns regarding consumer intake at the levels present in soybean 356043. Sub-chronic feeding studies with rats, repeated-dose toxicity studies with mice, as well as nutritional assessment trials with broilers and laying hens have not revealed adverse effects of soybean 356043. These studies indicate that soybean 356043 is nutritionally equivalent to and as safe as conventional soybean cultivars. The GAT4601 and GM-HRA proteins produced in soybean 356043 do not show sequence resemblance to known toxins or IgE-dependent allergens, nor has the whole GM plant been reported to cause changes in IgE-mediated allergic reactions in patients reactive to soybean or in non-ectopic control individuals. Soybean is not cultivated in Norway, and there are no cross-compatible wild or weedy relatives of soybean in Europe.

Based on current knowledge and considering the intended uses, which exclude cultivation, the VKM GMO Panel concludes that soybean 356043 with the GAT4601 and GM-HRA proteins:

- Is – with the exception of the novel traits and resulting increased content of the acetylated amino acids NAA and NAG, and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acids – compositionally, morphologically and agronomically equivalent to its conventional counterpart and other commercial soybean cultivars
- Are unlikely to introduce toxic or allergenic potentials in food or feed compared to conventional soybean cultivars
- Is nutritionally equivalent to and as safe as its conventional counterpart and other conventional soybean cultivars
- Does not represent an environmental risk in Norway.

Summary

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Scientific Committee for Food Safety (VKM) has been requested by the Norwegian Environment Agency (formerly Norwegian Directorate for Nature Management) and the Norwegian Food Safety Authority (NFSA) to conduct final food, feed and environmental risk assessments of all genetically modified organisms (GMOs) and products containing or consisting of GMOs that are authorised in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC. The request covers scope(s) relevant to the Gene Technology Act. The request does not cover GMOs that VKM already has conducted its final risk assessments on. However, the Agency and NFSA requests VKM to consider whether updates or other changes to earlier submitted assessments are necessary.

The herbicide-tolerant genetically modified soybean 356043 (Unique Identifier DP-356043-5) from Pioneer Hi-Bred International Inc. is approved under Regulation (EC) No 1829/2003 for food and feed uses, import and processing since 10 February 2012 (Application EFSA/GMO/UK/2007/43, Commission Implementing Decision 2012/84/EU).

Soybean 356043 has previously been assessed for use as food and feed by the VKM GMO Panel (VKM, 2008), as commissioned by the NFSA in connection with EFSA's public hearing of the application EFSA/GMO/UK/2007/43 in 2007.

The current food, feed and environmental risk assessment of the soybean 356043 is based on information provided by the applicant in the application EFSA/GMO/UK/2007/43, relevant peer-reviewed scientific literature, and scientific opinions and comments from EFSA (EFSA 2011b), VKM (VKM 2008) and other member states made available on the EFSA website GMO Extranet. Except for a synopsis of more recent literature, this draft opinion is to a large extent a summary of the above-mentioned VKM and EFSA reports, which are provided in Appendix I and II respectively, and readers are referred to these for details.

The VKM GMO Panel has evaluated soybean 356043 with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed. VKM has also decided to take account of the appropriate principles described in the EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA, 2006; EFSA, 2011d), the environmental risk assessment of GM plants (EFSA, 2010a), selection of comparators for the risk assessment of GM plants (EFSA, 2011b) and for the post-market environmental monitoring of GM plants (EFSA, 2011e).

The scientific risk assessment of soybean 356043 includes molecular characterisation of the inserted DNA and expression of novel proteins, comparative assessment of agronomic and phenotypic characteristics, nutritional assessments, toxicity and allergenicity, unintended effects on plant fitness, potential for gene transfer, interactions between the GM plant, target and non-target organisms, and effects on biogeochemical processes.

It is emphasised that the VKM mandate does not include assessments of contribution to sustainable development, societal utility or ethical considerations, according to the Norwegian Gene Technology Act and Regulations relating to impact assessment pursuant to the Gene Technology Act. These considerations are therefore not part of the risk assessment

provided by the VKM Panel on Genetically Modified Organisms. Likewise, the VKM mandate does not include evaluations of herbicide residues in food and feed from genetically modified plants.

Particle acceleration was used to insert the linear DNA fragment containing the two genes into the plant cells of the commercial cultivar "Jack". Soybean 356043 expresses two introduced traits: the *gat* gene encoding the enzyme N-acetyl transferase derived from the soil bacterium *Bacillus licheniformis*, as well as the *gm-hra* gene encoding the enzyme acetolactate synthase (ALS) derived from *Glycine max*. These render soybean 356043 tolerant to several active herbicidal substances, specifically glyphosate, chlorimuron, thifensulfuron and sulfonylureas.

Molecular characterisation

The soybean 356043 contains a DNA fragment with one functional copy each of the *gat4601* and *gm-hra* genes integrated in the soybean 356043 genome. No other functional vector genes were found. Southern and Western blot analyses, together with segregation studies show that the introduced genes are stably inherited and expressed over multiple generations. Bioinformatics comparisons of the amino acid sequence of the newly expressed GAT4601 protein and GM-HRA protein do not reveal similarities to known allergenic or toxic proteins.

The VKM GMO Panel concludes that the molecular characterisation of soybean 356043 does not indicate a safety concern.

Comparative assessments

Field studies were carried out to assess the composition of seed and forage, as well as agronomic and morphological characteristics of the GM soybean 356043 compared to the non-transgenic variety Jack (control) and other conventional soybean cultivars. Most likely due to the enzyme activities of the newly expressed proteins, soybean 356043 seeds contain increased levels of especially the acetylated amino acids N-acetylaspartate (NAA), but also N-acetylglutamate (NAG) and the odd-chain fatty acids heptadecanoic (C17:0), heptadecenoic (C17:1) and heptadecadienoic (C17:2) acid. Although these levels in soybean 356043 fell outside the ranges measured in its conventional counterpart and other conventional soybean cultivars, the sum of the acetylated amino acids and odd-chain fatty acids only made up a small proportion of total amino acids (<0.15%) and total fatty acids (<1%). Furthermore, the acetylated amino acids and odd-chain fatty acids are normal constituents of plant and animal-derived foods and feeds, and an in-depth toxicity and intake assessment did not reveal any safety concerns regarding consumer intake at the levels present in soybean 356043. With the exception of these changes, few biologically significant differences were observed between soybean 356043 and its corresponding conventional counterpart in the analysis of seed and forage and differences observed were only present in material from some of the locations. These were likely to reflect the natural variability observed in conventional soybean cultivars. The field studies investigating composition of soybean 356043 show no biologically relevant differences between GM crops treated and untreated with the target herbicides.

Based on current knowledge and excluding the novel traits and resulting increased content of the acetylated amino acids NAA and NAG, and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acids, the VKM GMO Panel concludes that soybean 356043 is compositionally, agronomically, and morphologically equivalent to its conventional counterpart and other conventional soybean cultivars.

Food and feed risk assessment

A subchronic, toxicity study in rat, repeated dose studies in mice, nutritional whole food studies in broilers and laying hens, and allergenicity assessment studies have been performed with soybean 356043. These studies have not revealed adverse effects or indicated any differences in the performance of animals fed soybean 356043 compared to conventional soybeans. Bioinformatics analysis of the amino acid sequence of GAT4601 and GM-HRA did not show sequence resemblance to known toxins or IgE-dependent allergens, nor have these proteins been reported to cause IgE-mediated allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that soybean 356043 is nutritionally equivalent to and as safe as its conventional counterpart and other conventional soybean cultivars. It is unlikely that the GAT4601 and GM-HRA proteins will introduce toxic or allergenic potentials in food or feed based on soybean 356043 compared to conventional soybean cultivars.

Environmental assessment

Considering the intended uses of soybean 356043, which excludes cultivation, the environmental risk assessment is concerned with accidental release into the environment of viable grains during transportation and processing, as well as indirect exposure to microorganisms in the gastrointestinal tract and soil, mainly via intestinal content and faeces from animals fed feeds containing soybean 356043.

With the exception of herbicide tolerances, soybean 356043 has no altered survival, multiplication or dissemination characteristics compared to conventional soybean, and there are no indications of an increased likelihood of spread and establishment of feral soybean plants in the case of accidental release into the environment of seeds from soybean 356043. Soybean is not cultivated in Norway, and there are no cross-compatible wild or weedy relatives of soybean in Europe. Plant to plant gene flow is therefore not considered to be an issue.

Considering the intended use of soybean 356043 as food and feed, interactions with the biotic and abiotic environment are not considered to be an issue in Norway.

Overall conclusion

Based on current knowledge and considering the intended uses, which exclude cultivation, the VKM GMO Panel concludes that soybean 356043 with the GAT4601 and GM-HRA proteins:

- Is – with the exception of the novel traits and resulting increased content of the acetylated amino acids NAA and NAG, and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acids – compositionally, morphologically and agronomically equivalent to its conventional counterpart and other commercial soybean cultivars
- Are unlikely to introduce toxic or allergenic potentials in food or feed compared to conventional soybean cultivars
- Is nutritionally equivalent to and as safe as its conventional counterpart and other conventional soybean cultivars
- Does not represent an environmental risk in Norway.

Key words

GMO, soybean (*Glycine max*), 356043, EFSA/GMO/UK/2007/43, herbicide tolerance, *gat4601*, *gm-hra*, food and feed safety, environmental risk evaluation, Regulation (EC) No 1829/2003, VKM, risk assessment, Norwegian Scientific Committee for Food Safety, Norwegian Environment Agency

Sammendrag på norsk

Som en del av forberedelsene til implementering av EU-forordning 1829/2003 i norsk rett, er Vitenskapskomiteen for mattrygghet (VKM) bedt av Miljødirektoratet (tidligere Direktoratet for naturforvaltning [DN]) og Mattilsynet om å utarbeide endelige helse- og miljørisikovurderinger av alle genmodifiserte organismer (GMOer) og avledete produkter som inneholder eller består av GMOer som er godkjent under forordning 1829/2003 eller direktiv 2001/18, og som er godkjent for ett eller flere bruksområder som omfattes av genteknologiloven. Miljødirektoratet og Mattilsynet har bedt VKM om endelige risikovurderinger for de EU-godkjente søknader hvor VKM ikke har avgitt endelige risikovurderinger. I tillegg er VKM bedt om å vurdere hvorvidt det er nødvendig med oppdatering eller annen endring av de endelige helse- og miljørisikovurderingene som VKM tidligere har levert.

Den genmodifiserte, herbicidtolerante soyalinjen 356043 (unik kode DP-356043-5) fra Pioneer Hi-Bred International Inc. ble godkjent til import, videreforedling og til bruk som mat og fôr under EU-forordning 1829/2003 den 10. februar 2012 (Kommisjonsbeslutning 2012/84/EU).

Soyalinjen 356043 ble første gang vurdert av VKMs faggruppe for GMO i 2008 ([VKM, 2008](#)). Helse- og miljørisikovurderingen ble utført på oppdrag av Mattilsynet i forbindelse med EFSAAs offentlige høring av søknad EFSA/GMO/UK/2007/43 i 2007.

Risikovurderingen av den genmodifiserte soyalinjen er basert på søkers dokumentasjon og uavhengige vitenskapelige publikasjoner, samt vitenskapelige vurderinger og kommentarer fra EFSA (EFSA, 2011c), VKM (VKM, 2008) og andre medlemstater som er gjort tilgjengelig på EFSAAs nettside EFSA GMO Extranet. Bortsett fra gjennomgang av nylig offentliggjort publikasjoner er resten av teksten i denne vurderingen en oppsummering av de tidligere VKM (2008) og EFSA (2011c) vurderingene, som er vedlagt i hhv. Appendix I og II. For utfyllende detaljer henvises leserne til disse.

Vurderingen er gjort i henhold til tiltenkt bruk i EU/EØS-området, og i overensstemmelse med Matloven, miljøkravene i Genteknologiloven med forskrifter, først og fremst forskrift om konsekvensutredning etter Genteknologiloven. Videre er kravene i EU-forordning 1829/2003/EF, utsettingsdirektiv 2001/18/EF (vedlegg 2, 3 og 3B) og veiledende notat til Annex II (2002/623/EF), samt prinsippene i EFSAAs retningslinjer for risikovurdering av genmodifiserte planter og avledete næringsmidler ([EFSA, 2006](#); [EFSA, 2010a](#); [EFSA, 2011b](#); [EFSA, 2011d](#); [EFSA, 2011e](#)) lagt til grunn for vurderingen.

Den vitenskapelige vurderingen omfatter transformeringsmetoden og vektorkonstruksjonen, karakterisering og nedarving av genkonstruksjonen, komparativ analyse av ernæringsmessig kvalitet, mineraler, kritiske toksiner, metabolitter, antinæringsstoffer, allergener og nye proteiner. Videre er agronomiske egenskaper, potensiale for utilsiktede effekter på fitness, genoverføring, målorganismer, ikke-målorganismer og biogeokjemiske prosesser vurdert.

Det presiseres at VKMs mandat ikke omfatter vurderinger av etikk, bærekraft og samfunnsnytte, i henhold til kravene i den norske genteknologiloven og dens konsekvensutredningsforskrift. Disse aspektene blir derfor ikke vurdert av VKMs faggruppe for genmodifiserte organismer. Vurderinger av mulige plantevernmiddelrester i den genmodifiserte planten som følge av endret sprøytemiddelbruk faller per i dag utenfor VKMs ansvarsområde og er derfor heller ikke vurdert.

Soya 356043 uttrykker to nye egenskaper: *gat4601*-genet fra jordbakterien *Bacillus licheniformis* som koder for enzymet N-acetyl transferase, og *gm-hra*-genet fra *Glycine max* som koder for enzymet acetolaktat syntase (ALS). De transgene plantene vil derfor tolerere høyere doser av herbicidene glyfosat og ALS-inhiberende herbicider som klorimuron, tifensulfuron og sulfonyleureaer sammenlignet med konkurrerende ugras.

Molekylær karakterisering

Soya 356043 har kun en funksjonell kopi av hver av genene *gat4601* og *gm-hra* og ingen andre funksjonelle vektorgener integrert i genomet. Homologisøk i databaser over kjente toksiner og allergener indikerer at genmodifiseringen ikke har ført til utilsiktet produksjon av skadelige proteiner eller polypeptider i soya 356043. Southern og Western blot og segresjons-analyser viser at det introduserte genet er stabilt nedarvet og uttrykt over flere generasjoner, og i samsvar med de fenotypiske egenskapene til soya 356043.

VKMs faggruppe for GMO konkluderer med at den molekylære karakteriseringen ikke indikerer noen helserisiko ved soya 356043.

Komparative analyser

Søker utførte feltforsøk med påfølgende analyse av næringsstoffer, antinæringsstoffer og andre relevante, biologisk aktive stoffer målt i bønner og øvrig plantemateriale. Registrering av agronomiske og morfologiske egenskaper ble også utført. Data fra soya 356043, dens konvensjonelle motpart og andre konvensjonelle soyasorter ble sammenlignet. Tilgjengelig data viser økt forekomst av særlig den N-acetylerede aminosyren N-acetylaspartat (NAA), men også N-acetylglutamat (NAG) samt de oddetalls-kjedede fettsyrene margarinsyre (C17:0), heptadekensyre (C17:1) og heptadekadiensyre (C17:2) i soya 356043, som ligger utenfor intervallet av verdier registrert for konvensjonelle soyatyper. Denne økte forekomsten er mest sannsynlig et resultat av genmodifiseringen med uttrykk av de to nye enzymene. Summen av disse aminosyrene og fettsyrene utgjør kun en liten del av de totale aminosyrene (<0.15%) og fettsyrene (<1%) i soyafrø. Dessuten finnes disse stoffene normalt i andre mat- og fôrråvarer, og en grundig toksikologisk og inntaksvurdering har ikke avslørt noen risiko for helse ved inntak av de nivåene målt i soya 356043. Det var ellers kun små tilfeldige variasjoner i enkeltparametere målt i bønner og øvrig plantemateriale. Disse ble vurdert som ikke biologisk relevante forskjeller mellom den genmodifiserte soyaen og konvensjonelle soyasorter. Feltstudier viste ingen ernæringsmessig effekt av sprøyting med glyfosat og ALS-inhiberende herbicider på soya 356043.

Ut i fra dagens kunnskap og med unntak av de introduserte egenskapene og dermed økt forekomst av de N-acetylerede aminosyrene NAA og NAG og oddetalls-kjedede fettsyrene C17:0, C17:1 og C17:2, konkluderer VKMs faggruppe for GMO at soya 356043 er vesentlig lik dens konvensjonelle motpart, samt andre konvensjonelle sorter i forhold til næringsstoffsammensetning, og agronomiske og morfologiske egenskaper.

Helserisiko

En subkronisk toksikologistudie med rotter, eksponeringsstudier med mus, ernæringsstudier med broilere og verpehøns, og allergenisitetstudier har blitt utført med soya 356043. Disse studiene har ikke vist negative effekter eller indikert forskjeller i ytelse hos dyr fôret med soya 356043 sammenlignet med konvensjonell soya. Med hjelp av bioinformatiske sammenligninger viser aminosyresekvensene av GAT4601 og GM-HRA proteinene ingen sekvenslikhet med kjente toksiner eller IgE-bundne allergener, og er heller ikke rapportert å ha forårsaket IgE-medierte allergiske reaksjoner.

Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO at soya 356043 er ernæringsmessig sammenlignbar og like trygg som dens konvensjonelle motpart og andre konvensjonelle sorter. Det er usannsynlig at GAT4601 eller GM-HRA proteinene vil føre til toksiske eller allergiske reaksjoner fra mat og fôr som inneholder 356043 sammenlignet med konvensjonelle soyatyper.

Miljørisiko

Miljøriskovurderingen av soyalinje 356043 er avgrenset til mulige effekter av utilsiktet spredning av spiredyktige frø i forbindelse med transport og prosessering, samt indirekte eksponering gjennom gjødsel fra husdyr føret med genmodifisert soya. Faggruppen har ikke vurdert mulige miljøeffekter knyttet til dyrking av soyalinjen.

Genmodifiseringen av soya 356043 har ikke medført endringer i egenskaper knyttet til overlevelse, oppformering eller spredning sammenlignet med konvensjonell soya, og det er ingen indikasjoner på økt sannsynlighet for spredning og etablering av ferals soyaplanter fra utilsiktet frøspill av soyalinjen. Soya dyrkes ikke i Norge, og arten har ikke viltvoksende populasjoner eller nærstående arter utenfor dyrking i Europa. Det er derfor ikke risiko for utkryssing med dyrkede sorter eller ville planter i Norge.

Med bakgrunn i tiltenkt bruksområde, som ekskluderer dyrking, konkluderer VKMs faggruppe for GMO at soya 356043 ikke vil medføre økt risiko for interaksjoner med det biotiske eller abiotiske miljøet i Norge.

Samlet vurdering

Ut i fra dagens kunnskap og ved tiltenkt bruksområde, som ekskluderer dyrking, konkluderer VKMs faggruppe for GMO at soya 356043 med GAT4601 og GM-HRA proteinene:

- Er med unntak av de introduserte egenskapene og dermed økt forekomst av de N-acetylerede aminosyrene NAA og NAG og oddetalls-kjedede fettsyrene C17:0, C17:1 og C17:2, vesentlig lik konvensjonelle soyasorter i forhold til næringsstoffsammensetning, og agronomiske og morfologiske egenskaper
- Vil ikke medføre økt fare for toksiske eller allergiske reaksjoner ved inntak av mat og fôr sammenlignet med konvensjonelle soyatyper
- Er ernæringsmessig lik og like trygg som dens konvensjonelle motpart og andre konvensjonelle soyasorter
- Vil ikke medføre noen økt miljørisiko i Norge.

Abbreviations and glossary

| | |
|-------------------|--|
| ALS | Acetolactate synthase |
| ARMG | Antibiotic resistance marker gene |
| <i>Bt</i> | <i>Bacillus thuringiensis</i> |
| bw | Body weight |
| <i>Cp4 epsps</i> | The <i>5-enolpyruvylshikimate-3-phosphate synthase</i> gene from <i>Agrobacterium tumefaciens</i> strain CP4 |
| CTP | Chloroplast transit peptide |
| DNA | Deoxyribonucleic acid |
| dw | Dry weight |
| EC | European Commission |
| EFSA | European Food Safety Authority |
| EPSP | 5-enolpyruvylshikimate-3-phosphate |
| EPSPS | 5-enolpyruvylshikimate-3-phosphate synthase |
| ERA | Environmental risk assessment |
| EU | European Union |
| fa | Fatty acid |
| FAO | Food and Agriculture Organisation |
| Fitness | Describes an individual's ability to reproduce successfully relative to that of other members of its population. |
| fw | Fresh weight |
| fwt | Fresh weight tissue |
| GAT | Glyphosate N-acetyltransferase |
| Glyphosate | Broad-spectrum systemic herbicide |
| GM | Genetically Modified |
| GM-HRA | <i>Glycine max</i> –derived, modified acetolactate synthase |

| | |
|----------------------------|---|
| GMO | Genetically Modified Organism |
| GMP | Genetically Modified Plant |
| MT/NFSA | Norwegian Food Safety Authority (Mattilsynet) |
| Near-isogenic lines | Term used in genetics/plant breeding, and defined genetic lines that are identical except for differences at a few specific locations or genetic loci. |
| NAA | N-acetylaspartate |
| NAG | N-acetylglutamate |
| OECD | Organisation for Economic Co-operation and Development |
| PCR | Polymerase chain reaction; a technique to amplify DNA by copying |
| RNA | Ribonucleic acid |
| Southern blot | Method used for transfer of electrophoresis-separated DNA fragments to a filter membrane and possible subsequent fragment detection by probe hybridisation |
| Western blot | Technique used to transfer proteins separated by gel electrophoresis by 3-D structure or denaturated proteins by the length of the polypeptide to a membrane, where they might be identified by antibody labelling. |

Background

On 11 April 2007, the European Food Safety Authority (EFSA) received from the Competent Authority of the United Kingdom an application (Reference EFSA/GMO/UK/2007/43) for authorisation of the genetically modified herbicide tolerant soybean 356043 (Unique Identifier DP-356043-5) with the trade name Optimum GAT™, submitted by Pioneer within the framework of Regulation (EC) No 1829/2003.

The scope of the application covers:

- Food
 - ✓ GM plants for food use
 - ✓ Food containing or consisting of GM plants
 - ✓ Food produced from GM plants or containing ingredients produced from GM
 - ✓ Plants
- Feed
 - ✓ GM plants for feed use
 - ✓ Feed containing or consisting of GM plants
 - ✓ Feed produced from GM plants
- GM plants for environmental release
 - ✓ Import and processing (Part C of Directive 2001/18/EC)

After receiving the application EFSA/GMO/UK/2007/43 and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed the EU- and EFTA Member States (MS) and the European Commission and made the summary of the dossier publicly available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of regulation (EC) No 1829/2003. Following receipt of additional information from the applicant, EFSA declared on 28 September 2007 that the application was valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the EC and consulted nominated risk assessment bodies of the MS, including the 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. Within three months following the date of validity, all MS could submit via the EFSA GMO Extranet to EFSA comments or questions on the valid application under assessment. The VKM GMO Panel assessed the application in connection with the EFSA official hearing, and submitted a preliminary opinion in March 2008 ([VKM, 2008](#)). EFSA published its scientific opinion 6 July 2011 ([EFSA, 2011c](#)), and soybean 356043 was approved for food and feed uses, import and processing 10 February 2012 (Commission Implementing Decision 2012/84/EU).

Terms of reference

The Norwegian Environment Agency (formerly the Norwegian Directorate for Nature Management) has the overall responsibility for processing applications for the deliberate release of genetically modified organisms (GMOs). This entails inter alia coordinating the approval process, and to make a holistic assessment and recommendation to the Ministry of the Environment regarding the final authorisation process in Norway. The Agency is responsible for assessing environmental risks upon the deliberate release of GMOs, and to assess the product's impact on sustainability, benefit to society and ethics under the Gene Technology Act.

The Norwegian Food Safety Authority (NFSA) is responsible for assessing risks to human and animal health upon the deliberate release of GMOs pursuant to the Gene Technology Act and the Food Safety Act. In addition, NFSA administers the legislation for processed products derived from GMO and the impact assessment on Norwegian agriculture according to sector legislation.

The Norwegian Environment Agency

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Environment Agency, by letter dated 13 June 2012 (ref. 2008/4367/ART-BI-BRH), requests VKM, to conduct final environmental risk assessments for all genetically modified organisms (GMOs) and products containing or consisting of GMOs that are authorised in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC. The request covers scope(s) relevant to the Gene Technology Act.

The request does not cover GMOs that VKM already has conducted its final risk assessments on. However, the Norwegian Environment Agency requests VKM to consider whether updates or other changes to earlier submitted assessments are necessary.

The basis for evaluating the applicants' environmental risk assessments is embodied in the Act Relating to the Production and Use of Genetically Modified Organisms etc. (the Norwegian Gene Technology Act), Regulations relating to impact assessment pursuant to the Gene Technology Act, the Directive 2001/18/EC on the deliberate release of genetically modified organisms into the environment, Guidance note in Annex II of the Directive 2001/18 (2002/623/EC) and the Regulation 1829/2003/EC. In addition, the EFSA guidance documents on risk assessment of genetically modified plants and food and feed from the GM plants ([EFSA, 2010a](#); [EFSA, 2011d](#)), and OECD guidelines will be useful tools in the preparation of the Norwegian risk assessments.

The risk assessments' primary geographical focus should be Norway, and the risk assessments should include the potential environmental risks of the product(s) related to any changes in agricultural practices. The assignment covers assessment of direct environmental impact of the intended use of pesticides with the GMO under Norwegian conditions, as well as changes to agronomy and possible long-term changes in the use of pesticides.

The Norwegian Food Safety Authority (NFSA/Mattilsynet)

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Environment Agency has requested NFSA to give final opinions on all GMOs and products

containing or consisting of GMOs that are authorised in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC within the Authority's sectoral responsibility. The request covers scope(s) relevant to the Gene Technology Act.

NFSA has therefore, by letter dated 13 February 2013 (ref. 2012/150202), requested VKM to carry out final scientific risk assessments of 39 GMOs and products containing or consisting of GMOs that are authorised in the European Union.

The assignment from NFSA includes food and feed safety assessments of GMOs and their derivatives, including processed non-germinating products, intended for use as or in food or feed.

In the case of submissions regarding genetically modified plants (GMPs) that are relevant for cultivation in Norway, VKM is also requested to evaluate the potential risks of GMPs to the Norwegian agriculture and/or environment. Depending on the intended use of the GMP(s), the environmental risk assessment should be related to import, transport, refinement, processing and cultivation. If the submission seeks to approve the GMP(s) for cultivation, VKM is requested to evaluate the potential environmental risks of implementing the plant(s) in Norwegian agriculture compared to existing cultivars (e.g. consequences of new genetic traits, altered use of pesticides and tillage). The assignment covers both direct and secondary effects of altered cultivating practices.

VKM is further requested to assess risks concerning coexistence of cultivars. The assessment should cover potential gene flow from the GMP(s) to conventional and organic crops as well as to compatible wild relatives in semi-natural or natural habitats. The potential for establishment of volunteer populations within the agricultural production systems should also be considered. VKM is also requested to evaluate relevant segregation measures to secure coexistence during agricultural operations up to harvesting. Post-harvest operations, transport and storage are not included in the assignment.

Evaluations of suggested measures for post-market environmental monitoring provided by the applicant, case-specific monitoring and general surveillance, are not covered by the assignment from NFSA. In addition, the changes related to herbicide residues of GMPs as a result of the application of plant-protection products fall outside the remit of the Norwegian VKM panels.

Assessment

1 Introduction

The food, feed and environmental risk assessment of the genetically modified soybean 356043 is based on information provided by the applicant in the application EFSA/GMO/UK/2007/43, relevant peer-reviewed scientific literature, and scientific opinions from VKM (VKM, 2008), EFSA (EFSA, 2011c) and other member states made available on the EFSA website GMO Extranet. Except for a synopsis of more recent literature, this draft opinion is to a large extent a summary of the above-mentioned VKM and EFSA reports, which are provided in Appendix I and II respectively, and readers are referred to these for details. These reports concluded that based on intended uses and data provided, soybean 356043 is as safe as its conventional counterpart with respect to potential effects on human and animal health.

Genetically modified soybean 356043 (Unique Identifier DP-356043-5) with the trade name Optimum GAT™ was developed to provide tolerance to multiple herbicides via introduction of both the *gat4601* and the *Glycine max-hra* (*gm-hra*) gene sequences. Thus soybean 356043 is tolerant to not only glyphosate, but also has heightened tolerance to so-called ALS (acetolactate synthase)-inhibiting herbicides such as chlorimuron, thifensulfuron and sulfonylureas. The DNA fragment containing the gene sequences for both traits were introduced by the particle acceleration method.

Glyphosate is phytotoxic to the majority of annual and perennial grasses and broadleaved weeds. Its mode of action is to inhibit the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an essential enzyme involved in aromatic amino acid synthesis in plants, bacteria and fungi. Blocking of the EPSPS enzyme results in a lack of synthesis of the aromatic amino acids; tyrosine, tryptophan and phenylalanine in glyphosate-treated grasses and weeds. The resulting deficiency in these key amino acids prevents growth and ultimately leads to the death of the treated weeds.

In soybean 356043, the introduced *gat4601* gene sequence is an optimised form of the glyphosate acetyltransferase (*gat*) coding sequence from *Bacillus licheniformis*. GAT proteins catalyse the acetylation of glyphosate, producing N-acetyl glyphosate, which has no herbicidal activity. The introduction of the optimised gene sequence *gat4601* into the genome of crops will therefore confer effective tolerance to herbicides containing the active ingredients glyphosate and glyphosate-ammonium.

Acetolactate synthase (ALS)-inhibiting herbicides, such as chlorimuron, thifensulfuron and sulfonylureas, cause growth retardation in seedlings by impairing branch chain amino acid synthesis in treated grasses and broadleaf weeds, but not in crops such as rice, wheat, barley, soybean, maize and others due to their high endogenous ALS expression. The herbicides have potency at extremely low concentrations, but rapid resistance development in weeds has limited their application (see review by [Tranel and Wright, 2002](#)).

In soybean 356043, the introduced *gm-hra* gene sequence is an optimised form of the endogenous *als* coding sequence from soybean (*Glycine max*; *gm*), conferring heightened tolerance to ALS-inhibiting herbicides.

The genetic modification in soybean 356043 is intended to improve agronomic performance only and is not intended to influence the nutritional properties, the processing characteristics or the overall use of soybean as a crop.

Soybean 356043 has been evaluated with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed.

VKM has also taken into account the appropriate principles described in the EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA, 2011d), the environmental risk assessment of GM plants (EFSA, 2010a), the selection of comparators for the risk assessment of GM plants (EFSA, 2011b), and for the post-market environmental monitoring of GM plants (EFSA, 2011e).

It is emphasised that the VKM mandate does not include assessments of contribution to sustainable development, societal utility or ethical considerations, according to the Norwegian Gene Technology Act and Regulations relating to impact assessment pursuant to the Gene Technology Act. These considerations are therefore not part of the risk assessment provided by the VKM Panel on Genetically Modified Organisms.

2 Molecular characterisation

Previously, the GMO panels of VKM ([VKM, 2008; Appendix I](#)) and EFSA ([EFSA, 2011c; Appendix II](#)) assessed the molecular characterisation of the event DP-356043-5 (356043; *gat4601* and *gm-hra* inserts) with regards to the following:

1. The transformation system and vector constructs
2. Characterisation of the transgene insertions and constructs
3. Information on the expression of the insert (open reading frames), and
4. Inheritance and the stability of the inserted DNA

Both the VKM (2008) and EFSA (2011c) GMO panels concluded that the applicant had provided sufficient analyses to characterise the DNA insert, number of inserts, integration site and flanking sequences in the soybean 356043 genome. The results show the presence of a DNA fragment containing one functional copy of each of the *gat 4601* and *gm-hra* genes only. No other functional vector genes were detected. Similarity searches with databases of known toxins and allergens did not indicate potential production of allergenic or toxic proteins or polypeptides as a result of the genetic modification (Technical Dossier; [Delaney et al., 2008](#)). Southern blot and segregation analyses show that the introduced gene elements were stably inherited and expressed over multiple generations in parallel with the observed phenotypic characteristics of soybean 356043. More recent literature concerning the molecular characterization of soybean 356043 has not been identified.

2.1 Conclusions

Based on the above considerations, the VKM GMO panel concludes that the molecular characterisation of soybean 356043 does not indicate a safety concern.

3 Comparative assessments

Previously, the GMO panels of VKM ([VKM, 2008; Appendix I](#)) and EFSA ([EFSA, 2011c; Appendix II](#)) assessed compositional and agronomic data provided by the applicant from various field trials conducted in North and South America in 2005-2006. A brief summary from these reports are provided below.

3.1 Production of material for comparative assessment

In the compositional and agronomic studies, seed and forage of the GM soybean 356043 were compared to the non-transgenic variety Jack (control), which is a conventional soybean cultivar with background genetics similar to soybean 356043, in replicated field trials conducted in 2005 and/or 2006 in USA and Canada and during the 2005/2006 growing season in Chile and Argentina. The two soybeans were grown under the same agronomic conditions. Plots were included in which soybean 356043 was treated with glyphosate and/or ALS-inhibiting herbicides. Data obtained were compared to ranges for agronomic and compositional characteristics obtained from other commercial non-GM soybean cultivars, both from the literature as well as from a separate study. In the separate study, four conventional soybean cultivars were grown in six locations in North America in 2005.

More recent field trials have apparently not been conducted. Therefore, only data from the above-mentioned field trials, which were conducted before more recent EFSA guidelines existed ([EFSA, 2011d](#)), form the basis for the risk assessment.

3.2 Compositional analysis

Both soybean seed and forage were analyzed. The analytes assessed for the compositional comparisons followed the recommendations by ([OECD, 2000](#)). In addition, compounds related to the activities of the newly expressed proteins were analysed in the seeds: acetylated amino acids, free amino acids, and some odd-chained fatty acids. For each analyte, the statistical analysis was conducted both within and across sites.

It was concluded that with the exception of the changes caused by the transgenetically introduced traits, few statistically or biologically significant differences were observed between soybean 356043 and conventional "Jack" varietal in the analysis of seed and forage. Most of the differences observed were only present in material from some of the locations and were likely to reflect the natural variability observed in conventional soybean cultivars. However, due to the enzyme activities of the new proteins expressed as a result of the inserted genes, higher levels of acetylated amino acids, especially N-acetylaspartate (NAA; >300 times higher than conventional soybean cultivars) but also N-acetylglutamate (NAG), and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid were measured in seed from soybean 356043 (Table 3.2-1). These levels fell outside the ranges measured for other conventional soybean cultivars, yet only made up a small proportion of total amino acids (<0.15%) and total fatty acids (<1%) in raw seeds.

Table 3.2-1 Levels of acetylated amino acids (in mg/kg dry weight) N-acetylaspartate (NAA) and N-acetylglutamate (NAG), and odd-chain fatty acids (as % of total fatty acids) heptadecanoic acid (C17:0), heptadecenoic acid (C17:1) and heptadecadienoic acid (C17:2) in raw seeds from soybean 356043, untreated or treated with target herbicides glyphosate and ALS-inhibiting herbicides, compared to seeds from the conventional soybean varietal "Jack" and the ranges reported in other conventional reference cultivars (adopted from [EFSA, 2011c](#)).

| Analyte | | Control soybean Jack, untreated with target herbicides | Soybean 356043, untreated with target herbicides | Soybean 356043, treated with target herbicides | Range reported for conventional reference cultivars |
|---------|-------|--|--|--|---|
| NAA | Mean | 1.92 | 653 | 681 | 0-2.27 |
| | Range | 1.10-3.67 | 490-870 | 502-994 | |
| NAG | Mean | 2.34 | 18.3 | 18.1 | 0-3.17 |
| | Range | 1.42-3.35 | 9.86-43.2 | 8.27-31.8 | |
| C17:0 | Mean | 0.129 | 0.326 | 0.330 | 0.085-0.146 |
| | Range | 0.105-0.304 | 0.207-0.408 | 0.152-0.423 | |
| C17:1 | Mean | 0.063 | 0.179 | 0.183 | 0.073-0.087 |
| | Range | 0.049-0.136 | 0.117-0.240 | 0.067-0.248 | |
| C17:2 | Mean | 0.056 | 0.150 | 0.153 | 0-0.068 |
| | Range | 0.045-0.121 | 0.099-0.203 | 0.061-0.211 | |

The applicant concluded that the biological significance of intake of NAA and NAG in soybean 356043 is minimal since they are normal constituents in mammalian metabolism, present in conventional food and feedstuffs, and mammals and humans possess deacetylase activity in their intestines. Furthermore toxicity testing (acute, repeated dose, subchronic, and reproductive, developmental and genotoxicity testing) and exposure assessments have not revealed any relevant safety concerns (see [EFSA, 2011c](#)).

The relative levels of the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acids in raw unprocessed soybean seeds 356043 are similar or lower than levels observed in plant oils, butter, cheese and meat, and have also been observed in human tissues. Considering intake information and exposure, EFSA ([EFSA, 2011c](#)) concluded that replacement of soybean oil from conventional soybeans with oil from soybean 356043 does not raise safety concerns.

VKM (2008) and EFSA (2011c) concluded that no differences were identified between soybean 356043, its conventional counterpart and other conventional soybean cultivars except for the newly expressed proteins, and for higher levels of the acetylated amino acids NAA and NAG, and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acids in seeds from soybean 356043. The levels of these acetylated amino acids and odd-chain fatty acids fall outside the natural ranges observed for conventional soybean cultivars.

For more details, the readers are referred to Appendix II ([EFSA, 2011c](#)).

3.3 Agronomic traits and GM phenotype

Based on the field trials described above (section 3.1), VKM (2008) and EFSA (2011c) GMO panels concluded that agronomic traits and morphological parameters observed for soybean 356043, fell within the ranges observed for conventional cultivars. Soybean 356043 was therefore considered agronomically and morphologically not different from conventional soybean cultivars.

3.4 Conclusion

The VKM GMO Panel has considered the available data concerning compositional, agronomic and morphological characteristics and confirms that except for increased levels of especially the acetylated amino acids NAA, but also NAG and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acids in soybean 356043 seeds, no biologically relevant differences were identified between soybean 356043 and its corresponding conventional counterpart and other conventional cultivars. The small intermittent variations in other analytes were only present in material from some of the locations, were within the range of values observed in conventional soybean cultivars, and are therefore considered to reflect the natural variability.

Based on current knowledge and excluding the novel traits with resulting increased content of the acetylated amino acids NAA and NAG, and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acids, the VKM GMO Panel concludes that soybean 356043 is compositionally, agronomically, and morphologically equivalent to its conventional counterpart and other conventional soybean cultivars.

4 Food and feed safety assessment

4.1 Previous evaluations by the VKM GMO panel and EFSA

Previously, the GMO panels of VKM ([VKM, 2008: Appendix I](#)) and EFSA ([EFSA, 2011c: Appendix II](#)) evaluated food and feed safety assessments of soybean 356043 based on existing information, which was limited to a 28-day repeated dose study with mice and a 42-day nutritional assessment with broilers. Data was provided in the initially submitted technical dossier. The VKM panel concluded that the toxicity and allergenicity tests performed by the applicant were not sufficient. The panel deemed it necessary that Pioneer Hi-Bred Int. should submit data from a 90-day sub-chronic feeding study of soybean 356043 in rats, since new proteins (GAT4601/GM-HRA) are expressed as a result of the genetic modification. The following assessment is therefore based on more recent submissions from the applicant and recent publications (see 4.5.2). Information regarding product description and intended uses (see 4.2), which was not a part of the previous VKM report ([VKM, 2008](#)), is also included in the current opinion.

4.2 Product description and intended uses

Product description and intended uses were not considered in the previous VKM assessment ([VKM, 2008](#)), but were in EFSA's evaluation ([EFSA, 2011c](#)) of soybean 356043. Therefore a summary, including considerations specific for Norwegian soybean use, are included below.

The genetic modification in soybean 356043 will not impact the existing post-harvest production processes used for soybeans. The major soybean commodity products are seeds, oil, meal and protein concentrates/isolates. Unprocessed soybeans are not suitable for food and their use in animal feed remains limited because they contain anti-nutritional factors such as saponins, trypsin inhibitors and lectins ([OECD, 2012](#)). Adequate heat processing inactivates most of the biological activity of these factors. The main soybean product fed to most animals is the defatted/toasted soybean meal. However, aspirated grain fractions, forage, hay, hulls, and silage are also used as feed to a limited extent, primarily for cattle ([OECD, 2012](#)).

Further processing of soybean seed to produce soybean protein concentrate is required for farmed salmonid fishes and is the most commonly used plant ingredient in salmonid feed formulations in Norway (www.mattilsynet.no). Since 2008, NFSA has given four fish feed producers in Norway extended exemption from seeking approval of GM products. The exemption applies to processed, non-viable feed products from 19 different GM varieties. In October 2014, this exemption was not extended. Whole soybeans are utilised to produce food products such as soy sprouts, baked soybeans, toasted soybeans, full fat soy flour and the traditional Asian soy foods (miso, soy milk, soy sauce, and tofu) ([OECD, 2012](#)). The processing steps used in food manufacturing of soybean are shown in Figure 4.2-1 adapted from the Technical dossier. The first step in processing most soybeans is to separate the oil, either by solvent extraction or by expelling.

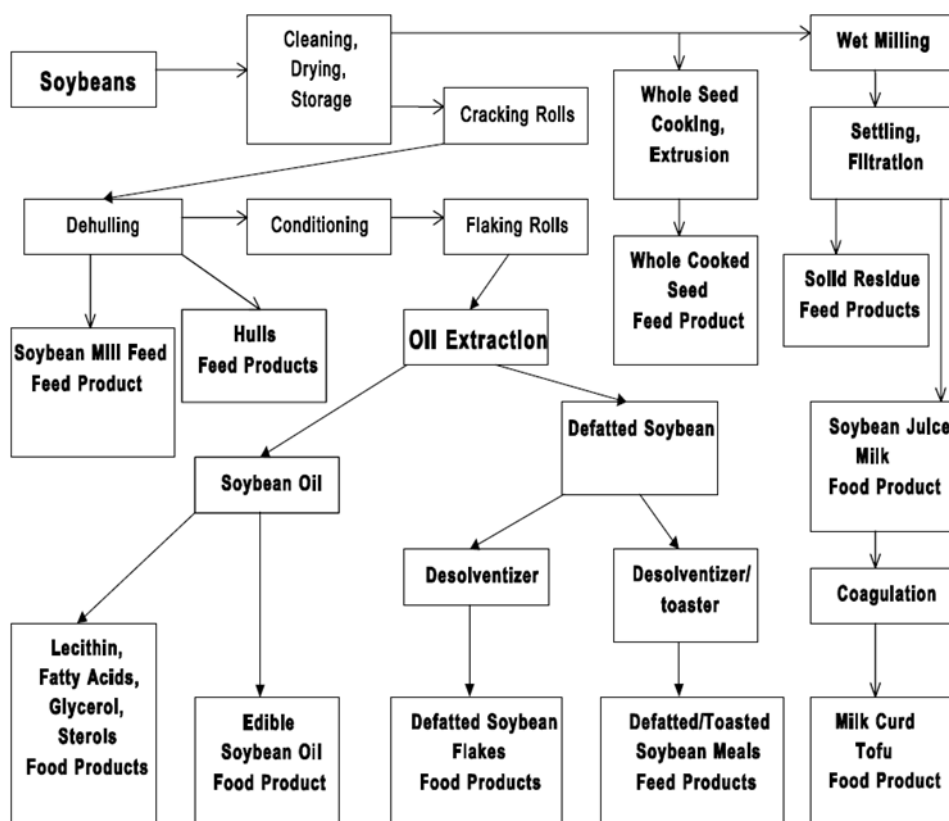


Figure 4.2-1. Processing of soybean, adapted from (OECD, 2012; Waggle and Kolar, 1979).

All GM soybean products are produced and processed for use in food, animal feed and industrial products in the same way as other commercial soybean and according to the applicant the commercial experience since 1996 has confirmed that this has been the case. The major soybean commodity products are seeds, oil, and meals.

The soybean 356043 and all food, feed and processed products derived thereof are expected to replace a portion of similar products from commercial soybean, with total consumption of soybean products remaining unchanged.

4.3 Effects of processing

The processing steps used to produce the various soy products are shown in Figure 4.2-1, above. Soybeans are first cracked and de-hulled, then heated to approximately 60°C, ground to flakes with rollers, and are then treated with solvent to remove the oil. The flakes are toasted, cooled and ground. During these processes, proteins in soy, including novel proteins, are subjected to harsh conditions, such as thermal processing, changes in pH, reducing agents, mechanical shearing, and so on, which will lead to denaturation and loss of protein function.

The applicant supplied data on the influence of temperature (36-60°C) and pH (5-9) on the enzyme activities for both GM-HRA and GAT4601 proteins produced in *Escherichia coli*. For GM-HRA, 15 min of exposure to 44°C reduced enzyme activity by ca. 50%, whereas nearly all activity was lost following exposure to 50°C for 15 min. The pH optimum for enzyme activity was in the range of 7.0-7.5. Below pH 6.0 and above pH 9.0, the enzyme was nearly inactivated. For the GAT4601 enzyme, exposure to 50°C for 15 min reduced activity by 40% while exposure to 56°C for 15 min nearly eliminated activity. The pH optimum for enzyme activity was in the range of 6.0-6.5. The enzyme activity was considerably reduced at pH 5 and 8.5.

Due to the compositional differences regarding the acetylated amino acids NAA and NAG and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acids in raw seeds and forage from soybean 356043 (see section 3.2), the applicant provided data on the levels of these components in processed products derived from soybean 356043, both untreated and treated with the target herbicides.

Compared to products derived from the conventional soybean "Jack", higher NAA and NAG levels were found in whole cooked seed, hull material, defatted raw flakes, defatted toasted meal, mill feed, defatted flour, and soy milk from soybean 356043. Higher NAA, but not NAG, were observed in aspirated seed fractions, crude lecithin, protein concentrate, okara and tofu. The NAA and NAG were below detection levels in protein isolate and degummed and refined, bleached and deodorised soybean oils.

In many processed products derived from soybean 356043, NAA and NAG levels were reduced or in the same range as in unprocessed soybean 356043. The exceptions were hull material and mill feed, in which NAA and NAG levels were higher, and in defatted raw flakes and defatted toasted meal, in which higher NAG levels were observed.

The odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acids in soybean oils from soybean 356043 compared to oil from the conventional soybean "Jack" (as % of total fatty acids) were fully in line with values observed in the respective seeds (see Table 3.2-1).

4.4 Toxicological assessment of soybean 356043

4.4.1 Toxicological assessment of the expressed novel proteins

4.4.1.1 Acute toxicity testing

A 14-day acute toxicity testing by single dose oral gavage with Crl:CD-1 mice at the limit dose of 2000 mg/kg bw of the pure GAT4601 protein was assessed ([Delaney et al., 2008](#)) following the OECD 423 Guidelines, ([OECD, 2001](#)). Control groups received vehicle (water) or 2000 mg/kg bw albumin. No clinical signs of systemic toxicity were observed and no gross lesions were observed at necropsy. All animals survived the duration of the study and weight gain was relative to day 0. It was therefore concluded that the GAT4601 protein is not acutely toxic.

A similar 14-day acute toxicity study with purified GM-HRA protein (obtained from a heterologous bacterial expression system) was conducted at a limit dose of 2000 mg/kg bw

via single oral gavage with CD-1 mice (5 mice /sex) ([Mathesius et al., 2009](#)). Control groups were administered water (vehicle) or bovine serum albumin (BSA) at 2000 mg/kg bw. Authors reported that no mortality or clinical signs of systemic toxicity occurred in any of the treatment groups. Mice gained weight relative to Day 0 of dosing and no gross lesions were evident at necropsy. Thus, the GM-HRA protein is not acutely toxic.

The VKM GMO Panel agrees with EFSA in the opinion that acute toxicity testing of the newly expressed proteins is of little additional value to the risk assessment of the repeated human and animal consumption of food and feed derived from GM plants (EFSA 2011), and is therefore not taken into account in this risk assessment.

4.4.1.2 Repeated-dose toxicity testing

Previous allergenicity and toxicity testing of the GAT4601 protein in a 27-day repeated-dose dietary administration with mice, together with *in silico* and *in vitro* assessments showed no adverse effects ([Delaney et al., 2008](#)). In the animal study, heterologously-produced GAT4601 protein was blended into rodent diets (PMI 5002) at doses corresponding to 10, 100, and 1000 mg/kg/day, whereas controls consumed only PMI 5002. Authors report that body/organ weights, clinical observations/chemistry as well as gross/microscopic lesions were assessed according to OECD 407 guidelines ([OECD, 1995](#)). None of these parameters showed adverse effects that were considered to be treatment related, although some statistically significant differences were observed in total protein, albumin and potassium values.

Similar to GAT4601 protein, the safety assessment of the GM-HRA protein was conducted employing the step-wise weight-of-evidence approach. Bioinformatics analysis of the amino acid sequence did not identify similarities to known allergenic or toxic proteins ([Mathesius et al., 2009](#)). In a 28-day repeated-dose toxicity assessment with CrI:CD-1 mice (25 mice/sex), the GM-HRA protein was blended into diets corresponding to daily doses of 100, 300, and 1000 mg/kg bw/day ([Mathesius et al., 2009](#)). No mortality, abnormal clinical/ophthalmological observations or adverse effects in the clinical chemistry variables were noted. With regards to organ weights, statistically significant decreases were observed in relative spleen and adrenal weights in male mice from some GM-HRA protein groups, compared to the control group, however, these effects were not considered to be treatment-related or adverse. *In vitro* studies showed that both proteins are acid and heat labile, and not glycosylated *in planta*.

4.4.1.3 Toxicological assessment of new constituents other than proteins

Other than the GAT4601 and GM-HRA proteins, the genetic modification led to production of N-acetylated amino acids NAA and NAG and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acids (see section 3.2) in soybean 356043. No other relevant changes in the composition of soybean 356043 were detected by the targeted compositional analysis. Additional toxicological and exposure assessment information of these constituents were provided by the applicant upon request from EFSA ([EFSA, 2011c](#) in Appendix II).

4.4.2 Toxicological assessment of the whole GM food/feed

A 93-day sub-chronic feeding study according to OECD 408 on CrI:CD (SD) rats was performed with soybean 356043 was published by Pioneer Hi-Bred Int. in 2008 (Appenzeller et al., 2008). The diet consisted of 20%(W/W) dehulled/defatted meal and 1.5% (W/W) toasted ground hulls prepared from untreated plants, herbicide-treated plants, non-transgenic isoline control and three commercial reference cultivars (93B86, 93B15 and 93M40) were formulated into individual diets in conformance to standard certified rodent chow formulation (Purina Rodent LabDiet® 5002). The study consisted of 6 experimental groups (12 rats/sex). Body weight/gain, feed consumption, clinical signs/pathology, mortality, ophthalmology, neurobehavioral examinations, organ weights and gross/microscopic pathology were assessed.

Generally, no biologically-relevant adverse effects were observed for the parameters measured. Of note, there were statistically significant differences ($p < 0.05$) in the mean corpuscular volume (MCV) and the mean corpuscular haemoglobin (MCH) values for female rats fed the herbicide-treated plants compared to the isoline control. However, the authors reported that:

1. The magnitude of the difference of 3% was small and as such negligible.
2. Changes in MCV and MCH values occur secondary to effects on mature red cell mass parameters (red blood cells (RBC) count, haemoglobin and haematocrit), which serve as indicators of an underlying pathogenesis but these parameters were not statistically different between the groups in question.
3. No statistical differences were observed for male rats in the same treatment group, or males/females in the untreated plants, compared with gender-matched isoline control.
4. All individual MCV and MCH values obtained for female rats in the herbicide-treated group for these response variables are within the range of natural variation since they were within the ranges of individual MCV and MCH values for females in the reference groups.

With regards to serum chemistry, the mean blood urea nitrogen (BUN) value for male rats in the herbicide-treated group was statistically significantly higher ($p < 0.05$) than the mean value for the matched isoline control. Again, the authors discussed that the difference was not adverse or considered to be diet-related for reasons that follow:

1. The magnitude of the difference was relatively small (13% higher than the control group) and within the performing laboratory's historical reference range for control male rats of similar strain and age (9-17 mg/dL).
2. A treatment-related increase in BUN would be expected to occur simultaneously with changes in other serum chemistry response variables related to glomerular filtration.
3. Neither male rats in untreated plants nor females in both test groups showed statistical differences in mean BUN values.
4. The individual BUN values for male rats in all groups were similar and ranged from 12 to 22 mg/dL.

A high occurrence of histiocytosis (increased tissue macrophages) was observed in the liver of rats fed both untreated and herbicide-treated GM soybeans compared to the non-transgenic isoline control, but the authors claim this observation is common in rats of the strain and age employed and consistent with normal background lesions.

4.4.3 Allergenicity

The strategies used when assessing the potential allergenic risk focus on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation or to elicit allergic reactions in already sensitised persons and whether the transformation may have altered the allergenic properties of the modified food. A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (Alimentarius, 2003; EFSA, 2006; EFSA, 2011d).

4.4.3.1 Assessment of allergenicity of the newly expressed proteins

As described earlier (Delaney et al., 2008; Mathesius et al., 2009), bioinformatics analysis of the amino acid sequence of GAT4601 and GM-HRA did not identify similarities to known IgE-dependent allergenic proteins. *In vitro* studies performed in simulated gastric fluid as well as intestinal fluid exhibited rapid degradation of both proteins. Additionally, both proteins are heat labile, and not glycosylated, as with most IgE-dependent allergenic proteins.

4.4.3.2 Assessment of allergenicity of the whole GM plant

Serum from soy allergic patients contains IgE antibodies that react with allergenic soy proteins. Such sera obtained from clinically reactive soy allergic patients were used to investigate the impact of the genetic modification in soybean from event DP-356043-5 (356043; *gat4601* and *gm-hra* genes) on allergenic proteins (Delaney et al., 2008). IgE immunoblot analysis and enzyme-linked immunosorbent assay (ELISA) inhibition analysis on protein extracts from 356043 and non-GM control demonstrated that soya 356043 does not produce new allergenic proteins. Similar protein/allergen profiles were observed, with no significant changes.

4.4.3.3 Assessment of allergenicity of proteins derived from the GM plant

Allergenicity of the soybean could be increased as an unintended effect of the random insertion of the transgene in the genome of the recipient, e.g. through qualitative or quantitative modifications of the expression of endogenous proteins. However, given that no biologically relevant agronomic or compositional changes (with the exception of the introduced traits; see 3.2 and 3.3) and no difference in allergenic potential of the whole plant (see 4.4.2.4) have been identified, no increased IgE-mediated allergenicity is anticipated for soybean 356043.

4.4.4 Assessment of adjuvanticity

According to the EFSA Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed from GM plants (EFSA, 2010b), adjuvants are substances that, when co-administered with an antigen increases the immune response to that antigen and therefore might increase the risk of allergic reactions. Adjuvanticity has not been routinely considered in the assessment of allergenicity or immunogenicity of GMOs. Literature review has not revealed any reports of adjuvant properties of the GM-HRA or GAT4601 proteins.

In cases when known functional aspects of the newly expressed protein or structural similarity to known strong adjuvants may indicate possible adjuvant activity, the possible role of these proteins as adjuvants should be considered. As for allergens, interactions with other constituents of the food matrix and/or processing may alter the structure and bioavailability of an adjuvant and thus modify its biological activity. The GAT and GM-HRA proteins have not been reported to have adjuvant properties.

“Bystander sensitisation” can occur when an adjuvant in food, or an immune response against a food antigen, results in an increased permeability of the intestinal epithelium for other components in food. Previously it was assumed that the epithelial cells of the intestine were permanently held together tightly by the so-called tight junctions. More recent knowledge shows that these complex protein structures are dynamic and can become less tightly joined, i.e. more “leaky”, by different stimuli.

Both *in vitro* and *in vivo* experiments have demonstrated that when an IgG response, which can result in a complement activation (among other reactions), is not balanced by an IgA response, the epithelial barrier can become leaky and unwanted proteins are able to enter the body (bystander-penetration) and lead to allergic sensitization ([Brandtzaeg and Tolo, 1977](#); [Lim and Rowley, 1982](#)).

4.5 Nutritional assessment of GM food and feed

Due to the genetic modification and the subsequent increased levels of the acetylated amino acids NAA and NAG, and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acids, soybean 356043 cannot be considered compositionally equivalent to conventional soybean cultivars. However, in the previous evaluations both EFSA (2011d) and VKM (2008) concluded that the presence and reported levels of these components do not raise safety concerns as they are present at low levels and found in other commonly ingested food and feed ingredients.

According to the updated version of the EFSA guidance for risk assessment of food and feed from genetically modified plants ([EFSA, 2011d](#)), the experimental design should always include the following test materials: the GM plant exposed to the target herbicide(s), the non-GM comparator treated with conventional herbicide management regimes, the GM plant treated with the conventional herbicide management regimes, as well as six conventional, commercial strains as reference groups. The peer-reviewed studies with broilers and laying hens summarized below (see 4.5.2) are not in accordance with the suggested experimental design in the last EFSA guidance document on risk assessment ([EFSA, 2011d](#)). The Norwegian GMO Panel is in agreement with the importance of including GM plants treated both with and without the target herbicide(s) in comparative analysis (composition, agronomic traits, food and feed safety assessments), but recognizes that the applicant submitted the application prior to the last guidance document from EFSA.

4.5.1 Intake information/exposure assessment

The human soybean oil consumption in Europe was calculated at 6.3-7.0 g/person/day, based on FAO Statistics from 1997 to 2001. Assuming that 54% of the soybean oil was derived from soybean 356043, the estimated average exposure of the European consumer to products of soybean 356043 would be approximately 3.4-3.7 g/person/ day (Technical dossier).

According to FAOSTAT databases (1961-2005), which was used as the source for exposure assessment of soybean oil by the applicant and reported in EFSA's scientific opinion concerning soybean 356043 (EFSA, 2011c), mean per capita intake of soybean oil was estimated to be 10.3 g/day, with the Netherlands consuming the highest levels of an average of 36.1 g/day. Using the consumption scenario in the Netherlands and assuming 100% replacement of oil derived from GM soybean 356043, it was calculated that the additional intake of heptadecanoic, heptadecenoic and heptadecadienoic acid would be 84, 60 and 42 mg/day, respectively.

Soybeans and their products are little used in the average Norwegian diet, with the exception of vegans and those with milk allergies. In Table 4.5.1-1 the mean intake of soy protein/day for an adult person in Norway eating either a vegan menu or a milk free diet are presented (Engeset & Lillegaard, 2014, unpublished results). The calculations were based on week menus. For the vegan menu a person who has previously eaten meat and is looking for meat substitutes like soy burgers and sausages were envisioned. In the milk free diet a 7 day week menu was composed where milk products were replaced with soy products. Both menus are included in Appendix III.

Table 4.5.1-1. Mean intake of soy products and soy protein for adult persons with milk allergy and vegans with high preference for soy products.

| Diet | MJ/day (mean) | Gram soy products/day (mean) | Gram soy protein/day (mean) |
|--------------|---------------|------------------------------|-----------------------------|
| Milk allergy | 9.7 | 538 | 19 |
| Vegan | 10.1 | 865 | 35 |

Average estimated energy requirement for children in different age groups, based on The Nordic Nutrition Recommendations (NNR), was used to adjust the numbers in table 4.5.1-1 according to age to give an estimate of how much soy protein children may consume if on the given diets (Table 4.5.1-2). We assumed that milk in coffee/tea in the menus is consumed as milk by the children.

Table 4.5.1-2. Estimated intake of soy products and soy protein for children in different age groups, with milk allergy and vegans, and with high preference for soy products.

| Diet | Estimated energy requirement MJ/day ¹ | Gram soy products/day | Gram soy protein/day |
|---------------------------------------|--|-----------------------|----------------------|
| Milk allergy | | | |
| 2-5 year | 5.3 | 294 | 10 |
| 6-9 year | 6.9 | 383 | 14 |
| 10-13 year (girls)² | 8.6 | 477 | 17 |
| 14-17 year (boys)² | 11.8 | 655 | 23 |
| Vegan | | | |
| 2-5 year | 5.3 | 454 | 18 |
| 6-9 year | 6.9 | 591 | 24 |
| 10-13 year (girls)² | 8.6 | 737 | 30 |
| 14-17 year (boys)² | 11.8 | 1011 | 41 |

1 Based on Nordic Nutrition Recommendations 2012

2 Boys 10-13 years and girls 14-17 years will have approximately the same consumption as adults; estimated energy requirement of 9,3 and 9,8 respectively.

EFSA conducted a scenario assessment for high consumers of soybeans assuming a daily consumption of 200 g of unprocessed soybeans (equivalent to approximately 70 g soy protein) for an individual with a bodyweight of 60 kg (EFSA, 2011c). Reports from the EFSA Comprehensive Food Consumption Database (EFSA, 2011a) confirmed that 200 g soybeans/day is a conservative assumption. The additional intake in the scenario was based on replacement of all soybeans with the GM soybean 356043, and gave an additional intake of NAA and NAG of 114 and 2.1 mg/day, respectively. The Norwegian soy scenario (table 4.5.1-1) is within the range of the EFSA assessment with the highest estimated soy protein intake of 35 g/day for vegans (half of the EFSA scenario).

Around 90% of the soybean defatted protein meal supply worldwide goes to animal feed, while there is limited use of soybean oil in feed. The applicant calculated, based on data from 2006, that the maximum inclusion levels (% of the diet) of soybean 356043 meal in the EU would be 21% for broilers, 18% for pigs and 12% for dairy cattle (Technical dossier).

In Norway, more than 1.6 mill tons of fish feed was produced in 2014 and soybean protein concentrate (SPC) is the major plant protein source in salmon feeds (Directorate of Fisheries, Biomass statistics 2015). The average inclusion level of SPC in feed for Atlantic salmon is 25%, total SPC used for fish feed production in 2013 was calculated to be approximately 375 000 tons (Skretting, 2013).

Assuming that 100% of the SPC was derived from soybean 356043, the estimated average exposure of Atlantic salmon (post smolt, 200 g) to products of soybean 356043 would be approximately 2 g/fish/day (assuming 3% growth per day and feed conversion ratio of 1).

Norwegian surveillance data show that imported SPC intended for feed production only contains trace amounts of GMO (*e.g* below 0.9%) (Spilsberg, 2014). Samples of all imported SPCs are analysed for the presence of five transgene sequences commonly found in GMOs. These five DNA specific targets are: 35S promoter (p35S), *Agrobacterium* nopaline synthase terminator (tNOS), *ctp2-cp4epsps*, the *bar* gene from *Streptomyces hygrosopicus* and the *pat* gene from *Streptomyces viridichromogenes*. The methodology is highly sensitive and capable of detecting minute amounts of GM-material. Additional analyses may also be carried out to determine the specific GMOs present in a sample.

4.5.2 Nutritional assessment of feed derived from the GM plant

Nutritional assessments of feed derived from soybean 356043 were not considered in the previous VKM assessment (VKM, 2008), but were in EFSA's evaluation (EFSA, 2011c). Therefore a summary, including considerations specific for Norwegian soybean use, are included above. More recent nutritional assessment studies (McNaughton et al., 2011a; McNaughton et al., 2011b) are summarised in addition.

The nutritional assessment studies were not conducted according to the latest EFSA guidelines (EFSA 2011c), but the VKM GMO panel recognizes that the applicant submitted the application prior to the last guidance document.

Comparison of the nutritional equivalence of soybean 356043 to non-transgenic soybeans was conducted in a 42-day feeding study with broilers (McNaughton et al., 2007). 720 Ross x Cobb broilers were divided into 6 groups (n=120/group, 50% female, 50% male). Diets were prepared using processed fractions from untreated soybean plants, herbicide-treated plants (Gly/SU; glyphosate, chlorimuron, and thifensulfuron mixture), non-transgenic near-isoline control (091) and three commercial reference cultivars (93B86, 93B15 and 93M40). Starter diets contained 30% soybean meal, grower diets 26% soybean meal, and finisher diets 21.5% soybean meal. Soybean hulls and oil were added at 1.0 and 0.5%, respectively, across all diets in each phase. No significant differences were observed in the nutritional proximate, growth performance variables, mortality, and carcass/organ yields consuming the different diets. However, relative liver weights in males were found to be higher ($p < 0.05$, but not statistically significant when the *P*-value was adjusted for false discovery rate) in the herbicide-treated plants compared to control. The authors pointed out that liver and kidney

weights in particular, are very sensitive to nutritional/dietary differences and as such indicators of overall broiler health.

More recently, a 42-day repeated-dose feeding study assessing broiler performance and carcass yields when fed a combination of processed fractions of soybean from event DP-356043-5 (356043; *gat4601* and *gm-hra* genes), and maize grain from event DP-Ø98140-6 (98140; *gat4621* and *gm-hra* genes) has been conducted and published in a peer-reviewed journal ([McNaughton et al., 2011a](#)). Five groups consisting of 120/group Ross 708 broilers (50% female, 50% male) were fed 356043 + 98140, controls with comparable genetic backgrounds or 3 other reference commercial non-transgenic soybean and maize combinations. The broilers were fed diets in 3 phases: starter (d 0 to 21), grower (d 22 to 35), and finisher (d 36 to 42). Starter diets contained (on average) 63% maize and 28% soybean meal, grower diets 66% maize and 26% soybean meal, and finisher diets 72% maize and 21% soybean meal; soybean hulls and oils were held constant at 1.0 and 0.5%, respectively, across all diets in all phases. Feed intake, weight gains and mortality-adjusted feed efficiency were analysed for the duration of the study and standard organ and carcass yields were collected on day 42. No significant differences were observed in the measured parameters, thus the authors concluded that 356043 + 98140 was nutritionally equivalent to non-transgenic soybean/maize and their corresponding controls.

The nutritional equivalence of soybean 356043, a similarly modified (inserted genes *gat4621* and *zm-hra*) maize grain 98140, or a combination of the two (356043 + 98140) were also evaluated in laying hens over three 4-week phases, in a total of 84 days ([McNaughton et al., 2011b](#)). Healthy pullets (Babcock B300 White Leghorn) were raised to 17 wk of age in cages at Slonaker Farms (Glengary, WV) under conditions common to commercial pullet rearing. The maize 98140 had apparently been treated with target herbicides, but it was not specified in the publication whether the soybean 356043 was herbicide-treated or not. Healthy pullets (n = 216) were randomly assigned to 9 dietary treatments (24 hens /treatment), including comparable background controls for 356043, 98140 and 356043 + 98140, as well as three reference commercially available maize-soybean meal source. Performance as measured by body weight, feed intake, and egg production as well as egg quality were examined. No observable differences were made between hens fed test diets or corresponding controls. Additionally, Haugh unit measures and egg component weights were comparable. It was concluded that the performance and egg quality of hens fed diets formulated with soybean 356043, maize grain 98140 or a combination of the two (356043 + 98140) were similar to that of hens fed diets with non-transgenic soybean meal and maize grain with comparable genetic backgrounds. Notably, the authors discuss that the presence of mycotoxins, namely fumonisins FB₁, FB₂, FB₃ in maize sources were well below the US FDA (2001) guideline for total fumonisins of 100 mg/kg and thus not of concern.

4.6 Conclusion

A subchronic toxicity study in rat, repeated dose studies in mice, nutritional whole food studies in broilers and laying hens and allergenicity assessment studies have been performed with soybean 356043. These studies have not revealed adverse effects or indicated any differences in the performance of animals fed soybean 356043 compared to conventional soybeans. Bioinformatics analysis of the amino acid sequence of GAT4601 and GM-HRA did not show sequence resemblance to known toxins or IgE-dependent allergens, nor have these proteins been reported to cause IgE-mediated allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that soybean 356043 is as nutritious and as safe as its conventional counterpart and other conventional soybean cultivars. It is unlikely that the GAT4601 and GM-HRA proteins will introduce toxic or allergenic potentials in food or feed based on soybean 356043 compared to conventional soybean cultivars.

5 Environmental risk assessment

Since the last assessments of soybean 356043 conducted by the GMO panels of VKM ([VKM, 2008](#)) and EFSA ([EFSA, 2011c](#)), VKM has broadened the scope of its environmental risk assessments in response to the Norwegian Environment Agency's request (see Terms of Reference). Therefore, further information is provided below.

Considering the scope of the application EFSA/GMO/UK/2007/43, which excludes cultivation, the environmental risk assessment is concerned with the accidental release into the environment of viable soybean 356043 seeds during transport and/or processing, and with indirect exposure to microorganisms in the gastrointestinal tract and soil/water, mainly via ingestion by animals, their intestinal content and faeces.

5.1 Unintended effects on plant fitness due to the genetic modification

Cultivated soybean, *Glycine max* (L.) Merr., is a member of the genus *Glycine* and belongs to the Fabaceae (Leguminosae) family. Soybean is an annual, subtropical plant, native to eastern Asia ([OECD, 2000](#)). The crop is, however, grown over a wide range of ecological zones, ranging from the tropics to the temperate zones ([Acquaah, 2012](#)). The major worldwide soybean producers are China, the United States, Brazil and Argentina ([FAOSTAT, 2013](#)). In Europe, soybean is mainly cultivated in Ukraine, the Russian Federation, Italy, France and Romania. There is no cultivation of soybean in Norway.

Despite accidental seed dispersal and extensive cultivation in many countries, seed-mediated establishment and survival of soybean outside cultivation or on disturbed land is rare ([OECD, 2000](#)). Establishment of feral soybean populations has never been observed in Europe. Soybean volunteers are rare throughout the world and do not effectively compete with the succeeding crop or primary colonisers ([OECD, 2000](#)).

Soybean is a highly domesticated crop and generally unable to survive in the environment without management intervention (Lu, 2005). The soybean plant is not weedy in character. As for all domesticated crops, soybean has been selected against seed shattering to reduce yield losses during harvesting. Cultivated soybean seeds rarely display any dormancy characteristics and have poor seed survivability in soils ([OECD, 2000](#)). Due to low frost tolerance, susceptibility to plant pathogens, rotting and germination, the seeds will normally not survive during the winter ([Owen, 2005](#)). The soybean seeds need a minimum soil temperature of 10 °C to germinate and the seedlings are sensitive to low temperatures ([Bramlage et al., 1978](#); [OECD, 2000](#)). Soybean is a quantitative short-day plant that needs short days for induction of flowering, and the growing season in Norway is too short for the soybean plant to reach full maturity. Potential soybean plants resulting from accidental release of viable seeds would therefore not be able to reproduce under Norwegian growing conditions.

There is no reason to assume that expression of the introduced characteristics in soybean 356043 will increase the potential to establish feral populations. A series of field trials with soybean 356043 was conducted by the applicant at several locations in 2005 and/or 2006 in USA and Canada, and during the 2005/2006 growing season in Chile and Argentina, to

compare the agronomic performance and field characteristics of soybean 356043 with its comparators (see section 3.1). With the exception of targeted responses to the presence of glyphosate and ALS-inhibiting herbicides, the agronomic and phenotypic field trial data did not show major changes in plant characteristics indicating altered fitness, persistence and invasiveness of soybean 356043 plants compared to its conventional counterpart.

In addition to the data presented by the applicant, the VKM GMO Panel is not aware of scientific reports indicative of increased establishment or spread of soybean 356043, or changes to its survivability (including over-wintering), persistence or invasive capacity. Because the general characteristics of soybean 356043 are unchanged, the herbicide tolerance is not likely to provide a selective advantage in Norway. The VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects based on establishment and survival of soybean 356043 will not differ from that of conventional soybean cultivars.

5.2 Potential for gene transfer

A prerequisite for 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 pollen or seed dispersal. Transgenic DNA is also a component of a variety of food and feed products derived from soybean 356043. This means that micro-organisms in the digestive tract in humans and animals (both domesticated animals and other animals feeding on fresh or decaying plant material from the transgenic soybean) may be exposed to transgenic DNA.

5.2.1 Plant to micro-organisms gene transfer

Experimental studies have shown that gene transfer from transgenic plants to bacteria rarely occurs under natural conditions and that such transfer depends on the presence of DNA sequence similarity between the DNA of the transgenic plant and the DNA of the bacterial recipient ([Bensasson et al., 2004](#); [de Vries and Wackernagel, 2002](#); [EFSA, 2004](#); [EFSA, 2009](#); [Nielsen et al., 2000](#); [VKM, 2005](#)).

Based on established scientific knowledge of the barriers for gene transfer between unrelated species and the experimental research on horizontal transfer of genetic material from plants to microorganisms, there is today little evidence pointing to a likelihood of random transfer of the transgene present in soybean 356043 to unrelated species such as bacteria.

It has, however, been pointed out that there are limitations in the methodology used in these experimental studies ([Nielsen and Townsend, 2004](#)). Experimental studies of limited scale should be interpreted with caution given the scale differences compared to commercial plant cultivation.

Experiments have been performed to study the stability and uptake of DNA from the intestinal tract in mice after M13 DNA was administered orally. The DNA introduced was detected in stool samples up to seven hours after feeding. Small amounts (<0.1%) could be traced in the blood vessels for a period of maximum 24 hours, and M13 DNA was found in the liver and spleen for up to 24 hours ([Schubbert et al., 1994](#)). Following oral intake, it has been shown that DNA from GM soybean is more stable in the intestine of persons with colostomy compared to a control group ([Netherwood et al., 2004](#)). No GM DNA was detected

in the faeces from the control group. Rizzi et al. ([Rizzi et al., 2012](#)) provides an extensive review of the fate of feed-derived DNA in the gastrointestinal system of mammals.

In conclusion, the VKM GMO Panel considers it is unlikely that the introduced genes from soybean 356043 will transfer to and establish itself in the genome of microorganisms in the environment or in the intestinal tract of humans or animals. In the rare, but theoretically possible case of transfer of the inserted genes from soybean 356043 to soil bacteria, no novel property would be introduced into or expressed in the soil microbial communities, as these genes are already present in other bacteria in soil. Therefore, no positive selective advantage, which would not have been conferred by natural gene transfer between bacteria, is expected.

5.2.2 Plant to plant gene flow

The genus *Glycine* has two distinct subgenera; *Glycine* and *Soya*. The subgenus *Glycine* contains 16 perennial wild species, whilst cultivated soybean (*G. max*) and its wild and semi-wild annual relatives, *G. soja* and *G. gracilis* are classified in the subgenus *Soja* ([OECD, 2000](#)). Wild soybean species are endemic to China, Korea, Japan, Taiwan and the former USSR, and while these species have not been reported in Europe or in North America.

Soybean is predominantly a self-pollinating species, propagated commercially by seed. The percentage of cross-pollinating is usually less than one percent ([Lu et al., 2005](#); [OECD, 2000](#)). The dispersal of pollen is limited because the anthers mature in the bud and directly pollinate the stigma of the same flower. Pollination and fertilisation are usually accomplished before the flower opens ([Acquaah, 2012](#)).

Since there is no cultivation of soybean in Norway and the species has no sexually compatible wild relatives in Europe, accidental seed spillage during transportation and/or processing of soybean 356043 will not present a risk of spread of transgenes to organic or conventionally grown cultivars, wild populations or closely related species in Norway.

5.3 Interactions between the GM plant and target organisms

The genetic modification in soybean 356043 confers herbicide tolerance only. Considering the intended uses of soybean 356043, which excludes cultivation, interactions with target organisms are therefore not considered an issue by the VKM GMO-panel.

5.4 Potential interactions between the GM plant and non-target organisms (NTOs)

The genetic modification in soybean 356043 confers herbicide tolerance only. Considering the intended uses of soybean 356043, which excludes cultivation, interactions with non-target organisms are therefore not considered an issue by the VKM GMO-panel.

5.5 Potential interactions with the abiotic environment and biochemical cycles

Considering the intended uses of soybean 356043, which exclude cultivation, and the low level of exposure to the environment, potential interactions of the GM plant with the abiotic environment and biogeochemical cycles were not considered an issue by the VKM GMO Panel.

5.6 Conclusion

Considering the intended uses of soybean 356043, which excludes cultivation, the environmental risk assessment is concerned with accidental release into the environment of viable grains during transportation and processing, and indirect exposure to microorganisms in the gastrointestinal tract and soil/water, mainly via intestinal content and faeces from animals fed feeds containing soybean 356043.

Soybean 356043 has no altered survival, multiplication or dissemination characteristics compared to conventional soybean, and there are no indications of an increased likelihood of spread to or establishment of feral soybean plants in the case of accidental release of seeds from soybean 356043 into the environment. Soybean is not cultivated in Norway, and there are no cross-compatible wild or weedy relatives of soybean in Europe. Plant to plant gene flow is therefore not considered to be an issue. Considering the intended use as food and feed, interactions with the biotic and abiotic environment are not considered to be an issue in Norway.

6 Post-market environmental monitoring

Directive 2001/18/EC introduces an obligation for applicants to implement monitoring plans, in order to trace and identify any direct or indirect, immediate, delayed or unanticipated effects on human health or the environment of GMOs as or in products after they have been placed on the market. Monitoring plans should be designed according to Annex VII of the Directive. According to Annex VII, the objectives of an environmental monitoring plan are 1) 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 (ERA) are correct, and (2) 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.

Post-market environmental monitoring is composed of case-specific monitoring and general surveillance (EFSA, 2011e). Case-specific monitoring is not obligatory, but may be required to verify assumptions and conclusions of the ERA, whereas general surveillance is mandatory, in order to take account for general or unspecific scientific uncertainty and any unanticipated adverse effects associated with the release and management of a GM plant. Due to different objectives between case-specific monitoring and general surveillance, their underlying concepts differ. Case-specific monitoring should enable the determination of whether and to what extent adverse effects anticipated in the environmental risk assessment occur during the commercial use of a GM plant, and thus to relate observed changes to specific risks. It is triggered by scientific uncertainty that was identified in the ERA.

The objective of general surveillance is to identify unanticipated adverse effects of the GM plant or its use on human health and the environment that were not predicted or specifically identified during the ERA. In contrast to case-specific monitoring, the general status of the environment that is associated with the use of the GM plant is monitored without any preconceived hypothesis, in order to detect possible effects that were not anticipated in the ERA, or that are long-term or cumulative.

No specific environmental impact of genetically modified soybean 356043 was indicated by the environmental risk assessment and thus no case specific monitoring is required. The VKM GMO Panel is of the opinion that the monitoring plan provided by the applicant is in line with the intended uses of soybean 356043.

7 Conclusions

Molecular characterisation

The applicant had provided sufficient analyses to characterise the DNA insert, number of inserts, integration site and flanking sequences in the soybean 356043 genome. The results show the presence of one fragment of the DNA insert containing one functional copy of each of the *gat 4601* and *gm-hra* genes only. No other functional vector genes were detected. Similarity searches with databases of known toxins and allergens did not indicate potential production of allergenic or toxic proteins or polypeptides as a result of the genetic modification. Southern blot and segregation analyses show that the introduced gene elements were stably inherited and expressed over multiple generations in parallel with the observed phenotypic characteristics of soybean 356043.

Based on the above considerations, the VKM GMO panel maintains the validity of previous assessments and concludes that the molecular characterisation of soybean 356043 does not indicate a safety concern.

Comparative assessments

The VKM GMO Panel considered the available literature on compositional, agronomic and morphological data. The compositional analysis revealed that the genetic modification most likely resulted in increased levels of especially acetylated amino acids NAA, but also NAG, and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acids in seeds of soybean 356043 compared to conventional soybean cultivars. These constituents are, however, present in other common food and feedstuffs. Small intermittent variations in other analytes were observed but were within the range observed in conventional soybean cultivars and therefore most likely a result of natural variability.

Based on current knowledge and excluding the novel traits with resulting increased content of the acetylated amino acids NAA and NAG, and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acids, the VKM GMO Panel concludes that soybean 356043 is compositionally, agronomically, and morphologically equivalent to its conventional counterpart and other conventional soybean cultivars.

Food and feed risk assessment

A subchronic, toxicity study in rat, repeated dose studies in mice, nutritional whole food studies in broilers and laying hens and allergenicity assessment studies have been performed with soybean 356043. These studies have not revealed adverse effects or indicated any differences in the performance of animals fed soybean 356043 compared to conventional soybeans. Bioinformatics analysis of the amino acid sequence of GAT4601 and GM-HRA did not show sequence resemblance to known toxins or IgE-dependent allergens, neither have these proteins been reported to cause IgE-mediated allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that soybean 356043 is nutritionally equivalent to and as safe as its conventional counterpart and other conventional soybean cultivars. It is unlikely that the GAT4601 and GM-HRA proteins will introduce toxic or allergenic potentials in food or feed based on soybean 356043 compared to conventional soybean cultivars.

Environmental assessment

Considering the intended uses of soybean 356043, which excludes cultivation, the environmental risk assessment is concerned with accidental release into the environment of viable grains during transportation and processing, and indirect exposure to microorganisms in the gastrointestinal tract and soil/water, mainly via intestinal content and faeces from animals fed feeds containing soybean 356043.

Soybean 356043 has no altered survival, multiplication or dissemination characteristics compared to conventional soybean, and there are no indications of an increased likelihood of spread to or establishment of feral soybean plants in the case of accidental release of seeds from soybean 356043 into the environment. Soybean is not cultivated in Norway, and there are no cross-compatible wild or weedy relatives of soybean in Europe. Plant to plant gene flow is therefore not considered to be an issue. Considering the intended use as food and feed, interactions with the biotic and abiotic environment are not considered to be an issue in Norway.

Overall conclusion

Based on current knowledge and considering the intended uses, which excludes cultivation, the VKM GMO Panel concludes that soybean 356043 with the GAT4601 and GM-HRA proteins:

- Is – with the exception of the novel traits and resulting increased content of the acetylated amino acids NAA and NAG, and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acids – compositionally, morphologically and agronomically equivalent to its conventional counterpart and other commercial soybean cultivars
- Are unlikely to introduce toxic or allergenic potentials in food or feed compared to conventional soybean cultivars
- Is nutritionally equivalent to and as safe as its conventional counterpart and other conventional soybean cultivars
- Does not represent an environmental risk in Norway.

8 Data gaps

Filling data gaps would confirm and strengthen the conclusions drawn based on currently available knowledge. With added knowledge, VKM and its commissioning agencies could thereby provide greater certainty when communicating the safety of the GM products.

Apparently a consequence of the genetic modification and the expression of the respective enzymes led to enhanced acetylation of endogenous amino acids and production of odd-chain fatty acids. The question arises of whether other components in soybean 356043 may have been acetylated or otherwise modified. More knowledge is needed regarding this, which may be illuminated with the use of untargeted assays.

Herbicide tolerant (HT) crops permit the use of broad-spectrum herbicides such as glyphosate, as an in-crop selective herbicide to control a wide range of broadleaf and grass weeds without sustaining crop injury. This weed management strategy enables post-emergence spraying of established weeds and gives growers more flexibility to choose spraying times in comparison with the pre-emergence treatments of conventional crops.

As the broad-spectrum herbicides are sprayed on the plant canopy and spraying often takes place later in the growing season than is the case with selective herbicides associated with conventional crops, the residue and metabolite levels of herbicides in plants with tolerance to glyphosate and ALS-inhibiting herbicides could be higher compared to plants produced by conventional farming practices. Limited data is available on pesticide residues in HT crops. In Argentina, however, HT soybean cultivars now cover 98% of the land used for soybean cultivation. The annual use of glyphosate for weed management in Argentina has increased from 1.3 million litres in 1991 to ca. 200 million litres in 2013, and residues have been reported in soil, water and sediment (Aparicio et al., 2013).

More research is also needed to elucidate whether the genetic modifications used to make a plant tolerant against certain herbicide(s) may influence the metabolism of this or other plant protection products, and whether possible changes in the spectrum of metabolites may result in altered toxicological properties.

Investigations into possible health effects of soybean 356043 or its constituents N-acetylated amino acids NAA and NAG and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acids in cultivated fish have apparently not been conducted and would be of value for the Norwegian aquaculture industry.

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Appendix I



**Uttalelse fra Faggruppe for genmodifiserte organismer i
Vitenskapskomiteen for mattrygghet**

13.03.08

**Helse- og miljørisikovurdering av genmodifisert soyalinje 356043
fra Pioneer Hi-Bred. International Inc.
(EFSA/GMO/NL/2007/43)**

BIDRAGSYTERE

Den som utfører arbeid for VKM, enten som oppnevnte medlemmer eller på *ad hoc*-basis, gjør dette i kraft av sin egen vitenskapelige kompetanse og ikke som representanter for den institusjon han/hun arbeider ved. Forvaltningslovens habilitetsregler gjelder for alt arbeid i VKM-regi.

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INNHALDSFORTEGNELSE

| | |
|---|----|
| BIDRAGSYTERE | 2 |
| VURDERT AV | 2 |
| INNHALDSFORTEGNELSE | 3 |
| SAMMENDRAG..... | 4 |
| NØKKEWORD..... | 5 |
| BAKGRUNN | 6 |
| OPPDRAG FRA DIREKTORATET FOR NATURFORVALTING OG MATTILSYNET | 6 |
| RISIKOVURDERING | 7 |
| 1. Innledning..... | 7 |
| 1.1. Beskrivelse av egenskaper og virkningsmekanismer..... | 7 |
| 2. Molekylær karakterisering | 7 |
| 2.1. Transformasjonssystem og vektorkonstruksjon | 7 |
| 2.2. Karakterisering av geninnsettingen/ genkonstruksjonen..... | 8 |
| 2.3. Informasjon vedr. uttrykk av introduserte gener, åpne leserammer (ORF) | 9 |
| 2.4. Nedarving og stabilitet av innsatt DNA | 10 |
| 2.5. Delkonklusjon | 10 |
| 3. Komparative analyser..... | 10 |
| 3.1. Forsøksdesign og valg av komparator..... | 10 |
| 3.2. Analyser av ernæringsmessige komponenter | 11 |
| 3.3. Agronomiske egenskaper | 12 |
| 3.4. Delkonklusjon | 13 |
| 4. Dokumentasjon av toksisitet og allergenisitet..... | 13 |
| 4.1. Toksisitet | 13 |
| 4.2. Allergenisitet | 14 |
| 4.3. Delkonklusjon | 14 |
| 5. Miljørisikovurdering | 14 |
| 5.1. Potensiale for ikke intenderte effekter på fitness relatert til genmodifiseringen..... | 14 |
| 5.2. Potensiale for genoverføring | 15 |
| 5.3. Miljøovervåkingsplan..... | 16 |
| 5.4. Delkonklusjon | 16 |
| KONKLUSJON | 18 |
| REFERANSER | 19 |

SAMMENDRAG

Helse- og miljørisikovurderingen av den herbicidtolerante soyalinjen 356043 fra Pioneer Hi-Bred International, Inc. (EFSA/GMO/NL/2007/43) er utført av Faggruppe for genmodifiserte organismer under Vitenskapskomiteen for mattrygghet. Mattilsynet og Direktoratet for naturforvaltning (DN) ber Vitenskapskomiteen for mattrygghet om å vurdere den genmodifiserte soyalinjen 356043 til bruk i næringsmidler og fôrvarer, men ikke for dyrking.

Vurderingen av den genmodifiserte soyaen er basert på dokumentasjonen som er gjort tilgjengelig på EFSA's nettside GMO EFSA-net. I tillegg er det benyttet informasjon fra uavhengige vitenskapelige publikasjoner i vurderingen. Soyalinjen 356043 er vurdert i henhold til tiltenkt bruk og i overensstemmelse med kravene i genteknologiloven, forskrift om konsekvens-utredning etter genteknologiloven, forordning 1829/2003/EF, samt kravene i EUs utsetningsdirektiv 2001/18/EF med annekser. Videre er EFSA's retningslinjer for risikovurdering av genmodifiserte planter (EFSA 2006) og Organisation for Economic Cooperation and Development (OECD) konsensusdokument for soya (OECD 2001) lagt til grunn for vurderingen. Den vitenskapelige vurderingen omfatter transformeringsprosess og vektor-konstruksjon, karakterisering og nedarving av genkonstruksjonen, komparativ analyse av ernæringsmessig kvalitet, mineraler, kritiske toksiner, metabolitter, antinæringsstoffer, allergener og nye proteiner, samt agronomiske egenskaper, genoverføring og potensiale for ikke-intenderte effekter på fitness.

Soyalinjen 356043 er fremkommet ved at soyaceller fra den kommersielle sorten 'JACK' er transformert ved hjelp av partikkelakselerasjon. Soyalinjen har fått innsatt en genkonstruksjon med en optimalisert form av *gat*-genet fra jordbakterien *Bacillus licheniformis*. Genet koder for GAT4601-proteinet, et N-acetyltransferase-enzym som medfører inaktivering av herbicider med virkestoff glyfosat. I tillegg uttrykker 356043 GM-HRA-proteinet, et acetolaktatsyntase enzym (ALS) som gir plantene toleranse mot herbicider med tifensulfuron og klorimuron. Produkter av 356043 vil bli markedsført under handelsnavnet OptimumTM GATTM Soybean.

Det er hovedsakelig olje, mel, proteinisolat og bønne fra soya som brukes som menneskeføde og fôr. I følge OECD nyttes om lag 93 % av soyaoljen som mat, mens ca. 97 % av melet brukes som fôr (OECD 2001). Analysene av ernæringsmessige viktige komponenter ble vurdert. Det er funnet statistiske forskjeller for enkelte komponenter. De statistiske forskjellene for disse komponentene er imidlertid ikke konsistente over forsøksfelt. Ingen av proteinene som blir uttrykt som følge av genmodifiseringen har likheter med kjente allergener eller egenskaper som tilsier at de er allergener.

Faggruppen ønsker å påpeke at det er kunnskapshull med hensyn på mulige helseeffekter ved soya 356043. Soyalinjen 356042 uttrykker et nytt protein og faggruppen etterlyser derfor bakgrunnsmaterialer med hensyn på mulige helseeffekter. Analyser av ernæringsmessige viktige komponenter i soya er utført i tråd med OECDs konsensusdokument (OECD 2001). Faggruppen påpeker at det i søknaden henvises til en undersøkelse av agronomiske karakterer. Denne undersøkelsen er ikke lagt ved søknaden. Faggruppen mener at når det i søknaden henvises til resultater av slike studier, skal resultatene fra undersøkelsene være

tilgjengelige. Videre mener Faggruppe for GMO at det bør kreves av søker å utføre et 90-dagers subkronisk fôringsforsøk på rotter.

Søknaden gjelder godkjenning av soyalinjen 356043 for import, prosessering og til bruk i næringsmidler og fôrvarer. Faggruppen har derfor ikke vurdert mulige miljøeffekter knyttet til dyrking av soyalinjen. Det er ingen indikasjoner på økt sannsynlighet for spredning, etablering og invasjon av soyalinjen i naturlige habitater eller andre arealer utenfor jordbruksområder som resultat av frøspill i forbindelse med transport og prosessering. Soya dyrkes ikke i Norge, og det er ingen stedegne eller introduserte viltvoksende arter i den europeiske flora som soya kan hybridisere med.

Samlet vurdering

Faggruppen konkluderer med at olje fra soyalinjen 356043 er vesentlig lik olje fra umodifisert soya, men påpeker betydelige kunnskapshull med hensyn på mulige helseeffekter knyttet til bruk av 356043 som næringsmiddel og fôrvarer. Faggruppen finner det lite trolig at bruk av soyalinjen 356043 vil medføre endret risiko for miljø i forhold til annen soya.

NØKKELORD

Soya, *Glycine max* (L.) Merr., genmodifisert soyalinje 356043, herbicidtoleranse, GAT4601-protein, glyfosatacetyltransferase (GAT), GM-HRA-protein (ALS protein), acetolactatsyntase (ALS), helsemessig trygghet, helse, miljørisiko, forordning 1829/2003/EF

BAKGRUNN

Faggruppe for genmodifiserte organismer under Vitenskapskomiteen for mattrygghet er blitt bedt av Mattilsynet og Direktoratet for naturforvaltning om å foreta en utredning av helse- og miljørisiko ved en eventuell godkjenning av den genmodifiserte soyalinjen 356043 fra Pioneer Hi-Bred International, Inc. (EFSA/GMO/UK/2007/43). Soyalinjen er søkt omsatt i EU/EØS-området under Forordning (EF) No. 1829/2003 om genmodifiserte næringsmidler og fôrvarer (artiklene 3(1) og 15(1)). Søknaden omfatter bruksområdene import, prosessering, næringsmidler og fôrvarer, og ble fremmet og anbefalt av britiske myndigheter i april 2007. Søknaden ble lagt ut på EFSA-nett 28. september 2007, med frist på 90-dager for innspill fra EU- og EØS/EFTA-landene. Norge har ikke tidligere uttalt seg om soya 356043.

Soyalinjen 356043 ble godkjent til bruk som mat og fôr i USA i 2007 (Agbios 2008), og er søkt notifisert i Canada for alle bruksområder inkludert dyrking. Det foreligger også søknad om godkjenning av 356043 for import til mat og fôr i Mexico.

OPPDRAK FRA DIREKTORATET FOR NATURFORVALTING OG MATTILSYNET

Vitenskapskomiteen for mattrygghet er blitt bedt av Mattilsynet og Direktoratet for naturforvaltning om å foreta en utredning av helse- og miljørisiko ved en eventuell godkjenning av den genmodifiserte soyalinjen 356043 (EFSA/GMO/UK/2007/43) til mat, fôr, import og industriell prosessering. Søknaden gjelder ikke dyrking. Vurderingen av 356043 skal utføres i henhold til tiltenkt bruk og i overensstemmelse med prinsippene som er nedfelt i EFSA's retningslinjer for vurdering av genmodifiserte planter ("Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed") (EFSA 2006).

I henhold til oppdragsbrev fra DN skal VKM primært fokusere på miljørisiko i EØS-området, og på miljørisiko som er spesifikke for Norge. Det skal også gis en samlet konklusjon om miljørisiko i tråd med kravene i forskrift om konsekvensutredning etter genteknologiloven, vedlegg 2 C.

Produktet som ønskes vurdert

Genmodifisert soya, EFSA/GMO/UK/2007/43 (356043).

Unik kode: DP-356043-5.

Status i EU: Søknad under forordning 1829/2003/EF. Frist for innspill til EFSA-nett er 28.12.07.

Ønsket svarfrist til Mattilsynet/DN: 28. desember 2007.

RISIKOVURDERING

1. Innledning

Helse- og miljøvurderingen av den transgene soyalinjen 356043 er gjort i henhold til tiltenkt bruk, basert på den dokumentasjonen som er gjort tilgjengelig på EFSAs nettside GMO EFSAnet. I tillegg er det benyttet uavhengige vitenskapelige publikasjoner med referee i vurderingen.

Vurderingen er gjort i overensstemmelse med kravene i genteknologiloven, forskrift om konsekvensutredning etter genteknologiloven, forordning 1829/2003/EF, samt kravene i EUs utsettingsdirektiv 2001/18/EF med annekser. Faggruppe for genmodifiserte organismer har på faggruppemøtet 02.02.05 vedtatt å bruke EFSAs retningslinjer som gruppens retningslinjer for vurdering av genmodifiserte planter. Prinsippene som er lagt til grunn for vurderingen, er derfor hentet fra EFSAs dokument "Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed" (EFSA 2006). I henhold til Vitenskapskomiteen for mattrygghets uttalelse på møtet 23. april 2004 har Faggruppe for genmodifiserte organismer (GMO) vedtatt at i de sakene hvor EFSA har kommet med sine uttalelser før Faggruppe for GMO får sakene til behandling, skal søknadene behandles på samme måte som i EU-landene. Det vil imidlertid bli tatt hensyn til særnorske forhold der slike kan påvises.

Det er kun medlemmene i faggruppen som har vurdert den genmodifiserte maisen.

1.1. Beskrivelse av egenskaper og virkningsmekanismer

Soyalinjen 356043 er fremkommet ved at soyaceller fra den kommersielle sorten "JACK" er transformert ved hjelp av partikkelakselerasjon. Soyalinjen har fått innsatt en genkonstruksjon med en optimalisert form av *gat*-genet fra jordbakterien *Bacillus licheniformis*. Genet koder for GAT4601-proteinet, et N-acetyltransferase-enzym som acetylerer det sekundære aminet i glyfosat og medfører inaktivering av glyfosat. I tillegg uttrykker 356043 GM-HRA-proteinet, et acetolaktatsyntase enzym (ALS), som gir plantene toleranse mot herbicider med virkestoffene tifensulfuron og klorimuron.

2. Molekylær karakterisering

2.1. Transformasjonssystem og vektorkonstruksjon

Til transformasjon er det brukt partikkelakselerasjonmediert transformasjon av soyaceller fra foreldresorten "Jack". Et lineært rekombinant DNA fragment PHP20163A ble ved hjelp av restriksjonsenzymene Not I og Asc I klippet ut av plasmidet PHP20163. Det rekombinante DNA-fragmentet inneholder to ekspresjonskassetter, og ble benyttet til å transformere celler fra den umodifiserte sorten. DNA-fragmentet inneholder en GAT4601- og en GM-HRA ekspresjonskasset. Transformanter ble selektert ved at de overlevde og vokste i nærvær av klorsulfuron. Den ene kassetten koder for GAT4601-protein. GAT4601 er et N-acetyltransferase-enzym og tilhører GCN5-acetyltransferasefamilien, også kalt GNAT-familien. GNAT-familien består av over 10 000 gener og er representert i alle riker. GAT4601

acetylerer det sekundære aminet i glyfosat, som medfører at glyfosat inaktiveres. GM-HRA ekspressjonskassetten danner GM-HRA-proteinet. GM-HRA er et syntetisk acetolaktat-syntase enzym (ALS) som ikke hemmes av herbicider som hemmer enzymer i ALS-familien. PHP20163A DNA-fragmentet inneholder ikke antibiotikaresistensgen.

2.2. Karakterisering av geninnsettingen/ genkonstruksjonen

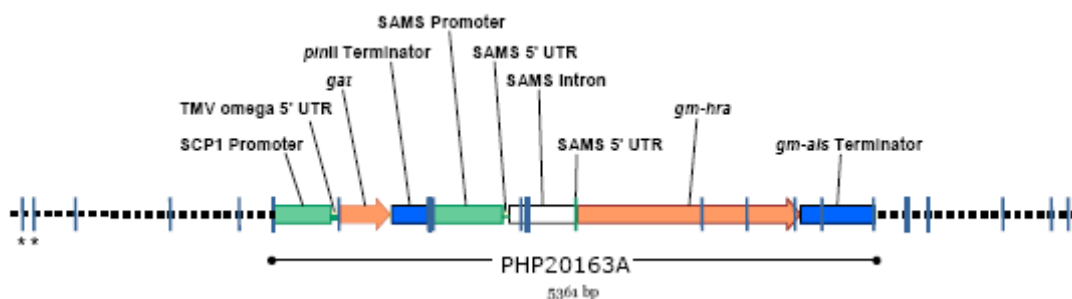
Southern blot og PCR har blitt brukt for å karakterisere det rekombinante DNA-fragmentet i planten. Molekylærbiologisk karakterisering viser at det er satt inn bare en kopi av DNA-fragmentet i soyaens genom. Dette fragmentet inneholder:

GAT4601 ekspresjonskassett

- a) *SCPI* syntetisk konstitutiv promoter fra blomkålmosaikkvirus (CaMV) 35S RNA og Rsyn7-Syn II Core konsensus promoter
- b) *TMV ω5'UTR* øker transkripsjonen, fra tobakk mosaikkvirus
- c) *Gat* *GAT4601* (glyfosatacetyltransferase) gen, en optimalisert form av *gat*-genet fra jordbakterien *Bacillus licheniformis*, fremkommet ved DNA-shuffling av *gat*-gener fra *B. licheniformis*.
- d) *T-pinII* 3' DNA sekvens som avslutter transkripsjonen, kommer fra proteinase hemmer II (*pinII*) terminator, stammer fra potet, uttrykkes ikke i planten

GM-HRA ekspresjonskassett

- a) *SAMS-P* promoter fra S-adenosyl-L-metioninsyntetase (SAMS) fra soya
- b) *SAMS-5'UTR* øker transkripsjonen, fra soya
- c) *SAMS-I* SAMS intron, fra soya
- d) *SAMS-5'UTR* øker transkripsjonen, fra soya
- e) *gm-hra* en optimalisert form fra endogent soya acetolaktatsyntase gen (*gm-als*), inneholder overføringssekvenser til kloroplaster,
- f) *gm-als-T* endogen terminator fra *als*-genet, fra soya.



Figur 1. Rekombinant PHP20163A DNA fragment i soyaens genom. Områdene utenfor PHP20163A er genomisk DNA.

Molekylærbiologiske analyser viser at det rekombinante fragmentet i planten inneholder de samme gener og genelementer som er på det tilsvarende DNA fragmentet i plasmidet PHP20163A. Både GAT4601- og GM-HRA- proteinet som uttrykkes i soya er undersøkt med Western-blot analyse og densitometri, SDS-PAGE og densitometri, trypsinbehandling av

proteinene og peptidkartlegging med MALDITOF massespektrometri, Southern blot, analyse av N-enden til proteinet, samt glykosyleringsanalyse. Proteinene er undersøkt for enzymaktivitet. Analysene viser at GAT4601 og GM-HRA proteinene er strukturelt og funksjonelt like de *E. coli*-produserte proteinene. Fordøyelighetstest av GAT4601 og GM-HRA viste at proteinet fordøyes raskt i simulert magesaft og tarmsaft. Det ble ikke påvist glykosyleringssteder på proteinene. Southern-blot analysene er utført på DNA rensset ut fra blad, og analysene er utført på generasjonene T3, T4 og T5.

PCR-analyser av det rekombinante DNA fragmentet på 5362 bp i 356043 viser at flankesekvensene til fragmentet er genomisk DNA fra soya. Flankerende sekvenser til dette rekombinante DNA-fragmentet er sekvensert, 3317 bp oppstrøms (5'-flankesekvens) og 2170 bp nedstrøms (3'-flankesekvens). Både 5'- og 3'-flankesekvenser ble undersøkt med BLASTn analyse for å undersøke egenskapen(e) og eventuelle funksjoner til flankesekvensene. I den genomiske 3'-enden er det påvist to offentlig tilgjengelige soyagenomsekvenser (CL86833.1 og CL867466.1) samt en proprietær genomsekvens (snelx.pk001.e1). Disse sekvensene er 97-99 % identiske til basesekvensene i 3'-enden. Et annet område i 3'-enden viste 92-94 % identitet til offentlige tilgjengelige mitokondrielle sekvenser fra hvete og gulrot (AP0008982.1, AF301604.1, AF301603.1) og til en genomsekvens fra hvete (CW510860.1). I 5'-enden ble det påvist to sekvenser som har 98 og 92 % likehet til et gen fra gruvesneglebelg (*Medicago truncatula*)(CR339131.1), 98 % til en proprietær soyagensekvens samt flere forskjellige soyagensekvenser med identitet fra 84-92 % (sbacm.pk071.a11.f, sbach.pk120.e3, sbacm.pk041.n22f, sbacm.pk082.n1). Ingen åpne leserammer (ORF) større eller lik 100 aminosyrer ble identifisert i 5' eller 3'-grenseområdet. PCR analyse av det rekombinante DNA fragmentet i soyagenomet viser at både *GAT4601* og *GM-HRA* DNA-sekvensene er identiske til de korresponderende sekvensene på plasmidet PHP20163.

2.3. Informasjon vedr. uttrykk av introduserte gener, åpne leserammer (ORF)

Søker har analysert prøver fra seks feltforsøk, fire utført i USA og to i Canada i 2005. Det er tatt ut fire prøver fra hvert forsøksfelt (se kapittel 3.1). Mengde GAT4601- og GM-HRA-protein i soyabønne er målt til henholdsvis $0,24 \pm 0,072$ µg/g tørrvekt (variasjonsbredde = 0,14 – 0,39) og $0,91 \pm 0,17$ µg/g tørrvekt (variasjonsbredde = 0,64 – 1,2), og i furasje til henholdsvis $1,6 \pm 0,32$ µg/g tørrvekt (variasjonsbredde = 20 – 56) og $27 \pm 8,0$ µg/g tørrvekt (variasjonsbredde = 15 – 55).

I tillegg er det foretatt analyser av proteininnhold i prøver fra seks feltforsøk i Sør-Amerika i vekstsesongen 2005-2006. I disse forsøkene ble mengde GAT4601- og GM-HRA-protein i bønne målt til henholdsvis $0,24 \pm 0,071$ µg/g tørrvekt (variasjonsbredde = 0,14 – 0,38) og $0,59 \pm 0,30$ µg/g tørrvekt (variasjonsbredde = 0 – 1,1), og i furasje $1,1 \pm 0,22$ µg/g tørrvekt og $15 \pm 4,2$ µg/g tørrvekt.

Teoretiske analyser av mulige polypeptider fra hver leseramme v.h.a. allergen (FARRP6 database fra Nebraska universitet)- og toksin (NCBI-proteindatabase, SWISS-PROT, PIR, PRF, PDB)-databaser viser ingen biologisk relevante strukturelle likheter til allergener og toksiner. Hvis noen av disse leserammene skulle bli transkribert viser resultatene fra disse teoretiske analysene at det er lite sannsynlig at det vil resultere i polypeptider som medfører potensielle toksiske eller allergene konsekvenser.

2.4. Nedarving og stabilitet av innsatt DNA

Krysning over fem generasjoner viser at det rekombinante GAT4601 og GM-HRA er stabilt inkorporert i soyagenomet.

2.5. Delkonklusjon

Faggruppen har vurdert de fysiske, kjemiske og funksjonelle karakteriseringene av proteinene og finner at informasjonen er tilstrekkelig. Faggruppen konkluderer med at karakteriseringen av det rekombinante innskuddet i 356043 er tilfredsstillende.

3. Komparative analyser

3.1. Forsøksdesign og valg av komparator

Prøvene som er analyserte stammer fra fire feltforsøk utført i USA og to feltforsøk utført i Canada i 2005 (Study Number: PHI-2005-056/000), samt to studier i Argentina og fire i Chile i vekstsesongen 2005-2006 (EU Final Report: PHI-2005-088/000). I tillegg henviser søker til studien PHI-2005-055/000, der det er foretatt observasjoner av agronomiske karakterer i feltforsøk i USA. Dokumentasjonen fra denne undersøkelsen er imidlertid ikke vedlagt søknaden.

I de amerikanske feltforsøkene bestod hvert forsøksfelt av et fullstendig randomisert blokkdesign med fire blokker. Det ble tatt ut prøver fra alle blokkene fra hvert felt. I blokkene i forsøksfeltene i USA og Canada ble noen av testlinjene (R2 fase) sprøytet to ganger med av en blanding av glyfosat, klorimuron og thifensulfuron, samt to ganger med glyfosat i R8 fase. Herbicidbehandlingen avvek for de argentinske og chilenske feltforsøkene. Soya 356043 ble her behandlet med klorimuron og/eller thifensulfuron, eller behandlet med glyfosat pluss klorimuron og/eller thifensulfuron. Alle seks feltene ble behandlet med herbicidet klorimuron. De fire feltene i Chile ble også behandlet med herbicidet thifensulfuron. Thifensulfuron ble ikke benyttet i Argentina fordi herbicidet ikke var merket for bruk eller kommersielt tilgjengelig. Som kontroll i alle feltforsøkene er det benyttet en umodifisert kontrollsort som hevdes å ha samme genetiske bakgrunn som de genmodifiserte plantene. Kontrollvarieteten er ikke angitt med navn.

Statistiske analyser

I Nordisk ministerråds rapport "Safety Assessment of Novel Food Plants: Chemical Analytical Approaches to the Determination of Substantial Equivalence" (TemaNord 1998), anbefales det at tilstrekkelig antall prøver må analyseres for å få adekvat sensitivitet for statistisk analyse. Spredning i enkeltparametre skal være sammenlignbare for genetisk modifisert plante og umodifisert plante. I rapporten er det anbefalt at spredningen i enkeltverdier bør ligge innenfor $\pm 20\%$.

3.2. Analyser av ernæringsmessige komponenter

Hovedkomponenter i soya og andre plantedeler

Valg av analyseparametere er gjort i henhold til OECDs konsensusdokument for soya (OECD 2001). Det er foretatt forskjellige analyser av hovedkomponenter for fôr og bønne. For fôrfraksjonen ble det analysert for aske, fett, protein, total fiber, ADF (acid detergent fiber), NDF (neutral detergent fiber), og karbohydrater. For bønne ble det analysert for protein, fett, aske, karbohydrater, ADF, NDF, total fiber, karbohydrater, aminosyrer, fettsyrer (C8-C22), fosfor, jern, kalium, kalsium, kobber, magnesium, mangan, natrium, sink, vitaminene B1, B2, B5, B6, totalmengde vitamin E, α -tokoferol β -tokoferol, δ -tokoferol, γ -tokoferol og folinsyre, isoflavonene genistin, genistein, malonylgenistin, acetylgenistin, daidzin, daidzein, malonyldaidzin, acetyldaidzin, glycitin, glycitein, malonylglycitin, acetylglycitin, oligosakkaridene sukrose, raffinose og staktyose, samt sekundære metabolitter og anti-næringsstoffene coumestrol, lektiner, trypsinhemmer og fytinsyre. Analysene ble utført under god laboratoriepraksis (GLP). Det ble ikke funnet statistiske forskjeller for komponentene aske, fett, protein, total fiber, ADF (acid detergent fibre), NDF (neutral detergent fibre), og karbohydrater.

Fettsyresammensetning i soya

Fettsyresammensetningen for 356043 er målt i henhold til OECDs konsensusdokument for soya. Det ble analysert for 25 fettsyrer. Av disse ble 10 ekskludert fra statistiske analyser fordi mengdene var lavere enn deteksjonsgrensene. For de tre fettsyrene palmitin-, heptadekan (C17:0)- og heptadekensyre (C17:1) er det funnet statistiske forskjeller. For tre fettsyrer er det funnet statistiske forskjeller for alle forsøksfeltene i USA, Canada, Argentina og Chile. Alle verdiene ligger innenfor typiske verdier for andre soyasorter som er rapportert i litteraturen. Pioneer har vurdert biologisk betydning og foretatt eksponeringsvurdering av C17:0 og C17:1 syrene fra olje fra 356043 og sammenlignet med generelt inntak av disse syrene i vegetabilsk olje, smør, ost og kjøtt. Pioneer konkluderer med at mengdene av disse syrene i 356043 er lik eller lavere enn i disse matvarene.

Aminosyrer i soya

Aminosyreinnholdet er målt i henholdt til OECDs konsensusdokument for soya. Både essensielle og ikke-essensielle aminosyrer ble analysert. Det er funnet statistiske forskjeller for to aminosyrer for forsøksfeltene i USA, Canada, Argentina og Chile. Asparagin- og glutaminsyre N-acetyleres av GAT-enzymet. Innholdet av N-acetylasparagin (NAA)- og N-acetyl glutaminsyre(NAG) er høyere enn kontroll. Mengde av NAA i 356043 og kontroll over alle feltene i USA og Canada er henholdsvis 580 (variasjonsbredde 434 til 958) og 2,52 (variasjonsbredde 1,06 til 12,6) $\mu\text{g/g}$ tørrvekt, og i Argentina og Chile er mengdene henholdsvis 653 (variasjonsbredde 490 til 870) og 1,92 (1,10 til 3,67) $\mu\text{g/g}$ tørrvekt. For NAG er mengdene for USA/Canada henholdsvis 11,6 (variasjonsbredde 4,84 til 21,2) og 1,53 (variasjonsbredde 0,876 til 2,35) $\mu\text{g/g}$ tørrvekt, og Argentina/Chile henholdsvis 18,3 (variasjonsbredde 9,86 til 43,2) og 2,34 (variasjonsbredde 1,42 til 3,35) $\mu\text{g/g}$ tørrvekt. Pioneer har vurdert biologisk betydning og foretatt eksponeringsvurdering av NAA- og NAG fra soyamel fra 356043 og sammenlignet med generelt inntak av disse syrene i matvarer som kylling, kyllingbuljong, egg og kjøtt. Pioneer konkluderer med at selv om 356043 soya skulle føre til økt inntak av disse N-acetylerede aminosyrene vil et slik økt inntak ikke være av helsemessig betydning fordi deacetylaser er utbredt i mennesker.

Vitaminer

OECDs konsensusdokument for soya har ikke satt opp vitaminer som komponenter det skal måles for. Vitaminer som er undersøkt for er B1, B2, B5, B6, totalmengde vitamin E, α -tokoferol, β -tokoferol, δ -tokoferol, γ -tokoferol, niacin og folinsyre. Det er ikke funnet statistiske forskjeller for vitaminer. For de fleste vitaminene som er målt ligger mengdene innenfor typiske verdier som er rapportert i litteraturen.

Mineraler

OECDs konsensusdokument for soya har ikke satt opp mineraler som komponenter det skal måles for. Det er målt for mineralene fosfor, jern, kalium, kalsium, kobber, magnesium, mangan, natrium og sink. Det er ikke funnet store statistiske forskjeller mht innhold av mineraler. Verdiene for alle mineralene ligger innenfor typiske verdier som er rapportert i litteraturen.

Sekundære metabolitter og anti-ernæringsstoffer

Sekundære metabolitter og anti-ernæringsstoffer er målt i henholdt til OECDs konsensusdokument for soya. Det er funnet statistiske forskjeller mellom soyalinjen 356043 og komparator for trypsinhemmer i et av forsøksfeltene i Argentina. Kombinerte analyser over lokaliteter viser ingen forskjeller mht denne variabelen. Mengden av coumesterol var lavere enn påvisningsgrensen. Det er ellers ikke funnet statistiske forskjeller for lektiner og fytinsyre.

Isoflavoner

Isoflavoner er målt i henholdt til OECDs konsensusdokument for soya. Det er ikke funnet statistiske forskjeller for isoflavonene genistin, genistein, malonylgenistin, acetylgenistin, daidzin, daidzein, malonyldaidzin, acetyldaidzin, glycitin, glycitein, malonylglycitin og acetylglycitin.

Oligosakkarider

Oligosakkarider er målt i henholdt til OECDs konsensusdokument for soya. Det er ikke funnet statistiske forskjeller for sukkerartene oligosakkaridene sukrose, raffinose og staktyose.

3.3. Agronomiske egenskaper

Søker opplyser om at det er foretatt observasjoner av agronomiske karakterer i feltforsøk med soyalinjen 356043 på 6 lokaliteter i USA og Canada i 2005, og 6 lokaliteter i Argentina og Chile i vekstsesongen 2005-2006. Hvert forsøksfelt bestod av et fullstendig randomisert blokkdesign med 4 gjentak. Foreldrelinjen cv. 'Jack' ble benyttet som kontrollsort. For øvrig beskrivelse av forsøksmetodikk og sprøyteregimer, se kap. 3.1. Tre av blokkene ble benyttet til vurdering av agronomiske ekvivalens. Det er foretatt registreringer av en rekke karakterer knyttet til reproduksjon, spredning, vegetativ vekst, samt sjukdoms- og insektsresistens. Det er foretatt statistiske analyser innen steder og separate kombinerte analyser over steder for forsøkene i henholdsvis Nord- og Sør-Amerika. Analyser fra forsøkene i Canada og USA viste signifikante forskjeller mellom den transgene linjen og kontrollsorten med hensyn på frøplantevitalitet og plantehøyde. Tilsvarende ble det funnet signifikante forskjeller mellom 356043 og komparator for plantehøyde og lengde i de søramerikanske forsøkene. I følge søker ligger imidlertid gjennomsnittsverdiene for disse parametrene innenfor forventet variasjonsområde for soya. For de øvrige karakterene ble det ikke registrert signifikante forskjeller. Spiretester av 800 frø fra henholdsvis den transgene linjen og kontrollsort viste ingen tegn på dormancy (frøkvile).

3.4. Delkonklusjon

Faggruppen påpeker at noe av referansedokumentasjonen mangler. Analysene av ernæringsmessige komponenter viser statistiske forskjeller i enkeltparametere, men verdiene for de enkelte komponentene ligger innenfor typiske verdier for andre soyasorter som er rapportert i litteraturen. Når det gjelder oljefraksjonen er det ikke funnet store statistiske forskjeller mellom genmodifisert og umodifisert kontrollsort i enkeltparametere. Faggruppen konkluderer derfor med at olje fra transgen plante er vesentlig lik olje fra umodifisert plante.

Resultatene fra undersøkelsene av agronomiske og morfologiske karakterer viser at, med unntak av herbicidresistens, det er ingen eller små forskjeller mellom soyalinje 356043 og kontrollsorten.

4. Dokumentasjon av toksisitet og allergisitet

4.1. Toksisitet

Akutt oral fôringsstudie på mus

Pioneer har i 2005 utført akutt oral fôringsstudier på mus med renfremstilt GAT4601 og GM-HRA produsert av *E. coli*. Studiene er utført i henhold til retningslinjene fra EPA (OPPTS 870.1100), EEC (B.1) og OECD (akutt toksisitetstest nr. 401). I studiene ble det benyttet 5 hann og 5 hunn mus. For kontroll ble det benyttet serumalbumin.

GAT4601

GAT4601 og serumalbumindosen var 2000 mg/kg kroppsvekt. Etter 14 dagers observasjonsperiode ble alle dyrene avlivet. Det er utført patologiske undersøkelser. Det er ikke påvist testrelaterte skader på dyrene.

GM-HRA

GM-HRA- og serumalbumindosen var henholdsvis 582 og 2000 mg/kg kroppsvekt. Etter 14 dagers observasjonsperiode ble alle dyrene avlivet. Det er utført patologiske undersøkelser. Det er ikke påvist testrelaterte skader på dyrene.

Fôringsforsøk på mus

Det er foretatt 28-dagers fôringsforsøk på mus med renfremstilt GAT4601 produsert av *E. coli*. Antall dyr og dosering (0, 10, 100, 1000 mg/kg kroppsvekt/dag) er i henhold til OECDs retningslinjer nr. 407 (Repeated Dose 28-day Oral Toxicity Study in Rodents). Det er foretatt undersøkelser av relevante organer, hematologiske parametere, fôrkonsum, klinisk-kjemiske parametere samt gross - og mikroskopisk patologiundersøkelser. For GAT4601 ble det påvist signifikante forskjeller i tre klinisk-kjemiske parametere, dvs. kaliummengde, total mengde protein og albumin i serum. Det ble imidlertid ikke påvist dose-respons forhold for disse parametrene. Ut fra dosene som ble benyttet i fôringsforsøkene har Pioneer beregnet NOAEL for GAT4601 til 1000 mg/kg kroppsvekt/dag basert på 28-dagers fôringsforsøk på mus. Pioneer hevder at fordi GM-HRA-enzymet er svært lik de fleste ALS-enzymene er det ingen grunn til å foreta et 28-dagers fôringsforsøk på mus.

Fôringsforsøk på broiler

Søknaden inneholder dokumentasjon fra 42-dagers fôringsforsøk på broilere. Forsøket omfattet 720 dyr, fordelt på seks grupper à 120 dyr. Dyrene ble føret med henholdsvis soyamel fra 356043, 356043 sprøytet med glyfosat/klorimuron og thifensulfuron, en umodifisert kontrollsort (091) og tre kommersielle umodifiserte referansesorter (93B86, 93B15, 93M40). Det ble ikke påvist vesentlige endringer ved fôring med soya fra 356043, kontroll og de tre referansesortene.

Subkronisk fôringsforsøk på rotter

Det er ikke utført et 13 ukers fôringsforsøk med fôr fra soya 356043.

4.2. Allergenitet

For å undersøke om transformasjonsprosessen kan ha ført til økning av endogene allergener i 356043 soya i forholdt til umodifiserte soyabønner ble det utført IgE immunoblotanalyse (SDS-PAGE) og ELISA-analyser med ekstrakter fra 356043- og umodifisert soya. Forsøket ble utført med sera fra soya-allergikere. Pioneer hevder at hemming av ELISA reaksjonen viser den samme bindingsprofilen for 356043- og umodifisert soyaekstrakt. Det ble konkludert med at 356043-transgen soya ikke er mer allergen enn umodifisert soya.

4.3. Delkonklusjon

Soyalinjen 356043 uttrykker et nytt protein og faggruppen mener derfor at det bør utføres sub-kronisk fôringsforsøk på rotter.

5. Miljørisikovurdering

Pioneer Hi-Bred Int. sin søknad om godkjenning av den transgene soyalinjen 356043 under forordning 1829/2003/EF omfatter bruksområdene næringsmidler, fôrvarer, import og prosessering. Søknaden gjelder ikke dyrking. Miljørisikovurderingen av 356043 er derfor avgrenset til mulige effekter av utilsiktet frøspredning i forbindelse med transport og prosessering til mat, fôr og industrielle formål. I tillegg vil indirekte eksponering gjennom gjødsel fra husdyr føret med genmodifisert soya representere en mulig kilde til uønsket genspredning.

5.1. Potensiale for ikke intenderte effekter på fitness relatert til genmodifiseringen

Soya (*Glycine max* (L.) Merr.) er stedegen i nordlige - og sentrale deler Kina, og regnes som en av verdens eldste kulturplanter (OECD 2000). Planten dyrkes kommersielt i over 35 land, med USA, Kina, Nord- og Sør-Korea, Brasil og Argentina som de dominerende produsentlandene (FAOSTAT 2006). I Europa dyrkes det soya først og fremst i Italia, Romania, Frankrike, Ungarn og Østerrike. Det er ingen produksjon av soya i Norge.

Dyrket soya er en ettårig art med nesten utelukkende selvbefruktning (~99 %) (Lu 2005). Frø av dyrkede former av soya har normalt ingen form for frøkvile. Lav frosttoleranse, predasjon, råte og spiring gjør at soyafrøene normalt ikke vil overleve til neste vekstsesong. Kravet til spiretemperatur er høyt og frøplantene er dessuten svært sensitive for lave temperaturer.

Planten krever lang vekstsesong for frømodning. Under norske vekstforhold vil derfor eventuell planter spirt fra spillfrø ikke kunne reproducere.

Til tross for omfattende dyrking over mange år i Europa og USA er det ikke påvist noen risiko knyttet til spredning, etablering og invasjon av naturlige habitater eller andre arealer utenfor jordbruksområder. Det er ingen stedeegne eller introduserte viltvoksende arter i den europeiske flora som soya kan hybridisere med (OECD 2000). Soya hybridiserer med andre ettårige arter i underslekten *Soya*, dvs. den viltvoksende arten *G. soja* og ugrasformen *G. gracilis*. Begge artene er endemiske i Asia, og det er ikke observert forekomster av naturaliserte populasjoner verken i Europa eller Amerika (OECD 2000). Det er ikke rapportert om spontant hybridisering mellom soya og flerårige arter i underslekten *Glycine*.

Spredning av soya til andre habitater i Europa er i hovedsak begrenset av manglende frøkvile, liten toleranse for lave temperaturer og dårlig konkurransevne. Det er ikke påvist forskjeller mellom soyalinje 356043 og konvensjonelle sorter med tilsvarende genetisk bakgrunn for disse karakterene, og det er ikke grunn til å anta at den introduserte egenskapen vil medføre økt fitness utenfor dyrkingsmiljø i forhold til ikke-transgene sorter av soya.

5.2. Potensiale for genoverføring

En forutsetning for genspredning er tilgjengelige veier for overføring av genetisk materiale, enten via horisontal genoverføring av DNA, eller vertikal genflyt i form av frøspredning og krysspollinering. Eksponering av mikroorganismer for rekombinant DNA skjer under nedbryting av plantemateriale på dyrket mark og/eller pollen i åkrer og omkringliggende arealer. Rekombinant DNA er også en komponent i en rekke mat- og fôrprodukter som er avledet av plantemateriale fra den transgene sorten. Dette medfører at mikroorganismer i fordøyelseskanalen hos mennesker og dyr kan eksponeres for rekombinant DNA.

5.2.1 Horisontal genoverføring

Data fra tilgjengelige eksperimentelle studier viser at genoverføring fra transgene planter til bakterier etter all sannsynlighet inntreffer svært sjelden under naturlige forhold, og at denne overføringen forutsetter sekvenshomologi mellom overført DNA og bakterien (EFSA 2004; VKM 2005).

Ut fra dagens vitenskapelig innsikt mht barrierer for genoverføring mellom ubeslektede arter og flere års forskning for om mulig å framprovosere tilfeldig overføring av genetisk materiale fra planter til mikroorganismer, dyr eller mennesker gjennom inntak eller eksponering, er det ingenting som tyder på at transgenene i soya 356043 skal kunne overføres til andre enn naturens kryssingspartnere dvs. annen dyrket soya i Europa. Det er blant annet gjort forsøk som ser på stabilitet og opptak av DNA fra tarmkanalen hvor mus er oralt tilført M13 DNA. Det tilførte DNAet var sporbart i avføring opp til syv timer etter fôring. Svært små mengder av M13 DNA (<0.1 %) kunne spores i blodbanene i en periode på maksimum 24 timer, mens M13 DNA ble funnet i opptil 24 timer i lever og milt (Schubbert *et al.* 1994). Ved oralt inntak av genmodifisert soya er det vist at DNA er mer stabilt i tarmen hos personer med utlagt tarm sammenlignet med kontrollgruppen (Netherwood *et al.* (2004). I kontrollgruppen ble det ikke påvist GM DNA i feces. Nielsen *et al.* (2000) og De Vries og Wackernagel (2002) har undersøkt persistens av DNA og opptak av GM DNA i jord. I disse laboratorieforsøkene ble det detektert svært små mengder DNA som var overført fra planter til bakterier. Forutsetningen for at dette kunne skje var sekvenshomologi mellom plantetransgenet og mottagerbakterien.

Med bakgrunn i opprinnelse og karakter/egenskaper av de innsatte genene og mangel på seleksjonspress i fordøyelseskanal og/eller miljøet, er sannsynligheten for at horisontal genoverføring vil gi selektive fordeler eller økt fitness på mikroorganismer svært liten (Nielsen 2003). Det er derfor usannsynlig at gener fra soya 356043 vil etableres stabilt i genomet til mikroorganismer i miljøet eller i fordøyelseskanalen hos mennesker eller dyr. Ut fra tilgjengelig kunnskap er det ikke grunn til å forvente at det vil skje horisontal genoverføring av DNA-materiale fra 356043.

5.2.2. Vertikal genoverføring

Soya dyrkes ikke i Norge, og arten har ikke viltvoksende populasjoner eller nærstående arter utenfor dyrking i Europa. Utsiktet frøspredning i forbindelse med transport, håndtering og prosessering vil derfor ikke medføre risiko for spredning av transgener til økologiske eller konvensjonelt dyrkede sorter, eller til ville populasjoner og arter utenfor jordbruksområder

5.3. Miljøovervåkingsplan

I følge direktiv 2001/18/EF, annekset VII er formålet med overvåkingsplanen å bekrefte at alle antagelser i miljørisikovurderingen som gjelder forekomst og omfang av potensielle skadevirkninger av den genmodifiserte organismen, eller bruken av den er korrekt. Videre skal den identifisere forekomsten av skadevirkninger på menneskers helse eller miljøet som skyldes den genmodifiserte organismen eller bruken av den, og som ikke ble forutsett i miljørisikovurderingen.

Overvåking er relatert til risikohåndtering og en totalvurdering av overvåkingsplanen er derfor utenfor VKMs mandat. I henhold til oppdrag fra DN, skal imidlertid VKM diskutere behovet for særskilt overvåking. Dette gjelder både i de tilfeller hvor søker ikke har foreslått særskilt overvåking og i de tilfeller hvor søkers risikovurdering avdekker behov for en spesiell overvåkingsplan. I sistnevnte tilfelle skal VKM gi en vurdering av kvaliteten på søkers overvåkingsplan, om denne er egnet til å avdekke så vel umiddelbare og direkte virkninger som forsinkede og indirekte virkninger påvist i miljørisikovurderingen. VKM skal ikke vurdere innretningen av den generelle overvåkingen.

Søknaden EFSA/GMO/NL/2007/43 omfatter ikke dyrking, og potensiell miljøeksponering av den transgene soyalinjen er derfor avgrenset til mulige effekter av utsiktet frøspredning i forbindelse med transport og prosessering til mat, fôr og industrielle formål. I tillegg vil indirekte eksponering gjennom gjødsel fra husdyr fôret med genmodifisert mais representere en mulig kilde til uønsket genspredning. Miljørisikovurderingen som er presentert av søker identifiserer ingen endret risiko for miljø i forhold til annen soya. Pioneer har derfor ikke utarbeidet spesifikke strategier for risikohåndtering eller en særskilt plan for overvåking av denne eventen.

Tatt i betraktning tiltenkt bruksområde for 356043 anser Faggruppe for GMO at det ikke er behov for å iverksette særskilt program for overvåking av soyalinjen.

5.4. Delkonklusjon

Søknaden gjelder godkjenning av soyalinjen 356043 for import, prosessering og til bruk i næringsmidler og fôrvarer, og omfatter ikke dyrking. Med bakgrunn i tiltenkt bruksområde er

miljøriskovurderingen avgrenset til mulige effekter av utilsiktet frøspredning i forbindelse med transport og prosessering, samt indirekte eksponering gjennom gjødsel fra husdyr fôret med genmodifisert soya.

Det er ingen indikasjoner på økt sannsynlighet for spredning, etablering og invasjon av soyalinjen i naturlige habitater eller andre arealer utenfor jordbruksområder. Soya dyrkes ikke i Norge, og arten har ikke viltvoksende populasjoner eller nærstående arter utenfor dyrking i Europa. Det er derfor ikke risiko for utkryssing med dyrkede sorter eller ville planter i Norge.

KONKLUSJON

Faggruppe for genmodifiserte organismer ønsker å påpeke at det er kunnskapshull med hensyn på mulige helseeffekter ved soya 356043. Soyalinjen 356042 uttrykker et nytt protein og faggruppen etterlyser derfor bakgrunnsmaterialer med hensyn på mulige helseeffekter. Analyser av ernæringsmessige viktige komponenter i soya er utført i tråd med OECDs konsensusdokument (OECD 2001). Faggruppen påpeker at i søknaden henvises det til en undersøkelse av agronomiske karakterer. Denne undersøkelsen er ikke lagt ved søknaden. Faggruppen mener at når det i søknaden henvises til resultater fra slike undersøkelser, skal resultatene fra undersøkelsene være tilgjengelige. Videre mener faggruppen at det bør kreves av søker å utføre et 90- dagers subkronisk fôringsforsøk på rotter. Faggruppen konkluderer imidlertid med at olje fra transgen plante er vesentlig lik olje fra umodifisert plante.

Søknaden gjelder godkjenning av soyalinjen 356043 for import, prosessering og til bruk i næringsmidler og fôrvarer. Faggruppen har derfor ikke vurdert mulige miljøeffekter knyttet til dyrking av soyalinjen. Det er ingen indikasjoner på økt sannsynlighet for spredning, etablering og invasjon av soyalinjen i naturlige habitater eller andre arealer utenfor jordbruksområder som resultat av frøspill i forbindelse med transport og prosessering. Soya dyrkes ikke i Norge, og arten har ikke viltvoksende populasjoner eller nærstående arter utenfor dyrking i Europa. Det er derfor ikke risiko for utkryssing med dyrkede sorter eller ville planter i Norge.

Samlet vurdering

Faggruppen konkluderer med at olje fra soyalinjen 356043 er vesentlig lik olje fra umodifisert soya, men påpeker betydelige kunnskapshull med hensyn på mulige helseeffekter knyttet til bruk av 356043 som næringsmiddel og fôrvare. Faggruppen finner det lite trolig at bruk av soyalinjen 356043 vil medføre endret risiko for miljø i forhold til annen soya.

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Appendix II

SCIENTIFIC OPINION

Scientific Opinion on application (EFSA-GMO-UK-2007-43) for the placing on the market of herbicide tolerant genetically modified soybean 356043 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Pioneer¹

EFSA Panel on Genetically Modified Organisms (GMO)^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This scientific opinion is an evaluation of a risk assessment for the genetically modified herbicide tolerant soybean 356043 for food and feed uses, import and processing. Soybean 356043 contains a single copy of intact *gat4601* and *Glycine max-hra* cassettes at a single insertion locus. The results of the bioinformatic analyses of the insert and the flanking regions, and the levels of newly expressed proteins did not raise a safety concern. The comparative analysis of phenotypic and agronomic characteristics indicated that soybean 356043 is not different from its conventional counterpart. In the composition, differences were identified between 356043 soybean and its conventional counterpart in the newly expressed proteins *Glycine max-HRA* and GAT4601, and the levels of the fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid and the acetylated amino acids N-acetylaspartate (NAA) and N-acetylglutamate (NAG). The safety assessment of the newly expressed proteins *Glycine max-HRA* and GAT4601 identified no concerns regarding potential toxicity and allergenicity. Heptadecanoic, heptadecenoic and heptadecadienoic acid are present in the diet and the intake of small amounts of these fatty acids via food or feed is not expected to produce adverse effects. NAA and NAG are normal constituents in the mammalian metabolism and the estimated increases in their intake are considered low when related to the normal intake of L-aspartic acid and L-glutamic acid. Further toxicological, allergenicity and nutritional analysis provided no indications of adverse effects. There are no indications of an increased likelihood of establishment and spread of feral soybean plants, except in the presence of the glyphosate and ALS-inhibiting herbicides neither a risk caused by a possible transfer of the recombinant gene from soybean 356043 to environmental micro-organisms. The EFSA GMO Panel considers that the information available for soybean 356043 addresses the scientific comments raised by the Member States and states that the soybean 356043, as

1 On request from the Competent Authority of the UK on an application (EFSA-GMO-UK-2007-43) submitted by Pioneer, Question No EFSA-Q-2007-087, adopted on 6 July 2011.

2 Panel members: Hans Christer Andersson, Salvatore Arpaia, Detlef Bartsch, Josep Casacuberta, Howard Davies, Patrick du Jardin, Gerhard Flachowsky, Lieve Herman, Huw Jones, Sirpa Kärenlampi, Jozsef Kiss, Gijs Kleter, Harry Kuiper, Antoine Messéan, Kaare Magne Nielsen, Joe Perry, Annette Pötting, Jeremy Sweet, Christoph Tebbe, Atte Johannes von Wright, and Jean-Michel Wal. Correspondence: gmo@efsa.europa.eu

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, Thoams Frenzel, Gerd Neemann and Joachim Schiemann as external experts and EFSA's staff member Anna Christodoulidou, Diveki Zoltan, Karine Lheureux and Davide Arcella for the support provided to this EFSA scientific opinion.

described in this application, 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.

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KEY WORDS

GMO, soybean, 356043, risk assessment, food and feed safety, environment, import and processing, Regulation (EC) No 1829/2003, GAT, *Glycine max*-HRA, NAA, NAG.

SUMMARY

Following the submission of an application (EFSA-GMO-UK-2007-43) under Regulation (EC) No 1829/2003 from Pioneer, the EFSA Panel on Genetically Modified Organisms was asked to deliver a scientific opinion on the herbicide tolerant genetically modified (GM) soybean 356043 (Unique identifier DP-356043-5) for food and feed uses, import and processing.

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-UK-2007-43, additional information supplied by the applicant and scientific comments submitted by Member States. The scope of application EFSA-GMO-UK-2007-43 is for food and feed uses, import and processing of soybean 356043 and all derived products, but excludes cultivation in the EU. The EFSA GMO Panel assessed soybean 356043 with reference to the intended uses and 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, 2006a). The scientific assessment included molecular characterisation of the inserted DNA and expression of 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 plan were undertaken.

The molecular characterisation data establish that the genetically modified soybean 356043 contains one copy of an intact *gat4601* expression cassette and a *Glycine max-hra* (*gm-hra*) cassette in a single locus. No other parts of the plasmid used for transformation are present in the transformed plant. Bioinformatic analysis of the open reading frames spanning the junctions between the inserted DNA and soybean genomic DNA did not raise safety concerns. The stability of the inserted DNA and the herbicide tolerance trait were confirmed over several generations. Analyses of the levels of newly expressed proteins in various plant tissues collected from field trials performed in South- and North America did not raise safety concerns.

The EFSA GMO Panel concludes that no differences were identified between 356043 soybean and its conventional counterpart, except for the newly expressed proteins, for higher levels of the acetylated amino acids N-acetylaspartate (NAA) and N-acetylglutamate (NAG), and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid in seed from 356043 soybean. The levels of these acetylated amino acids and odd chain fatty acids fall outside the natural ranges observed for other commercial non-GM soybean varieties. The overall level of NAA and NAG (taken together) in soybean 356043 was found to be less than 0.15 % of the total amino acids. The total level of odd chain fatty acids amounts to less than 1% of total fatty acids. No statistically significant differences in total amino acid contents in seed were observed between the 356043 soybean and its conventional counterpart. Levels of major fatty acids in 356043 soybean seed were found to be comparable to those observed in the conventional counterpart.

No toxicity of the GAT4601 and the *Glycine max*-HRA proteins was observed in acute oral toxicity studies and repeated-dose (28 days) feeding studies using mice. The studies on *in vitro* digestibility of the proteins showed that most of the proteins were degraded. In bioinformatics studies the proteins showed no homology to known toxic proteins and allergens.

The odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid are normal constituents of plants and animals and have also been identified in human tissues. There is no information indicating that the intake of small amounts of these fatty acids via food or feed causes adverse effects. The EFSA GMO Panel is of the opinion that the estimated increases in intake levels of heptadecanoic, heptadecenoic and heptadecadienoic resulting from replacement of conventional soybean oil with oil from soybean 356043 do not raise safety concerns.

NAA and NAG are normal constituents in the mammalian metabolism. They are also present in conventional foodstuffs and thus consumed as part of a normal diet. The available scientific information indicates that under normal conditions NAA and NAG, like other N-acetylated amino acids, are deacetylated in the intestine to form the corresponding L-amino acids, which are further metabolised in the body. The oral toxicity of NAA and NAG has been tested in acute and subacute (28 days) studies using rats. In addition, NAA was tested in a subchronic (90 days) feeding study and in a study on reproductive and developmental toxicity (two generation study) using rats. Considering the outcome of a conservative intake assessment, the estimated increase in intake of NAA is more than 100 fold lower than the NOEL observed in the 90-day rat feeding study with NAA. Furthermore, in relation to the normal intake of L-aspartic acid and L-glutamic acid resulting from consumption of food protein, the estimated increases in the intake of NAA and NAG are considered low. Considering all the available information, the EFSA GMO Panel is of the opinion that the estimated increases in intake levels of NAA and NAG resulting from replacement of food products derived from conventional soybeans by the respective products derived from soybean 356043 do not raise safety concerns. The same conclusion applies to the use of feed materials derived from this genetically modified soybean.

Furthermore, a subchronic 92-day feeding study in rats using diets including meal and hulls derived from soybean 356043 provided no indications of adverse effects. Testing of extracts from soybeans 356043 with sera from patients allergic to soybean showed that the overall allergenicity of the whole plant had not been changed. A 42-day feeding study using broiler chickens demonstrated that soybean 356043 is nutritionally equivalent to its conventional counterpart and commercial non-GM soybean varieties included in this study. Therefore, the EFSA GMO Panel is of the opinion that soybean 356043 is as safe as its conventional counterpart with respect to potential effects on human and animal health in the context of its intended uses.

The application EFSA-GMO-UK-2007-43 is for food and feed uses, import and processing. Therefore, there is no requirement for scientific information on possible environmental effects associated with the cultivation of soybean 356043. 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 seeds of soybean 356043 (e.g. during transportation and processing), except in the presence of glyphosate and ALS-inhibiting herbicides. Taking into account the scope of the application, 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. In the context of its intended uses, the theoretically possible transfer of the recombinant genes from soybean 356043 to gut or other environmental bacteria has not been identified to be a risk due to the lack of any selective advantage. 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 356043 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 356043 addresses the scientific comments raised by the Member States and that the soybean 356043, as described in this application, 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.

TABLE OF CONTENTS

| | |
|---|----|
| Abstract | 1 |
| Summary | 3 |
| Table of contents | 5 |
| Background | 6 |
| Terms of reference..... | 6 |
| Assessment | 8 |
| 1. Introduction | 8 |
| 2. Issues raised by the Member States | 8 |
| 3. Molecular characterisation..... | 8 |
| 3.1. Evaluation of the relevant scientific data..... | 8 |
| 3.1.1. Transformation process and vector constructs | 8 |
| 3.1.2. Transgene constructs in the genetically modified plant..... | 9 |
| 3.1.3. Information on the expression of the insert | 9 |
| 3.1.4. Inheritance and stability of inserted DNA | 10 |
| 3.2. Conclusion | 10 |
| 4. Comparative analysis..... | 10 |
| 4.1. Evaluation of relevant scientific data..... | 10 |
| 4.1.1. Choice of comparator and production of material for the compositional assessment | 10 |
| 4.1.2. Compositional analysis..... | 11 |
| 4.1.3. Agronomic traits and GM phenotype | 13 |
| 4.2. Conclusion | 13 |
| 5. Food/Feed safety assessment..... | 13 |
| 5.1. Evaluation of relevant scientific data..... | 13 |
| 5.1.1. Product description and intended use | 13 |
| 5.1.2. Effect of processing | 14 |
| 5.1.3. Toxicology..... | 15 |
| 5.1.4. Allergenicity | 26 |
| 5.1.5. Nutritional assessment of GM food/feed..... | 27 |
| 5.1.6. Post-market monitoring of GM food/feed | 28 |
| 5.2. Conclusion | 28 |
| 6. Environmental risk assessment and monitoring plan | 29 |
| 6.1. Environmental risk assessment | 29 |
| 6.1.1. Unintended effects on plant fitness due to the genetic modification | 29 |
| 6.1.2. Potential for gene transfer..... | 30 |
| 6.1.3. Interactions of the GM plant with target organisms | 32 |
| 6.1.4. Interactions of the GM plant with non-target organisms..... | 32 |
| 6.1.5. Interactions with the abiotic environment and biogeochemical cycles | 32 |
| 6.1.6. Monitoring..... | 32 |
| 6.2. Conclusion | 33 |
| Overall Conclusions and Recommendations..... | 33 |
| Documentation provided to EFSA | 35 |
| References | 36 |

BACKGROUND

On 11 April 2007, the European Food Safety Authority (EFSA) received from the Competent Authority of the United Kingdom an application (Reference EFSA-GMO-UK-2007-43), for authorisation of the herbicide tolerant genetically modified (GM) soybean 356043 (Unique Identifier DP-356043-5), submitted by Pioneer within the framework of Regulation (EC) No 1829/2003 on genetically modified food and feed. After receiving the application EFSA-GMO-UK-2007-43 and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed Member States and the European Commission, and made the summary of the application publicly available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 11 September 2007, EFSA received additional information requested under completeness check (requested on 06 August 2007). On 28 September 2007, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application 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 following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member State bodies had three months after the date of acknowledgement of the valid application (28 December 2007) within which to make their opinion known.

The Scientific Panel on Genetically Modified Organisms of EFSA (EFSA GMO Panel) carried out a scientific assessment of the GM soybean 356043 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. When carrying out the safety assessment, the EFSA GMO Panel took 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, 2006a), the scientific comments of Member States and the additional information provided by the applicant.

On 20/12/2007, 27/02/2008, 22/07/2008, 08/09/2008, 14/01/2010, 28/05/2010 and 22/10/2010 the EFSA GMO Panel requested from the applicant additional information. The applicant provided the requested information on 12/02/2008, 15/04/2008, 06/10/2009, 08/09/2008, 15/10/2008, 05/03/2010, 12/07/2010 and 07/12/2010. Complementary information was submitted spontaneously by the applicant on 21/04/2010 and 11/03/2011. After receipt and assessment of the full data package the EFSA GMO Panel finalised its risk assessment on soybean 356043.

In giving its scientific opinion on GM soybean 356043 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 application. 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 EFSA overall opinion in accordance with Articles 6(5) and 18(5).

TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific risk assessment of soybean 356043 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. 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/environment 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 to the Cartagena Protocol. Furthermore, the EFSA GMO Panel did also not consider proposals for labelling and methods of detection (including sampling and the identification of the specific transformation 356043 in the food/feed and/or food/feed produced from it), which are matters related to risk management.

ASSESSMENT

1. Introduction

The genetically modified (GM) soybean 356043 (Unique Identifier DP-356043-5) was evaluated with reference to its intended uses, taking account of 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, 2006a). The evaluation of the risk assessment presented here is based on the information provided in the application, as well as additional information from the applicant, scientific comments submitted by the Member States and relevant scientific publications.

2. Issues raised by the Member States

The issues raised by the Member States are addressed in Annex G of the EFSA overall opinion⁴ and have been considered in this scientific opinion.

3. Molecular characterisation

3.1. Evaluation of the relevant scientific data

3.1.1. Transformation process and vector constructs⁵

The 356043 soybean has been genetically modified for herbicide tolerance. This was achieved by the introduction of the *gat4601* and the *Glycine max-hra* (*gm-hra*) coding sequences surrounded by their necessary regulatory components.

- *gat4601* is an optimized form of the glyphosate acetyltransferase (*gat*) coding sequence from *Bacillus licheniformis* that confers tolerance to glyphosate- and glyphosate-ammonium based herbicides. Glyphosate inhibits the enzyme enolpyruvulshikimate-3-phosphate synthase (EPSPS), which is involved in the biosynthesis of aromatic amino acids. GAT proteins acetylate glyphosate giving rise to N-acetyl glyphosate, which has no herbicidal activity. The synthetic *gat4601* coding sequence was obtained after seven rounds of DNA shuffling using three distinct alleles of the *gat* gene isolated from three different strains of *B. licheniformis* as well as the introduction of changes *via* PCR. The native GAT enzymes were capable of acetylating glyphosate, but at a very slow rate. The GAT4601 protein is 84% homologous at the amino acid level to each of the three GAT enzymes from *B. licheniformis* from which it was derived but with 2400-fold increased catalytic efficiency. The GAT proteins are members of the GNAT family of N-acetyltransferases. GNAT proteins have a number of metabolic functions (Dyda et al. 2000). The studies on substrate specificity of GAT4601 concluded that it acetylates aspartic and glutamic acids, and has very low affinity to serine, threonine, glycine and some aminophosphonates.
- *gm-hra* is an optimized form of the endogenous acetolactate synthase (*als*) coding sequence from soybean (*Glycine max*), that confers tolerance to ALS-inhibiting herbicides, such as chlorimuron, thifensulfuron or sulfonyleureas. The synthetic *gm-hra* coding sequence was obtained by introducing a synthetic start codon together with twelve nucleotides from the endogenous *als* 5' untranslated region at the 5' end of the endogenous *als* gene. In addition, two nucleotide changes were made within the coding sequence with the purpose of introducing two point mutations (A183 and L560) in the protein sequence. As a result, the *Glycine max*-HRA protein is tolerant to ALS-inhibiting herbicides.

⁴ <http://registerofquestions.efsa.europa.eu/roqFrontend/questionLoader?question=EFSA-Q-2011-00856>

⁵ Technical Dossier / Sections C and D1

The 356043 soybean was produced by the particle acceleration method. A linear DNA fragment containing the *gat4601* and *gm-hra* expression units was inserted into soybean plant cells from the commercial cultivar “Jack”. The DNA fragment (PHP20163A) introduced into 356043 soybean was obtained from plasmid PHP20163 following digestion of the plasmid DNA with restriction enzymes. PHP20163A contains the *gat4601* and *gm-hra* expression units in tandem orientation.

- The *gat4601* coding sequence is under the regulation of the synthetic constitutive promoter (SCP1) comprising a portion of the *Cauliflower mosaic virus* 35S promoter and the Rsyn7-Syn II core consensus promoter, with translation enhanced by omega 5' untranslated region translational enhancer element from the *Tobacco mosaic virus*, and with transcription terminated by the proteinase inhibitor II (*pinII*) terminator from potato (*Solanum tuberosum*).
- The *gm-hra* coding sequence is under the regulation of the constitutive S-adenosyl-L-methionine synthetase (SAMS) promoter from soybean, and with transcription terminated by the endogenous *als* gene terminator from soybean.

3.1.2. Transgene constructs in the genetically modified plant⁶

Molecular analyses were undertaken on T₄ plants produced after four generations of self-pollination of the original transformation event DP-356043-5. Southern blot, PCR, sequencing and inheritance studies established that a single, intact PHP20163A fragment was inserted into the soybean nuclear genome to produce 356043 soybean. The absence of additional DNA sequences from the PHP20163 plasmid in 356043 plants has been confirmed by Southern analysis using probes that cover the entire sequence of the plasmid backbone (including the hygromycin resistance gene used to maintain the plasmid in bacteria).

The DNA sequence of the insert contains 5362 base pairs spanning the entire PHP20163A fragment. Flanking genomic sequences extending 3317 base pairs at the 5' end and 2169 base pairs at the 3' end of the insert were determined. Both flanking regions were shown to be soybean genomic sequences. The applicant carried out further bioinformatics analysis (BLASTn, BLASTx) in order to identify the nature and potential function of the soybean flanking sequences⁷. The outcome was that genetic modification did not interrupt any known genes.

The applicant performed a bioinformatic analysis of all twelve open reading frames spanning the insert – genomic DNA junction regions in order to assess the similarity of their putative translational products to known toxins and allergens^{7,8}. No similarities were found.

3.1.3. Information on the expression of the insert⁹

The expression levels of GAT4601 and *Glycine max*-HRA were measured by ELISA in several samples of 356043 soybean cultivated in field trials at six locations during one season in South America (2005/2006) and at one location during one season in North America (2005). The expression levels were determined from forage, root and grain at different growth stages and from plants treated and non-treated with herbicides. The expression level of GAT4601 varied between 0.09 and 1.0 µg/g dry weight (dw)¹⁰ for grain and between 0.72 and 2.3 µg/g dw¹¹ for forage. The *Glycine max*-HRA expression level ranged between the level of detection and 1.2 µg/g dw¹⁰ for grain and between 5.6 and 46 µg/g dw¹¹ for forage. Mean levels of the newly expressed proteins in treated and non-treated

⁶ Technical Dossier / Section D2

⁷ Additional information, April 2008

⁸ Additional information, October 2008

⁹ Technical Dossier / Section D3

¹⁰ Technical Dossier, Tables 4-5

¹¹ Technical Dossier, Annexes 4-5

plants were very similar. The expression ranges of the newly expressed proteins are summarised in Table 1.

Table 1: Ranges of GAT4601 and *Glycine max*-HRA levels in soybean 356043 (µg/g dw)

| Site / season | tissue | GAT4601 | <i>Glycine max</i> -HRA |
|---------------------------|--------|-------------|-------------------------|
| South America (2005/2006) | grain | 0.09 – 0.43 | < LOD – 1.1 |
| | forage | 0.72 – 1.9 | 5.6 – 34 |
| North America (2005) | grain | 0.12 – 1.2 | < LOD – 1.2 |
| | forage | 1.1 – 2.3 | 11 – 46 |

LOD: limit of detection

3.1.4. Inheritance and stability of inserted DNA¹²

Genetic stability of 356043 soybean was investigated by Southern and Western analyses in two populations of plants, one segregating (F₃) and one not segregating (T₅) for the insert. Southern analysis of 92 F₃ individual plants spanning both 5' and 3' on the insert showed that the insert was genetically stable and followed the Mendelian inheritance pattern of a single locus. A further study across two generations (T₄-T₅) confirmed the genetic and phenotypic stability of the insert. The EFSA GMO Panel is of the opinion that, should instability leading to loss of the trait(s) occur, no safety concern would arise.

3.2. Conclusion

Appropriate molecular and bioinformatic analyses of the 356043 soybean insert and its flanking genomic regions have been undertaken. The expression of the genes introduced has been sufficiently analysed and the stability of the genetic modification has been demonstrated over several generations. The molecular characterisation provided for the transformation event 356043 soybean is sufficient for the safety assessment. The GMO panel considers this to be an adequate analysis and the molecular characterisation does not indicate a safety concern.

4. Comparative analysis

4.1. Evaluation of relevant scientific data

4.1.1. Choice of comparator and production of material for the compositional assessment¹³

In the compositional studies, the 356043 soybean was in replicated field trials compared to the non-transgenic variety Jack, which is a conventional soybean variety with a history of safe use and with background genetics similar to 356043 soybean. The 356043 soybean and its conventional counterpart were grown under the same agronomic conditions. In addition plots were included where 356043 soybean was treated with glyphosate herbicides and/or ALS inhibiting herbicides. The field trials were in the season 2005-2006 carried out in Chile and Argentina and in year 2005 in USA and Canada, each season/year at six different geographical sites. Additional field trials for agronomic and compositional analyses were performed at four locations in the USA and two locations in Canada in 2006. Five of the six locations in North America were planted both in 2005 and 2006. Data obtained for 356043 soybean and its conventional counterpart were compared to ranges for agronomic and compositional characteristics obtained from other commercial non-GM soybean varieties. Data to define natural ranges were derived from literature and from data collected under a separate study, in which four commercially available soybean varieties grown at six locations in North America (2005) were planted, harvested, processed, and analyzed using the same methods employed in the comparative

¹² Technical Dossier / Section D5

¹³ Technical dossier/ Section D7.1 and D7.2 and additional information, April 2008 and March 2010

analysis of 356043 soybean. All the locations used in the field trials over the three seasons are representative for the environmental conditions of commercial soybean production in North and South America.

4.1.2. Compositional analysis¹⁴

Soybean seeds were analysed for proximates, fibre fractions, minerals, amino acids, fatty acids, vitamins, 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. The selection of compounds followed the recommendations by OECD (2000). In addition, compounds related to the activities of the proteins newly expressed in 356043 soybean were analysed in soybean seeds, i.e. acetylated amino acids, free amino acids, and minor odd chain fatty acids. The data on each analyte were statistically analysed for potential differences in their levels in 356043 soybean compared to those in its conventional counterpart within-site and across-sites (data from all sites combined).

Consistent statistically significant compositional differences between 356043 soybean and its conventional counterpart were found for the odd chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid, independently of the herbicide treatment regime. Levels determined for 356043 soybean were around two to three times higher than those observed for the conventional counterpart and outside the ranges observed for other commercial soybean varieties (Table 2). The range for heptadecadienoic acid in commercial soybeans was determined by the applicant by analysis of material obtained from eight soybean varieties grown in three field studies in the US and Canada (2007, 2009).

Table 2: Levels [% of total fatty acids] of heptadecanoic acid (C17:0), heptadecenoic acid (C17:1) and heptadecadienoic acid (C17:2) in seeds from 356043 soybean untreated or treated with glyphosate and ALS-inhibiting herbicides (i.e. target herbicides) compared to those in seeds from the conventional counterpart Jack (North American locations, 2006)

| Analyte | | Control soybean Jack untreated with target herbicides | 356043 soybean untreated with target herbicides | 356043 soybean treated with target herbicides | Reference varieties |
|---------|-------|---|---|---|---------------------|
| C17:0 | Mean | 0.129 | 0.326 | 0.330 | 0.085 - 0.146 |
| | Range | 0.105 - 0.304 | 0.207 - 0.408 | 0.152 - 0.423 | |
| C17:1 | Mean | 0.063 | 0.179 | 0.183 | 0.073 - 0.087 |
| | Range | 0.049 - 0.136 | 0.117 - 0.240 | 0.067 - 0.248 | |
| C17:2 | Mean | 0.056 | 0.150 | 0.153 | 0 - 0.068 |
| | Range | 0.045 - 0.121 | 0.099 - 0.203 | 0.061 - 0.211 | |

As an explanation for this effect the applicant considered that odd chain fatty acid biosynthesis starts with the conversion of 2-ketobutyrate to propionyl-CoA followed by subsequent addition of C2 moieties. One of the specific amino acid changes introduced into the *Glycine max*-ALS enzyme to form the *Glycine max*-HRA enzyme conferring herbicide tolerance (i.e. replacement of tryptophan 560 by leucine), is expected to increase the 2-ketobutyrate pool available for odd chain fatty acid biosynthesis due to decreased affinity to that intermediate. Studies on the odd chain fatty acids showed increased levels of the C17 long fatty acids but the contents of the longer odd chain fatty acids C19:0, C21:0 and C23:0 have not been altered in 356043 soybean seeds and that they are comparable to the levels in the conventional counterpart. Statistically significant differences occasionally observed for other fatty acids were considered to be small and not biologically relevant. Levels of major fatty acids

¹⁴ Technical dossier/ Section D7.2 and D7.3 and additional information of April 2008, September 2008, March 2010 and December 2010

in 356043 soybean seeds were found to be comparable to those observed in the conventional counterpart.

In tests for substrate specificity, the newly expressed GAT4601 protein was shown to acetylate aspartic acid and glutamic acid. The protein was found to have a very low affinity for serine, threonine and glycine. (see section 5.1.3.2.). The levels of the acetylated amino acids N-acetylaspartate (NAA) and N-acetylglutamate (NAG) were measured in seeds of 356043 soybean, its conventional counterpart and commercial soybean varieties. The mean values for NAA and NAG in 356043 soybean were consistently statistically significantly different from those of its conventional counterpart and markedly outside natural ranges determined for commercial soybean varieties. This effect was observed independently of the herbicide treatment regime (Table 3).

Table 3: Levels [mg/kg dry weight] of N-acetylaspartate (NAA) and N-acetylglutamate (NAG) in seeds from 356043 soybean untreated or treated with glyphosate and ALS-inhibiting herbicides (i.e. target herbicides) compared to seeds from control soybean (South American locations, 2005-2006)

| Analyte | | Control soybean Jack untreated with target herbicides | 356043 soybean untreated with target herbicides | 356043 soybean treated with target herbicides | Reference varieties |
|---------|-------|---|---|---|---------------------|
| NAA | Mean | 1.92 | 653 | 681 | 0 - 2.27 |
| | Range | 1.10 - 3.67 | 490 - 870 | 502 - 994 | |
| NAG | Mean | 2.34 | 18.3 | 18.1 | 0 - 3.17 |
| | Range | 1.42 - 3.35 | 9.86 - 43.2 | 8.27 - 31.8 | |

The applicant was requested by the GMO Panel to quantify acetylated derivatives of serine, threonine and glycine in 356043 soybean and its conventional counterpart. It was demonstrated that the levels in seed from 356043 soybean and its conventional counterpart are comparable and within natural ranges calculated for these compounds in non-genetically modified soybean seed.

Considering the modes of action of the GAT4601 and *Glycine max*-HRA proteins newly expressed in 356043 soybean, comprehensive comparative analyses of total and free amino acids were carried out. No statistically significant differences in total amino acid contents in seed were observed between the 356043 soybean and its conventional counterpart for any of the eighteen proteinogenic amino acids tested. In addition, the levels of free amino acids in seed from 356043 soybeans are comparable to the levels of free amino acids in seed from its conventional counterpart, regardless of the treatment with glyphosate and ALS-inhibiting herbicides. The overall level of NAA and NAG (taken together) in soybean 356043 was found to be less than 0.15 % of the total amino acids.

For other key constituents, recommended by OECD, including anti-nutrients and other secondary metabolites (isoflavones), no consistent alteration in the level of the studied components in 356043 soybeans as compared to the conventional counterpart was found between sites and between growing seasons. Furthermore, the differences were generally small and fell within the range of natural variation calculated from the occurrence of these constituents in other commercial soybean varieties.

Compositional analysis of soybean forage did not reveal consistent alterations in the level of studied components in 356043 soybean as compared to the conventional counterpart. The composition of forage obtained from 356043 soybean fell within the range of natural variation. The applicant did not provide information on the levels of acetylated amino acids in forage.

The Panel considered the total compositional data supplied and the observed compositional differences between 356043 soybean and its conventional counterpart in the light of the measured biological variation and the level of the studied compounds in other commercial non-GM soybean varieties. The EFSA GMO Panel concludes that no differences were identified between 356043 soybean and its conventional counterpart, except for the newly expressed proteins, for higher levels of the acetylated

amino acids N-acetylaspartate (NAA) and N-acetylglutamate (NAG), and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid in seed from 356043 soybean. The levels of these acetylated amino acids and odd chain fatty acids fall outside the natural ranges observed for other commercial non-GM soybean varieties.

4.1.3. Agronomic traits and GM phenotype¹⁵

The applicant provided information on agronomic performance, phenotypic characteristics and ecological interaction of 356043 soybean and its conventional counterpart from field trials performed in the USA, Canada, Chile and Argentina in 2005 and 2006. The characteristics evaluated were early population, final population, seedling vigour, lodging, shattering, disease incidence, insect damage, plant height, days to maturity, yield, flower colour, pod wall colour, and hila colour. When analysed across locations, statistically significant differences were observed for some agronomic parameters, i.e. lodging, seedling vigour, final population, and plant height. However, when analysed by site, statistically significant differences for seedling vigour and plant height were observed at one and four of the six locations situated in North America, respectively. Statistically significant differences were observed at one of the six locations in the individual location analysis for both lodging and plant height in South America. As the magnitudes of the differences were small, and parameters fell within the ranges observed for conventional soybean, the GMO Panel found these differences to be of no biological relevance.

The EFSA GMO Panel assessed the provided data and considers 356043 soybean to be agronomically not different from its conventional counterpart with the exception of the newly introduced traits.

4.2. Conclusion

The EFSA GMO Panel concludes that no differences were identified between 356043 soybean and its conventional counterpart, except for the newly expressed proteins, for higher levels of the acetylated amino acids N-acetylaspartate (NAA) and N-acetylglutamate (NAG), and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid in seed from 356043 soybean. The levels of these acetylated amino acids and odd chain fatty acids fall outside the natural ranges observed for other commercial non-GM soybean varieties. The overall level of NAA and NAG (taken together) in soybean 356043 was found to be less than 0.15% of the total amino acids. The total level of odd chain fatty acids amounts to less than 1% of total fatty acids. No statistically significant differences in total amino acid contents in seed were observed between the 356043 soybean and its conventional counterpart. Levels of major fatty acids in 356043 soybean seed were found to be comparable to those observed in the conventional counterpart. The observed differences are further evaluated in the following Food/Feed safety assessment (section 5).

5. Food/Feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Product description and intended use

The scope of application EFSA-GMO-UK-2007-43 is for food and feed uses, import and processing of soybean 356043. Thus soybean 356043 will be used for the production of soybean products as any commercial soybean variety. The main product for human use is soybean oil. In addition, soybean is used for the production of soybean milk, protein isolate, flour, sprouts, baked or roasted soybeans, tofu, soybean sauce and other products for human consumption. Defatted soybean meal is used as a source of protein in animal feed, often in combination with soybean hulls. There is also a limited direct use of soybeans as an animal feed.

¹⁵ Technical dossier/ Section D7.4

The genetic modification of soybean 356043 is intended to improve agronomic performance only and is not intended to influence the nutritional properties, processing characteristics and overall use of soybean as a crop.

5.1.2. Effect of processing¹⁶

The applicant has studied the influence of temperature (36–60°C) and pH-value (5–9) on the enzyme activity of the *Glycine max*-HRA protein produced in *Escherichia coli* (see 5.1.3.1.) using an ALS activity assay based on the production of acetolactate from pyruvate. After incubation at 44°C for 15 minutes approximately 50% of the activity was lost, and the enzyme was practically inactivated after incubation at 50°C for 15 minutes. The pH optimum of the enzyme activity was in the range of pH 7–7.5, whereas there was practically no activity at or below pH 6.0 as well as at pH 9.0.

The influence of temperature (36–60°C) and pH-value (5–9) on the enzyme activity of the GAT4601 protein produced in *E. coli* (see 5.1.3.1.) was studied using a glyphosate acetyltransferase assay. After incubation at 50 °C for 15 minutes approximately 40% of the activity was lost, and the enzyme was practically inactivated after incubation at 56 °C for 15 minutes. The pH optimum of the enzyme activity was in the range of pH 6–6.5, whereas the activity was considerably reduced at pH 5 and pH 8.5.

Considering the significant compositional differences observed for the raw agricultural commodity (see section 4.1.2), the applicant has provided data on the levels of the acetylated amino acids NAA and NAG, determined by HPLC/MS in whole soybeans and processed products derived from soybean 356043 (untreated and treated with the target herbicides) and its conventional counterpart (Jack).

Compared with the conventional counterpart higher levels of NAA and NAG were present in whole cooked seed, hull material, defatted raw flakes, defatted toasted meal, mill feed, defatted flour, and soy milk from soybean 356043. Higher levels of NAA only, were found in aspirated seeds fraction, crude lecithin, protein concentrate, okara and tofu. NAA and NAG were not detected or below the limit of quantification in protein isolate, and degummed and refined/bleached/deodorised soybean oil.

In processed products the levels of these acetylated amino acids were generally reduced or in the same ranges as in whole unprocessed soybeans except for higher levels in hull material and mill feed (NAA and NAG), defatted raw flakes and defatted toasted meal (slightly higher levels of NAG only).

In summary, processing of whole 356043 soybeans can lead to lower as well as higher levels of the acetylated amino acids NAA and NAG in processed products compared with unprocessed 356043 soybeans.

Since soybean oil is the primary source of human exposure, the applicant determined the compositions of crude and refined bleached deodorized soybean oil obtained from 356043 soybean (treated and untreated with target herbicides) and its conventional counterpart. Soybean samples were collected from six separate field trials in 2006, four located in the United States and two located in Canada. Oil from 356043 soybean differed from oil from its conventional counterpart in the levels of heptadecanoic and heptadecenoic acid. Results obtained for oil were fully in line with those determined for seeds. Upon request of the EFSA GMO Panel to assess the potential intake of the odd-chain fatty acids, the applicant also provided information on the levels of heptadecadienoic acid in refined bleached deodorized soybean oil derived from 356043 soybeans and its conventional counterpart (see section 5.1.5.3).

¹⁶ Technical dossier/ Section D7.6 and additional information April 2008

5.1.3. Toxicology¹⁷

5.1.3.1. Proteins used for safety assessment

Given the low levels of the proteins GAT4601 and *Glycine max*-HRA expressed in soybean 356043 and the difficult task to isolate a sufficient quantity of purified proteins from this soybean, proteins produced in recombinant *Escherichia coli* strains were used for the safety testing.

The equivalence of the GAT4601 protein produced in *E. coli* to that produced in leaf tissue of soybean 356043 was shown by SDS-PAGE, Western analysis, MALDI-MS analysis of tryptic peptides, N-terminal amino acid sequence analysis and glycosylation analysis. The identity of the microbial protein was further confirmed using electrospray mass spectroscopy, amino acid composition analysis and an enzyme activity assay.

In the case of the *Glycine max*-HRA protein the mature form (604 amino acids), which does not contain the chloroplast transit peptide that is cleaved from the protein during processing in the plant, was produced in *E. coli* in the form of a fusion protein. Due to the cleavage of this fusion protein with thrombin during the purification process, the resulting microbial *Glycine max*-HRA protein has an additional glycine residue at the N-terminus compared to the *Glycine max*-HRA protein expressed in soybean 356043. The equivalence of the *Glycine max*-HRA protein produced in *E. coli* to that produced in leaf tissue of soybean 356043 was shown by Western analysis, N-terminal amino acid sequence analysis, MALDI-MS analysis of tryptic peptides and glycosylation analysis. In addition, the identity of the microbial protein was corroborated using electrospray mass spectroscopy, analysis of the amino acid composition and determination of the enzyme activity.

The EFSA GMO Panel therefore accepts the test materials derived from *E. coli* as appropriate substitute test materials for the GAT4601 and *Glycine max*-HRA proteins present in soybean 356043 in the safety studies.

5.1.3.2. Toxicological assessment of expressed novel proteins in soybean 356043¹⁸

The *Glycine max*-HRA protein expressed in soybean 356043 is an acetolactate synthase (ALS) encoded by a modified *als* gene from soybean (*Glycine max*). ALS enzymes are key enzymes in the biosynthesis of the essential branched-chain amino acids, where they catalyse the first common step in the biosynthesis of isoleucine, leucine and valine starting from pyruvate (LaRossa and Falco, 1984; Duggleby and Pang, 2000). The enzyme catalyses two reactions, these being the conversion of two molecules of pyruvate into 2-acetolactate, used in the synthesis of leucine and valine, and the condensation of pyruvate with 2-ketobutyrate producing 2-acetohydroxybutyrate, used in the synthesis of isoleucine. ALS enzymes are widespread in nature, and occur e.g. in plants, algae, yeast and bacteria (Friden et al., 1985; Falco and Dumas, 1985; Mazur et al., 1987; Mazur and Falco, 1989; Reith and Mulholland, 1995). The *Glycine max*-HRA protein (656 amino acids) expressed in soybean 356043 is a modified version of the endogenous ALS precursor protein. Compared with the endogenous ALS precursor protein in soybean, it includes 5 additional amino acid residues at the N-terminus and two internal amino acid changes. The amino acid sequence of the mature form (after cleavage of the chloroplast transit peptide) differs from that of the mature endogenous soybean protein in two out of 604 amino acids (see section 3.2.1). These changes confer tolerance to ALS-inhibiting herbicides to the modified soybean.

The GAT4601 protein (146 amino acids, molecular mass ca. 17 kDa) is an optimised form of the enzyme glyphosate acetyltransferase (GAT) from *Bacillus licheniformis*, which acetylates glyphosate using acetyl-CoA as acetyl-donor. The coding sequence was obtained after seven rounds of DNA shuffling using three distinct alleles of the *gat* gene isolated from three different strains of *B. licheniformis* (see section 3.2.1.). The GAT4601 protein shows an 84% sequence homology at the

¹⁷ Technical dossier/ Section D7.8

¹⁸ Technical dossier/ Section 7.8.1/additional information April 2008 and September 2008

amino acid level to each of the three GAT enzymes from *B. licheniformis* from which it was derived and has an increased catalytic efficiency. The enzymatic acetylation of glyphosate produces N-acetylglyphosate and renders the plant tolerant to glyphosate herbicides. The GAT proteins are members of the GCN5-related family of N-acetyltransferases (GNAT family), consisting of more than 10 000 representatives from all kingdoms of life. Members of the GNAT family contain a highly conserved GNAT motif but show high sequence diversity in other parts of the protein and have diverse functions (Vetting et al., 2005; Dyda et al., 2000).

The substrate specificity of the GAT4601 protein was studied *in vitro* using 21 amino acids, 11 antibiotics and 20 different agrochemicals. In these studies GAT4601 acetylated aspartic acid and glutamic acid with relatively low efficiency compared with the acetylation of glyphosate. The affinity of the protein for serine, threonine and glycine was so low that a K_M value could not be estimated. The enzyme did not show detectable activity on the other tested substances.

(a) Acute toxicity testing¹⁹

The proteins GAT4601 and *Glycine max*-HRA produced in *E. coli* were tested separately for acute oral toxicity using mice and did not induce adverse effects after administration of single doses of 1596 and 582 mg/kg bw, respectively.

(b) Repeated-dose testing²⁰

The applicant provided a repeated-dose feeding study using the GAT4601 protein produced in *E. coli* as test material. Groups of 5 male and 5 female mice (CD-1) received the GAT4601 protein at dietary doses of 7.8, 76.7 or 783.1 mg/kg bw/day (males) and 9.2, 94.4 or 926.9 mg/kg bw/day (females) for 27 days. The diets were not adjusted for protein content, and the control group received a standard rodent diet without additional protein. Throughout the treatment period there was no mortality, and the regular observations of the animals revealed no clinically relevant findings that were considered related to the test material. There were no relevant differences in feed consumption between the groups and no statistically significant changes in mean body weights (except for one value at day 1 of the treatment period) as well as body weight gains compared with the control group. In ophthalmic examinations no abnormalities were noted. In haematology examinations no statistical significant differences compared with the control group were observed. Clinical-chemistry analyses showed statistically significantly lower levels of plasma total protein and albumin in females of the high-dose group compared with the control group. The differences were small and the mean values fell within the ranges of the historical controls. In the absence of differences in related parameters indicating liver or kidney toxicity these differences, which were not observed in male animals, are not considered toxicologically relevant. Males of the low and high-dose groups had lower plasma potassium levels. However, this effect was not dose-related and no differences in the plasma levels of other electrolytes were observed. Therefore the differences are considered as incidental. Determination of the weights of selected organs and tissues did not reveal statistically significant differences except for reduced mean absolute spleen weight as well as spleen weight in relation to brain weight but not in relation to body weight in females of the low- and high-dose groups. Furthermore microscopic examinations of organs and tissues, including the spleen, revealed no gross lesions and no relevant differences in microscopic findings between the groups. The EFSA GMO Panel concludes that there were no indications of adverse effects up to the highest dose tested.

On request of the EFSA GMO Panel the applicant provided the complete report on a repeated-dose feeding study using the *Glycine max*-HRA protein²¹, which was described in a scientific publication (Mathesius et al., 2009). Groups of 5 male and 5 female mice (CD-1) were fed diets containing the *Glycine max*-HRA protein produced in *E. coli* with the diet for 27 days. The actual doses administered

¹⁹ Additional information of March 2010

¹⁹ Additional information of September 2008 and March 2010

²¹ Additional information July 2010

were 107.4, 301.8 and 991.7 mg/kg bw/day for males and 123.2, 382.6 and 1247.1 mg/kg bw/day for females. One control group received a standard rodent diet, and another was fed a diet containing bovine serum albumin (BSA) at a dose of 1066.4 mg/kg bw/day (males) and 1337.0 mg/kg bw/day (females). The study was not carried out according to OECD guideline 407 since haematology data were not provided. No mortality occurred during the treatment period and general clinical condition, performance in functional observational tests and motor activity measurements were not affected by the treatment. Ophthalmoscopy results were unremarkable. Feed consumption and body weight development were comparable in all groups. Clinical-chemistry analyses showed no statistically significant differences in groups fed diets with the *Glycine max*-HRA protein compared with the control groups. Organ weight determinations showed statistically significantly lower adrenal weights (absolute and in relation to body weight) in male animals of the mid-dose group only, which was thus considered as an incidental finding. No difference in spleen weight was observed in female animals. Statistically significantly lower mean spleen weights (absolute and in relation to body weight) were observed in males of the medium and high dose groups. The absence of a dose effect relationship as well as the absence of relevant findings in the histopathological examinations of this organ and other tissues of the reticulo-endothelial system suggests that the difference in spleen weight in males is not a result of *Glycine max*-HRA treatment. Macroscopic and histopathological examinations at necropsy did not reveal relevant changes in the other organs and tissues examined. The available results do not indicate adverse effects.

(c) Degradation in simulated digestive fluids

The digestibility of the proteins GAT4601 and *Glycine max*-HRA produced in *E. coli* were studied *in vitro* using pepsin-containing simulated gastric fluid (SGF) and pancreatin-containing intestinal fluid (SIF).

After incubation of the protein *Glycine max*-HRA in SGF (pH 1.2) for 30 seconds no intact protein was detected using SDS-PAGE and protein staining. At this time point a faint band (ca. 3 kDa) was visible, which further decreased in intensity until the last time point in this study (60 minutes). After incubation of the protein *Glycine max*-HRA in SIF (pH 7.5) no intact protein was detectable after 30 seconds using SDS-PAGE and protein staining. Using Western analysis intact protein was still detectable after 30 seconds but not after 1 minute. Several bands, which probably represent degradation products of the *Glycine max*-HRA protein were detected after 30 seconds and 1 minute but not at later time points.

No intact protein was detected by SDS-PAGE and protein staining after incubation of the GAT4601 protein in SGF for 30 seconds. A faint band (ca. 3 kDa) was visible, which was still detectable after 60 minutes of incubation. After incubation in SIF for 30 seconds and 1 minute intact GAT4601 protein was still detectable by SDS-PAGE and protein staining. Using Western analysis the intact protein was detectable after incubation for up to 2 minutes, but at later time points, neither the intact protein nor fragments were detectable.

The *in vitro* digestion experiments demonstrated that the proteins *Glycine max*-HRA and GAT4601 are degraded by digestive enzymes.

(d) Bioinformatic studies²²

Bioinformatics-supported comparison of the amino acid sequence of the protein GAT4604 with the sequences stored in a general protein sequence database, revealed homology of the GAT4604 protein with other acetyltransferases but not with known toxic proteins.

²² Additional information April 2008

Analysis of the amino acid sequence of the *Glycine max*-HRA precursor protein showed homology to related acetolactate synthase proteins (ALS) and acetoxyacid synthase (AHAS) proteins as well as other functionally related proteins, but not with known toxic proteins.

5.1.3.3. Toxicological assessment of changed levels in natural constituents²³

The comparative compositional analysis has shown that in seeds from soybean 356043 the levels of the acetylated amino acids N-acetylaspartate (NAA) and N-acetylglutamate (NAG) as well as the levels of the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid are higher than in seeds derived from the conventional counterpart and from other commercial soybean varieties (see section 4.1.2).

5.1.3.4. Information on NAA and NAG

NAA and NAG are normal constituents in the mammalian metabolism. N-acetylation is a widespread process in metabolism and is mediated by a number of both specific and unspecific N-acetyltransferases.

NAA is synthesised from acetyl-CoA and L-aspartic acid by acetyl-CoA_L-aspartate N-acetyltransferase (E.C. 2.3.1.2) in neurons only. NAA is the second most abundant free amino acid in brain after glutamate (Tallan et al., 1956, Miyake et al., 1981, Alonso et al., 19991, Tsai and Coyle, 1995). After transport to oligodendrocytes NAA is split into aspartate and acetate by the enzyme aspartoacylase or aminoacylase-2 (E.C.3.5.1.15) which preferentially hydrolyses NAA. In contrast, all other acetylated amino acids are cleaved by aminoacylase-1 (EC 3.5.1.14). The functions of NAA in the central nervous system are still under investigation but include the provision of acetate for myelin lipid and for steroid synthesis, and a precursor role for the neuron specific dipeptide N-acetylaspartylglutamate (NAAG), the most concentrated neuropeptide in human brain which when split by NAAG peptidase delivers NAA. It was shown that intraperitoneally injected NAA does not reach the central nervous system (Berlinguet and Laliberté, 1965).

Low concentrations of NAA were also detected in other organs, e.g. the liver and kidneys. Ingested N-acetylated amino acids are presumably deacetylated by aminoacylase-1 the most abundant of the aminoacylases and expressed in all nucleated human cells, including the intestine and the kidneys, which means that under normal conditions acetylated amino acids are not absorbed in the gut or excreted in the urine to great extent. Deficiency of aminoacylase-1, an inborn error of metabolism, is characterised by considerable urinary excretion of several acetylated amino acids (Sass et al., 2006). Studies in mice using radiolabelled NAA and L-aspartic acid showed that after intraperitoneal injection both substances were metabolised at a similar rate (as determined by measurement of expired radioactive CO₂) indicating a rapid hydrolysis of the N-acetyl group.

NAG is intramitochondrially produced from L-glutamate by N-acetylglutamate synthase (NAGS) using acetyl-CoA. High concentrations of this enzyme are present in the liver and the epithelial cells of the small intestine (Uchiyama et al., 1981; Caldovic et al., 2002 a and b) and correspondingly, high concentrations of NAG were found in these tissues (Shigesada and Tatibana, 1971). NAG is an obligatory allosteric activator of mitochondrial carbamoyl phosphate synthase I (CPSI) (Hall et al., 1958; Caldovic and Tuchmann, 2003), the rate-limiting first step in the mammalian urea cycle. Intramitochondrially formed NAG is transported into the cytosol where it is cleaved by aminoacylase. Mitochondrial uptake of cytosolic NAG is not possible. Ingested NAG will be deacetylated like NAA by aminoacylase-1 and studies in rats, dogs and pigs with orally, enterally or parenterally administered N-acetylglutamine as a substitute for glutamine have shown that the nutritional value of N-acetylglutamine was comparable to that of L-glutamine (Neuhäuser-Berthold, et al., 1988; Gouttebel et al., 1992; Arnaud et al., 2004; Lopez-Pedrosa et al., 2007).

²³ Technical Dossier/Section 7.8.3

Toxicological information on NAA and NAG²⁴

On request of the EFSA GMO Panel the applicant provided toxicological information on NAA and NAG including the full reports of the available toxicological studies. A summary of these studies has been published by Harper et al. (2009), Delaney et al. (2008) and Karaman et al. (2009).

According to information from the scientific literature, injection of NAA into the brains of rats caused seizures, altered EEG recordings and abnormal behaviour (Akimitsu et al., 2000; Kitada et al., 2000). However, there are no reports of adverse effects after oral intake of NAA.

(a) Acute toxicity testing²⁵

In acute oral toxicity studies using male and female Sprague-Dawley rats there were no indications of adverse effects after administration of N-acetyl-L-aspartate and N-acetyl-L-glutamic acid at doses of 2000 mg/kg bw. When N-acetyl-L-aspartate was tested at a dose of 5000 mg/kg bw four of five female animals in the test group died and the surviving female as well as all male rats showed signs of toxicity including ataxia, abnormal gait, breathing noise or diarrhoea.

(b) Repeated-dose toxicity testing²⁶

Subacute (28-day) feeding study with NAA

N-acetyl-L-aspartic acid was administered in the diet to groups of 10 male and 10 female Sprague-Dawley rats for 28 days. The study was conducted under Good Laboratory Practice (GLP) compliance and in accordance with OECD guideline 407 apart from the selection of dose levels. During the first 14 days the animals received target doses of 10, 100 or 1000 mg/kg bw and during the remaining period the target doses were 100, 500 or 1000 mg/kg bw. The control group received a standard rodent diet. There were no deaths during the treatment period. Clinical signs as well as effects identified in ophthalmological examinations were not related to the test material. Although no statistically significant differences were observed at the end of the treatment period, males and females of the high-dose group showed a tendency of lower body weight gain as well as a slightly lower absolute feed intake and feed efficiency in relation to the control group. A functional observation battery (FOB) and motor activity evaluations did not reveal relevant differences between groups. Urine analyses showed differences in ketone concentrations for male rats, which can be considered as normal variation. Haematology and clinical-chemistry analyses showed several statistically significant differences in the high-dose group compared with the control group, i.e. a lower eosinophil count, lower levels of plasma creatinine and blood urea nitrogen and a higher plasma glucose level in males as well as a lower neutrophil count in females. These differences are not considered toxicologically relevant and most likely represent incidental findings. Other statistically significant differences, each observed in only one of the lower dose groups and in one sex, are also regarded as incidental. Organ weight determinations as well as macroscopic and microscopic examination of organs and tissues at necropsy did not reveal relevant differences in findings between the test and control groups. Therefore in this study no adverse effects were observed up to the highest dose administered. The no observed adverse effect level (NOAEL) in this study was the highest dose administered which corresponds to an actual average dose of 852.3 mg/kg bw for males and 890.1 mg/kg bw for females.

Subacute (28-day) feeding study with NAG²⁷

Applying a similar study design N-acetyl-L-glutamate was administered to Sprague-Dawley rats at target doses of 0, 100, 500 or 1000 mg/kg bw/day for 28 days. An additional group received L-glutamate at a target dose of 1000 mg/kg bw/day for comparison. All animals survived during the

²⁴ Additional information April 2008, March 2010 and April 2010

²⁵ Additional information March 2010

²⁶ Additional information March 2010

²⁷ Additional information March 2010

treatment period and there were no relevant differences between groups regarding body weight development, feed intake and feed efficiency. A functional observation battery (FOB) and motor activity evaluations as well as eye examinations did not reveal relevant findings. In haematology and clinical-chemistry examinations, the only statistically significant differences in relation to the control group were a higher calcium level in females of the mid-dose group, which is considered incidental, as well as higher white blood cell and absolute lymphocyte counts in male rats of the high-dose group. Similar changes in blood cell counts were also observed in male rats receiving L-glutamate. In the absence of any other relevant findings in the other examinations (urinalysis, organ weight determinations, macroscopic and microscopic examinations), these differences, which were not observed in females, are most likely not attributable to administration of NAA (and L-glutamate, respectively). The NOAEL in this study was the highest dose administered, which corresponds to an actual average dose of N-acetyl-L-glutamate of 914.2 mg/kg bw/day for males and 1006.6 mg/kg bw/day for females.

Subchronic (90-day) feeding study with NAA²⁸

The applicant also provided a subchronic (90 days) feeding study with N-acetyl-L-aspartic acid using Sprague-Dawley rats. Groups of 10 male and 10 female animals received diets containing N-acetyl-L-aspartic acid at target doses of 100, 250 and 500 mg/kg bw/day. The control group received a standard rodent diet. An additional group was administered L-aspartic acid at a target dose of 500 mg/kg bw/day. The study was conducted in accordance with OECD guideline 408 and under Good Laboratory Practice (GLP) compliance. All animals survived the treatment period. Clinical observations as well as ophthalmological examinations did not reveal relevant differences between the treatment groups and the control group. A functional observation battery (FOB) and motor activity evaluations also did not show relevant differences. Body weight, body weight gain, feed consumption and feed efficiency were comparable in all groups. Haematology examinations showed statistically significantly higher red blood cell counts in females of the high-dose group in relation to the control group. The difference was small and, in the absence of changes in related parameters, not considered toxicologically relevant. Other statistically significant differences observed at lower dose levels were unrelated to the dose and thus regarded as incidental findings (higher white blood cell counts and lymphocyte counts in males; prolonged activated partial thromboplastin time (APTT) in females). Clinical-chemistry examinations showed lower blood urea nitrogen levels in males of the high-dose group, which is not considered as an indication of toxicity. This also applies to a lower creatinine level in males of the mid-dose group. Urine analyses showed no relevant differences. In organ weight determinations carried out at necropsy, males of the mid-dose group showed a higher relative heart weight (in relation to brain), which was not dose-related and not observed in relation to bodyweight and therefore regarded as incidental. A similar conclusion can be drawn for differences in thymus weights observed in female animals (thymus weight in relation to bodyweight was higher in the mid-dose group, whereas thymus weight in relation to brain weight was a lower in the low-dose group). Females of all dose groups showed lower relative liver weight in relation to bodyweight, which was also not related to the dose level and, in the absence of other findings indicating liver toxicity, probably attributable to a relatively high value of the control group. Macroscopic examinations revealed numerous red areas of the thymus of a number of animals in all groups, in particular in female animals of the group administered L-aspartic acid, which was not further explained by the author of the study report but is not considered treatment-related. Microscopic examinations did not reveal relevant differences between groups except for an increased incidence and severity of hypertrophy of the mucus-secreting cells (acinar cells) in the submandibular salivary gland of male and female rats of the high-dose NAA group (but not in the group administered 500 mg L-aspartic acid /kg bw/day). The cells were enlarged with an increased amount of pale, basophilic cytoplasm but there was no evidence of injury or cytotoxicity, e.g. inflammation, degeneration, necrosis or hyperplasia. This effect was observed at a lower incidence and intensity also in the parotid salivary glands (high-dose males and females) and in the sublingual salivary gland (high-dose males). The

²⁸ Additional information April 2010, July 2010 and December 2010

effect was not observed at a target dose of 250 mg NAA/kg bw/day. The EFSA GMO Panel concludes that the no observed effect level (NOEL) in this study is the mid dose administered, corresponding to an actual average dose of 229.5 and 253.2 mg NAA/kg bw/day for male and female rats, respectively.

Considering the intake of NAA resulting from consumption of 200 g unprocessed 356043 soybeans per day, which can be considered as a conservative assumption for high consumers in the EU (see below the assessment of the EFSA DATEX Unit), the increase in NAA intake, when compared with consumption of 200 g conventional soybeans, would be 1.9 mg/kg bw/day (114 mg/day for a person with 60 kg bw). This is more than 100 fold lower than the NOEL in the 90-day rat feeding study.

Reproductive and developmental toxicity study with NAA²⁹

N-acetyl-L-aspartic acid was also tested in a two-generation reproduction toxicity study in rats, which was carried out according to OECD guideline 416 and under GLP compliance. The test material was administered in the diet at target doses of 100, 250 and 500 mg/kg bw/day. The control group received a standard rodent diet (carrier control group), and an additional group was administered L-aspartic acid at a target dose of 500 mg/kg bw/day (comparative control group). Groups of 25 male and 25 female Sprague-Dawley rats (P1 generation) continuously received the test substance in the diet starting 70 day before mating, through mating and continuing until sacrifice. F1 generation rats received the same diet concentrations from weaning until sacrifice or for at least 70 days before mating, through mating and continuing until sacrifice. F2 generation rats received the same diet concentrations from weaning until scheduled sacrifice. Several deaths occurring prior to scheduled sacrifice in the P1, F1 or F2 generation were considered incidental. Regular observations of P1, F1 and F2 animals did not reveal clinically relevant effects, and body weights, body weight changes, feed consumption and feed efficiency were comparable in all groups. Organ weight determinations, macroscopic and microscopic examinations at necropsy did not show relevant differences between groups except for hypertrophy of acinar cells of salivary glands in male and female rats in the F1 generation and male rats from the F2 generation of the high-dose NAA group. Neurohistopathological evaluation provided no evidence that NAA had any effects on brain development. Delivery or litter observations for the P1 or F1 generation females were not affected. There were also no signs of reproductive effects on the P1 or F1 generation males or females or effects on the viability and growth in the F1 or F2 generation offspring. Reproductive parameters evaluated in the F1 and F2 generation rats after weaning were not affected. The EFSA GMO Panel noted a decreased motor activity of male and female animals receiving NAA or L-aspartic acid in one specific subset of the F2 generation (examined on day 22 postpartum). This was not observed in the F1 generation and in older animals (examined on day 61 postpartum) of the F2 generations. Therefore the EFSA GMO Panel considers it unlikely that the observed difference in motor activity is attributable to NAA.

(c) Genotoxicity testing³⁰

Tests on induction of gene mutations in bacteria (Ames test) were conducted in accordance with OECD guideline 471. Using the plate incorporation method N-acetyl-L-aspartate and N-acetyl-L-glutamic acid did not induce gene mutations in *Salmonella enterica* var. Typhimurium strains TA98, TA100, TA1535 and TA1537 and *E. coli* strain WP2uvrA up to the highest tested concentration of 5000 µg/plate both in the absence and presence of tissue homogenate with metabolic activity (S9-mix).

N-acetyl-L-aspartate and N-acetyl-L-glutamic acid were also tested in the mouse bone marrow micronucleus test. Groups of 10 male and 10 female mice received by gavage N-acetyl-L-aspartate at doses of 500, 1000 or 2000 mg/kg bw as well as N-acetyl-L-glutamic acid at doses of 0 (vehicle control), 333, 1000 or 2000 mg/kg bw. In these tests, which were conducted in accordance with OECD

²⁹ Additional information, April 2010, July 2010 and July 2010

³⁰ Additional information April 2010

guideline 474, the test materials did not increase the frequency of micronucleated polychromatic erythrocytes up to the highest dose level tested.

On the basis of this information the EFSA GMO Panel concludes that there is no concern with regard to genotoxicity.

Intake information / Exposure assessment³¹

NAA and NAG are present in conventional foodstuffs and are thus normal constituents of the human diet. In the original application the applicant provided analytical data for a range of foodstuffs, which were selected because they have relatively high concentrations of aspartic acid and glutamic acid. In the study NAA and NAG were determined in yeast extract (ca. 12.6 and 159.8 mg/kg fresh weight (fw), respectively; average values from two samples), chicken bouillon (12.1 and 0.36 mg/kg fw, respectively), whole egg (1.38 and 0.05 mg/kg fw, respectively), ground beef (1.1 and 1.5 mg/kg fw, respectively), ground turkey (4.0 and 0.8 mg/kg fw, respectively) and other products. Additional studies were provided showing that NAA and NAG are also present in other foodstuffs including sardines, apples, oranges, spinach, rice, barley, wheat, walnuts, beer, coffee beans and brewed coffee, tea, cocoa powder and chocolate.

Furthermore, the normal intake of L-aspartic acid and L-glutamic acid resulting from consumption of food protein is 7.3 g aspartic acid plus asparagine and 8.5 g L-glutamic acid per day (Health Council of the Netherlands, 1999).

On request of the EFSA GMO Panel the applicant provided a dietary exposure assessment for NAA and NAG considering the substitution of conventional soybean by 356043 soybean. Separate studies were carried out using data provided by the US Dietary Exposure Evaluation Model – Food Commodity Intake Database (DEEM - FCID) and the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) Consumption Cluster Diets, respectively. The processed soybean products included in these calculations were defatted flour, protein isolate, soymilk and refined soybean oil. Considering the categorisation of soybean products in the DEEM-FCID and GEMS/Food databases, the applicant has applied specific factors in the assessment, which take into account the impact of processing on the levels of NAA and NAG in the above mentioned processed products.

Using DEEM-FCID, which was based on food consumption surveys conducted in 1994 and 1998, mean and 90th percentile intakes of NAA and NAG were calculated for the US population, including several sub-populations, at baseline (0 %), 45 % and 100 % replacement of materials derived from conventional soybean by materials from soybean 356043. The intake of NAA was estimated to increase from 9.4 µg/kg bw/day at baseline to 16.8 µg/kg bw/day (mean), and from 21.9 to 34.6 µg/kg bw/day (90th percentile) at a 100 % inclusion rate. Regarding a person with a bodyweight of 60 kg, this corresponds to an increase from ca. 570 to 1000 µg/person/day (mean) and from 1310 to 2080 µg/person/day (90th percentile). Thus, the estimated additional intake of NAA at a 100 % inclusion rate considering high consumption is 770 µg/day. For comparison, this amount would also be contained in ca. 290 g ground turkey or 75 g sardines. The intake of NAG was estimated to increase from 2.5 µg/kg bw/day at baseline to 2.7 µg/kg bw/day (mean), and from 5.8 to 6.2 µg/kg bw/day (90th percentile) at a 100 % inclusion rate. For a person with a bodyweight of 60 kg, the latter corresponds to an increase in intake from 348 to 372 µg/person/day. The estimated additional intake of NAG at a 100 % inclusion rate (90th percentile) is thus 24 µg/day, an amount which is also contained in ca. 115 g sardines or 2 g dark chocolate.

An intake estimate concerning infants was also provided. Considering all infants, the intake of NAA was estimated to be increased from 4.3 µg/kg bw/day at baseline to 7.0 µg/kg bw/day (mean), and

³¹Additional information April 2008

from 15.6 to 19.5 µg/kg bw/day (90th percentile) at a 100 % inclusion rate. At a 100 % inclusion rate the NAG intake would thus increase from 13.8 to 14.4 µg/kg bw/day (90th percentile).

The intake assessment based on GEMS/Food Consumption Cluster Diets made use of Food Balance Sheet data compiled by the Food and Agriculture Organisation of the United Nations (FAO). With regard to European countries, the applicant selected the country cluster with the highest predicted dietary exposure for the assessment. The intake of NAA was estimated to increase from 22.8 µg/kg bw/day at baseline to 393.0 µg/kg bw/day at a 100 % inclusion rate, corresponding to an increase from ca. 1.4 to 23.6 mg NAA/day for a 60 kg person. The intake of NAG would increase from 6.6 µg/kg bw/day at baseline to 13.0 µg/kg bw/day, corresponding to an increase from approximately 400 to 780 µg/kg day for a 60 kg person. For the US the intake of NAA was estimated to increase from 26.1 µg/kg bw/day at baseline to 1.2 mg/kg bw/day at a 100 % inclusion rate. The intake of NAG would increase from 8.0 µg/kg bw/day to 28.3 µg/kg bw/day. For an individual with a bodyweight of 60 kg this corresponds to an additional intake of NAA and NAG of ca. 70 and 1.7 mg/day, respectively. Compared with the results of the DEEM/FCID assessment for the US population, the estimated increases in the intakes of NAA and NAG obtained in the GEMS/Food assessment are considerably higher. This can be anticipated considering the differences in the databases and the methodology applied in the assessment.

With regard to the situation in EU countries, the DATEX Unit of EFSA has conducted an additional assessment for high consumers of soybeans assuming a daily consumption of 200 g of unprocessed soybeans (equivalent to 440 g of cooked soybean due to water absorption) for an individual with a bodyweight of 60 kg bw. Analysis of the EFSA Comprehensive Food Consumption Database (EFSA, 2011) has confirmed that 200 g/day is a conservative assumption for unprocessed soybeans. Under this assumption, the intake of NAA would increase from 0.008 mg/kg bw/day (assuming an NAA content in soybeans of 2.52 mg/kg) to 1.9 mg/kg bw/day (assuming an NAA content in soybeans of 580 mg/kg). The intake of NAG would increase from 0.005 mg/kg bw/day (assuming an NAG content in soybeans of 1.53 mg/kg) to 0.04 mg/kg bw/day (assuming an NAG content in soybeans of 11.6 mg/kg). Considering an individual with a bodyweight of 60 kg, the additional intake of NAA and NAG would thus be ca. 114 and 2.1 mg/day, respectively.

On the basis of data from the EFSA Comprehensive Food Consumption Database (EFSA, 2011) it can be anticipated that the daily intake of soybeans by toddlers (12 months - 3 years) and children (3 – 9 years) is lower than that of adults. On a bodyweight basis this may give rise to intake levels, which are lower or slightly higher than those of adults. Therefore the anticipated increases in intake levels of NAA and NAG on a bodyweight basis would be similar to those of adults.

Regarding the intake assessment for infants consuming infant formula, the relevant soybean-derived products to be considered are protein isolate and soybean oil. According to the information provided by the applicant, NAA and NAG were not detected or below the limit of quantification in protein isolate and degummed and refined/bleached/deodorised (RBD) soybean oil (see section 5.1.2). Therefore no increase in the intake of these constituents by infants due to consumption of infant formula containing protein isolate and RBD soybean oil derived from soybean 356043 is expected.

Regarding farm animals the EFSA GMO Panel has estimated the intake of NAA and NAG, which may result from the use of meal derived from soybean 356043 in animal feed. This assessment was based on the assumptions that the diets fed to ruminants and non-ruminants contain high amounts of soybean meal, (i.e. 20% and 15 % of dry mass for dairy cows and beef cattle respectively and 20-30, 20 and 30-40 % of dry mass for growing pigs, laying hens and broilers, respectively) and soy protein (i.e. 30% of soy protein is applied in milk replacer for suckling calves) exclusively derived from soybean 356043. Based on the maximum levels of NAA and NAG in soybean seed (according to the data provided in the application) and assuming that ca. 20 % of the total seed mass is removed by processing, the maximum levels of NAA and NAG in oil extracted meal would amount to approximately 1,300 mg/kg dry weight and 60 mg/kg dry weight, respectively. It was thus estimated that the intake of NAA would vary from 3.7 mg/kg bw/day for beef cattle to 160 mg/kg bw/day for

young broilers (age up to one week). In relation to the estimated intake of NAA, the intake of NAG would be negligible (below 1 mg/kg bw/day for each animal category).

Conclusion

NAA and NAG are normal constituents in the mammalian metabolism. They are also present in conventional foodstuffs and thus consumed as part of a normal diet. The available scientific information indicates that under normal conditions NAA and NAG, like other N-acetylated amino acids, are deacetylated in the intestine to form the corresponding L-amino acids, which are further metabolised in the body. Regarding the exposure assessment the Panel has considered all available data but focused on data from EU countries for soybean consumers. Considering the outcome of a conservative intake assessment (assuming an intake of 200 g unprocessed 356043 soybeans per day instead of unprocessed conventional soybeans), the estimated increase in intake of NAA (114 mg/day) is more than 100 fold lower than the NOEL in the 90-day rat feeding study. Furthermore, in relation to the normal intake of L-aspartic acid and L-glutamic acid resulting from consumption of food protein (see section 5.1.3.3/*Intake information*) the estimated increases in the intake of NAA and NAG are considered low. Considering all the available information, the EFSA GMO Panel is of the opinion that the estimated increases in intake levels of NAA and NAG resulting from replacement of food products derived from conventional soybeans by the respective products derived from soybean 356043 do not raise safety concerns. The same conclusion applies to the use of feed materials derived from this genetically modified soybean.

5.1.3.5. Information on heptadecanoic, heptadecenoic and heptadecadienoic acid

*Intake information / Exposure assessment*³²

The levels of the odd chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid in seeds from soybean 356043 soybean were found to be about two to three times higher than those in its conventional counterpart and outside the ranges for seeds from other commercial non-GM soybean varieties (see section 4.1.2). The contents of heptadecanoic acid, heptadecenoic acid and heptadecadienoic are less than 0.5 %, less than 0.3 %, and about 0.2 %, respectively, of total fatty acids in soybean 356043.

Heptadecanoic, heptadecenoic and heptadecadienoic acid are normal constituents of the human diet. According to the applicant, there are no published studies on the catabolism of heptadecanoic, heptadecenoic and heptadecadienoic acid in mammals. It can be anticipated, however, that these fatty acids are metabolised in a similar way as even chain fatty acids by β -oxidation generating acetyl-CoA, the entry molecule for the citric acid cycle. The terminal metabolite is expected to be propionyl-CoA (instead of acetyl-CoA), which can be converted to succinyl-CoA, an intermediate of the citric acid cycle. Heptadecanoic and heptadecenoic acid are also found in human tissues, namely heptadecanoic acid in skeletal muscle (Andersson et al., 2002) and subcutaneous adipose tissue (Baylin et al., 2002), and heptadecenoic acid in myocard tissue (Shenolikar, 1980). Both fatty acids are found in human breast milk and erythrocyte membrane lipids (Wendel, 1989). The information on heptadecadienoic acid is more limited.

According to the information provided in the application, the heptadecanoic acid content was 0.54 g/100 g tofu, 0.56 g/100 g butter, 0.29 g/100 g pork, 0.32-0.34 g/100 g beef and 0.3-1.16 g/100 g cooked lamb. The heptadecenoic acid content was 1.09 g/100 g tofu, 0.16-0.2 g/100 g beef, 0.15 g/100 g cheese and 0.13 g/100 g olive oil. Additional information based on a literature search, which was provided on request of the EFSA GMO Panel, showed that heptadecanoic and heptadecenoic acid are present in a wide variety of foodstuffs from plant and animal sources. The applicant has identified this fatty acid in various soy products, shortening, margarine, walnuts as well as several oils (walnut,

³² Additional information April 2008, December 2010

flaxseed, wheat germ, grapeseed and safflower oil). Heptadecadienoic acid was identified in pecan oil (Senter and Horvat, 1978), cuttlefish oil and beef tallow (Kurata et al., 2005).

On request of the EFSA GMO Panel the applicant provided an exposure assessment for the odd-chain fatty acids, which was based on the consumption of soybean oil, the major product for human consumption. In refined-bleached-deodorized (RBD) 356043 soybean oil the mean levels of heptadecanoic, heptadecenoic and heptadecadienoic acid were 0.343, 0.193, and 0.144 g/100 g, respectively. Using data from the FAOSTAT databases as well as annual production and trade data (1961-2005), the daily consumption of soybean oil was assessed on a per capita basis for 18 European countries, the EU and the USA. The EU average per capita daily soybean oil consumption was estimated to be 10.3 g/day, with the Netherlands showing the highest consumption level (36.1 g/day).

Regarding the potential increase in consumption of the odd-chain fatty acids through replacement of soybean oil with oil derived from soybean 356043, the applicant considered two scenarios compared with the baseline situation, i.e. 45 % and 100 % replacement. For the Netherlands the intake of heptadecanoic acid was estimated to rise from 40 mg/day at baseline to 78 mg/day at a 45 % inclusion rate, and 124 mg/day at a 100 % inclusion rate. For comparison, the additional amount consumed in the worst case situation (84 mg/day) would also be contained in ca. 22 g butter or 43 g pork. In the case of heptadecenoic acid the intake was estimated to increase from 10 mg/day to 37 mg/day at the 45 % inclusion rate, and 70 mg/day at the 100 % inclusion rate. The additional maximum amount consumed (60 mg) would also be contained in 40 g cheese or 33 g beef. Regarding heptadecadienoic acid the intake was estimated to rise from 10 mg/day to 29 mg/day at a 45 % inclusion rate, and 52 mg/day at a 100 % inclusion rate.

Conclusion

The odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid are normal constituents of plants and animals and have been identified in human tissues. There is no information in the scientific literature indicating that the intake of small amounts of these fatty acids via food or feed causes adverse effects. The EFSA GMO Panel is of the opinion that the estimated increases in intake levels of heptadecanoic, heptadecenoic and heptadecadienoic resulting from replacement of conventional soybean oil with oil from soybean 356043 do not raise safety concerns. The same conclusion applies to the use of feed materials derived from this genetically modified soybean.

5.1.3.6. Toxicological assessment of the whole GM food/feed³³

The applicant has provided the report on a subchronic (92 days) rat feeding study, which was also published in the scientific literature (Appenzeller et al., 2008). Groups of 12 male and 12 female rats (CrI:CD(SD)) were fed diets containing 20% (w/w) dehulled/defatted toasted meal and 1.5% (w/w) toasted ground hulls derived from soybean 356043 treated or not treated with glyphosate, chlorimuron and thifensulfuron (two test groups). The control group received diets formulated with processed meal and hulls from the conventional counterpart (Jack). Three additional groups were fed diets containing corresponding quantities of the respective feed materials derived from other commercial non-GM soybean varieties (reference groups). In the statistical analysis the data obtained for both test groups were compared separately with the data for the non-GM control group.

Throughout the treatment period there was no mortality except for one male animal in one of the reference groups, and no clinically relevant reactions were noted in the regular observations of the animals. Food consumption was comparable in all groups and there were no relevant differences in food efficiency and body weight development. In ophthalmic examinations as well as quantitative assessments of body functions and motor activity measurements, no statistically significant differences between the groups were detected. In haematology examinations female rats of the test group receiving materials derived from soybean 356043 treated with the target herbicides showed

³³ Technical Dossier/D 7.8.4 and additional information September 2008

statistically significantly higher mean MCV (mean corpuscular volume) and MCH (mean corpuscular haemoglobin) values compared with the control group. In the absence of differences in other red blood cell parameters these relatively small differences in MCV and MCH values are not considered toxicologically relevant. In addition, no differences were observed in male and female animals fed meal and hulls derived from soybean 356043 not treated with the target herbicides. Clinical-chemistry and urine analyses did not reveal significant differences except for a higher mean BUN (blood urea nitrogen) value for male rats of the test group receiving materials derived from soybean 356043 treated with the target herbicides. Since there were no changes in other parameters related to kidney function this difference, which was attributable to one animal in the group, can be considered as an incidental finding. Furthermore, no differences were noted for male and female rats fed materials from soybean 356043 not treated with the target herbicides. Determination of the weights of selected organs and tissues did not reveal statistically significant differences. In macroscopic and microscopic examinations no differences between the test groups and the control group were detected, which are related to administration of the test materials. The EFSA GMO Panel concludes that there are no indications of adverse effects in this subchronic feeding study.

5.1.4. Allergenicity³⁴

The strategies used when assessing the potential allergenic risk focus on the characterisation of the source of the recombinant protein(s), the potential of the newly expressed protein(s) to induce sensitisation or to elicit allergic reactions in already sensitised persons and whether the transformation may have altered the allergenic properties of the modified food. A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (CAC, 2003; EFSA, 2011)

5.1.4.1. Assessment of allergenicity of the newly expressed proteins

The gene encoding the *Glycine max*-HRA protein originates from soybean meaning that the source is a common allergenic food. This issue is addressed when assessing the allergenicity of the whole plant since the recipient of the genetic modification is also soybean. The amino acid sequence of the mature form of this protein differs from that of the endogenous acetolactate synthase (ALS) protein in two out of 604 amino acids.

Bacillus licheniformis, the source of the gene encoding the GAT4601 protein, is a common soil bacterium which is not known to cause allergy.

Bioinformatics-supported comparisons of the amino acid sequence of the *Glycine max*-HRA precursor protein and the GAT4601 protein with the sequences of known allergens were performed. These analyses included both an overall search for sequence alignments using the FASTA algorithm and a search for short identical stretches of at least eight contiguous amino acids. No similarity applying a criterion of 35% identity over a window of 80 amino acids was identified and no identical stretches of at least eight contiguous amino acids were detected.

The studies on *in vitro* digestibility of the proteins (see section 5.1.3.2.) showed that most of the proteins were degraded.

Based on this information the EFSA GMO Panel considers that the proteins *Glycine max*-HRA and GAT4601 present in soybean 356043 are unlikely to be allergenic in the intended conditions of use of 356043 soybean.

³⁴ Technical Dossier/ Section D7.9 and Additional information April 2008 and October 2009

5.1.4.2. Assessment of allergenicity of the whole GM plant³⁵

Allergenicity of the whole crop could be increased as an unintended effect of the random insertion of the transgene in the genome of the recipient, for example through qualitative or quantitative modifications of the pattern of expression of endogenous proteins.

Because the soybean is a recognised allergenic food, the applicant has performed *in vitro* allergenicity studies with extracts of seeds from soybean 356043 and its conventional counterpart (Jack). On the basis of one-dimensional (1-D) IgE immunoblot analysis and ELISA inhibition tests using pooled sera from 5 patients reactive to soybean (both children and adults) and pooled sera from non-atopic individuals as a negative control (number of individuals not given), the applicant concluded that the extracts of seeds from soybean 356043 and its conventional counterpart Jack had very similar protein/allergen profiles and inhibition patterns.

On request of the EFSA GMO Panel the applicant provided additional information. A 2-D immunoblot analysis and a quantitative ELISA analysis for soybean specific IgE were conducted using individual sera from 8 subjects with clinically confirmed allergy to soybeans, 5 negative control sera and one positive control serum. Besides extracts from soybeans 356043 and its conventional counterpart (Jack) extracts from 8 commercial non-GM soybean varieties were analysed in this study. In the 2-D immunoblot analysis no meaningful qualitative and quantitative differences in the IgE binding patterns were detected between extracts of soybean 356043 and its non-GM comparator. In the quantitative ELISA analysis extracts from soybeans 356043, its conventional counterpart and the 8 commercial non-GM soybean varieties had similar IgE binding capacity when tested using the sera from all allergic patients.

Based on the information provided, the EFSA GMO Panel concludes that the overall allergenicity of the whole GM soybean 356043 is unlikely to be different from that of its conventional counterpart and commercial soybean varieties.

5.1.5. Nutritional assessment of GM food/feed³⁶

A 42-day feeding study using broiler chickens (Ross x Cobb broilers) was performed according to the ILSI (2003) recommendations. The full report of this study was provided and the results were also published by McNaughton et al. (2007). Groups consisting of 60 male and 60 female animals (12 pens with 5 male and 12 pens with 5 female animals per group (initial body weight ca. 51 g/chick) were fed with diets containing meal from soybean 356043 not treated with herbicides or treated with glyphosate, chlorimuron and thifensulfuron (two test groups). The inclusion rate of soybean meal in the starter, grower and finisher diets was approximately 30%, 26% and 21.5%, respectively. Hulls and oil derived from soybean 356043 (not treated or treated with the target herbicides) were added to all diets at 1% and 0.5%, respectively. The control group received diets formulated with meal and hulls from the conventional counterpart (Jack). Three additional groups were fed diets containing the respective feed materials derived from commercial soybean varieties (reference groups). The diets were adjusted for their contents in protein, specific amino acids and minerals according to NRC (1994). Birds were provided feed and water *ad libitum*. Animal performance on the various diets was evaluated by measuring mortality, weight gain (overall final weight ca. 1910 g/animal), feed consumption, feed conversion ratio (FCR ca. 1.87 g/g bw), organ (kidney, liver) and carcass (breast, thigh, leg, wing, abdominal fat) yields.

There were no statistically significant differences in mortality, weight gain, feed conversion ratio (corrected for mortalities) and carcass yields between the two test groups and the control group, and overall survival was >98%. The only statistically significant difference, a higher mean liver weight only in males fed meal from soybean 356043 treated with the target herbicides, was not considered biologically relevant since the difference was small, not observed in the group fed meal from untreated

³⁵ Additional information October 2009

³⁶ Technical Dossier D.7.10.1 and D7.10.2

soybean 356043, and the values were within the ranges determined for three additional groups fed meal from other commercial soybean varieties. Thus, the broiler feeding study shows that diets formulated with meal, hulls and oil derived from soybean 356043 are as nutritious as diets formulated with the respective materials derived from the conventional counterpart and non-GM references soybean varieties included in the study.

5.1.6. Post-market monitoring of GM food/feed³⁷

An evaluation of the risk assessment concluded that no data have emerged to indicate that soybean 356043 is any less safe and nutritious than its conventional counterpart and commercial soybean varieties. Therefore, and in line with the EFSA GMO Panel guidance document (EFSA, 2011), the Panel is of the opinion that post-market monitoring of the food/feed derived from soybean 356043 is not necessary.

5.2. Conclusion

No toxicity of the GAT4601 and the *Glycine max*-HRA proteins was observed in acute oral toxicity studies and repeated-dose (28 days) feeding studies using mice. The studies on *in vitro* digestibility of the proteins showed that most of the proteins were degraded. In bioinformatics studies the proteins showed no homology to known toxic proteins and allergens.

The odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid are normal constituents of plants and animals and have also been identified in human tissues. There is no information indicating that the intake of small amounts of these fatty acids via food or feed causes adverse effects. The EFSA GMO Panel is of the opinion that the estimated increases in intake levels of heptadecanoic, heptadecenoic and heptadecadienoic resulting from replacement of conventional soybean oil with oil from soybean 356043 do not raise safety concerns.

NAA and NAG are normal constituents in the mammalian metabolism. They are also present in conventional foodstuffs and thus consumed as part of a normal diet. The available scientific information indicates that under normal conditions NAA and NAG, like other N-acetylated amino acids, are deacetylated in the intestine to form the corresponding L-amino acids, which are further metabolised in the body. Regarding the exposure assessment the Panel has considered all available data but focused on data from EU countries for soybean consumers. Considering the outcome of a conservative intake assessment, the estimated increase in intake of NAA is more than 100 fold lower than the NOEL in the 90-day rat feeding study with NAA. Furthermore, in relation to the normal intake of L-aspartic acid and L-glutamic acid resulting from consumption of food protein, the estimated increases in the intake of NAA and NAG are considered low. Considering all the available information, the EFSA GMO Panel is of the opinion that the estimated increases in intake levels of NAA and NAG resulting from replacement of food products derived from conventional soybeans by the respective products derived from soybean 356043 do not raise safety concerns. The same conclusion applies to the use of feed materials derived from this genetically modified soybean.

Furthermore, a subchronic 92-day feeding study in rats using diets including meal and hulls derived from soybean 356043 provided no indications of adverse effects. Testing of extracts from soybeans 356043 with sera from patients allergic to soybean showed that the overall allergenicity of the whole plant had not been changed. A 42-day feeding study using broiler chickens demonstrated that soybean 356043 is nutritionally equivalent to its conventional counterpart and commercial non-GM soybean varieties included in this study. Therefore, the EFSA GMO Panel is of the opinion that soybean 356043 is as safe as its conventional counterpart with respect to potential effects on human and animal health in the context of its intended uses.

³⁷ Technical Dossier D.7.11

6. Environmental risk assessment and monitoring plan

6.1. Environmental risk assessment

The scope of application EFSA-GMO-UK-2007-43 is for food and feed uses, import and processing and does not include cultivation. Considering the intended uses of soybean 356043, the environmental risk assessment is concerned with the exposure through manure and faeces from animals feeding seed produced by soybean 356043 and with the accidental release into the environment of viable seeds of soybean (e.g. during transportation and processing).

As the scope of the present application excludes cultivation, environmental concerns related to the use of glyphosate and ALS-inhibiting herbicides on soybean 356043 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 (herbicides) falls within the scope of Directive 91/414/EEC concerning the placing of plant protection products on the market.

6.1.1. Unintended effects on plant fitness due to the genetic modification³⁸

Cultivated soybean (*Glycine max* (L.) Merr.) is a species belonging to the subgenus *Soja* of the genus *Glycine*. The species originated from eastern Asia and is a highly domesticated crop (Lu, 2005). The major worldwide soybean producers are the United States (US), Brazil, Argentina, China, North Korea and South Korea. In European Union (EU), soybean is mainly cultivated in Austria, Italy, France, Hungary and Romania (Dorokhov et al., 2004). (EUROSTAT³⁹). 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 North and South America during the years 2005 (6 locations) and 2005-2006 (6 locations). These field trials did not show changes in plant characteristics that indicate altered fitness and invasiveness of GM soybean 356043 compared to its conventional counterpart, except in the presence of glyphosate and ALS-inhibiting herbicides. 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 soybean 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 and ALS-inhibiting herbicides tolerance traits introduced by the genetic modification result in increased invasiveness of any crop species, except when glyphosate and ALS-inhibiting herbicides are applied. Thus, the accidental release of GM soybean 356043 seeds would not result in 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 fitter in the presence of glyphosate and ALS-inhibiting herbicides.

Survival of soybean plants outside cultivation 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 356043, it can be considered that soybean 356043 has no altered survival, multiplication or dissemination characteristics. Therefore, the EFSA GMO Panel is of the opinion that the likelihood of unintended environment effects of the soybean 356043 in Europe will not be different to that of conventional soybean varieties.

³⁸ Technical dossier / section D9.1

³⁹ <http://epp.eurostat.ec.europa.eu/portal/page/portal/agriculture/data/database>

6.1.2. Potential for gene transfer⁴⁰

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 356043 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 micro-organisms) is not expected to occur at detectable frequencies under natural conditions (see EFSA, 2009 for more details).

A successful horizontal transfer would require stable insertion of the transgene sequences into a bacterial genome enabling it to multiply 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 exposure of bacterial communities to the recombinant genes in soybean 356043 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 inserted DNA includes the glyphosate detoxifying glyphosate acetyltransferase (*gat*) gene which originates from the soil bacterium *Bacillus licheniformis*. It has been subjected to an extensive previous gene shuffling process (Castle et al., 2004) for structural optimization. Sequence similarities between the recombinant gene and its natural counterparts in bacteria may however still be sufficient to increase the likelihood of HR. However, such a hypothesised horizontal gene transfer event would only replace an existing gene. Theoretically, a recombined *gat* gene may cause altered enzyme activities towards glyphosate and other amino acids of a recipient bacterium. However, it is unlikely that this enzyme would increase the fitness of the recipient in context of its natural habitat.

Soybean 356043 also contains the recombinant *Glycine max*-HRA gene encoding for an acetolactate synthase (ALS). This gene, however, is a sequence modified version of the *als* gene of *Glycine max* which decreases the likelihood for homologous recombination with bacterial genes. The unlikely case of a successful transfer of the *Glycine max*-HRA gene from soybean 356043 to gut or other environmental bacteria would not confer a new trait, because genes encoding for acetolactate synthase (ALS) are expected to be widespread in bacteria and fungi that produce the amino acids leucine, isoleucine and valine.

In addition to homology-based recombination processes, illegitimate recombination that does not require the presence of DNA similarity between the recombining DNA molecules is theoretically possible. However, the transformation rates for illegitimate recombination were considered to be 10¹⁰-fold lower than for homologous recombination (Hülter, 2008, EFSA, 2009). Illegitimate recombination events have not been detected in studies that have exposed bacteria to high

⁴⁰ Technical dossier / section D9.2

concentrations of GM plant DNA (EFSA, 2009). In the extreme unlikely event of such a horizontal gene transfer, expression of the recombinant genes in bacteria would be limited by the plant-specific promoters of soybean 356043.

In the context of its intended uses as food and feed, there is no direct exposure of bacteria to the herbicidal compound glyphosate and ALS-inhibiting herbicides. The selective advantage of glyphosate and ALS-inhibiting herbicides resistance in bacteria is therefore predicted to be limited. The hypothetical rare acquisition of the genes encoding for GAT4601 as well as of the *Glycine max*-HRA from soybean 356043 is therefore not considered to confer an advantage that would allow bacteria to enhance their viability or to alter their habitat range.

The EFSA GMO Panel concludes that the recombinant DNA in soybean 356043 does not represent an environmental risk in relation to its potential for horizontal transfer to bacteria in the context of its intended uses.

(b) Plant to plant gene transfer

Considering the intended uses of soybean 356043 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 mainly 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 the subgenus *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, 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 and the occasional soybean plant 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 356043 seeds (while most soybean 356043 seeds will be processed in countries of production), processing outside of importing ports, transportation in regions of soybean production in Europe, spillage of GM seeds mainly during transportation, germination and development of spilled seeds within soybean fields or in very close vicinity of cultivated soybean fields, overlap of flowering periods and environmental conditions favouring cross-pollination. The likelihood of all these conditions occurring and thereby resulting in cross-pollination between GM soybean plants and cultivated soybean is therefore extremely low. Apart from seed production areas, GM plants and plants 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 (e.g.; 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 do they 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 this application does 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 seed 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.

In conclusion, since soybean 356043 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 356043 in Europe will not differ from that of conventional soybean varieties.

6.1.3. Interactions of the GM plant with target organisms

Due to the type of trait (glyphosate and ALS-inhibiting herbicides with no target organisms) and the intended uses of soybean 356043, which exclude cultivation, this was not considered an issue by the EFSA GMO Panel.

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

Due to the intended uses of soybean 356043, 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.

6.1.5. Interactions with the abiotic environment and biogeochemical cycles

Due to the intended uses of soybean 356043, which exclude cultivation and due to the low level of exposure to the environment, potential interactions of the GM plant with the abiotic environment and biogeochemical cycles were not considered an issue by the EFSA GMO Panel.

6.1.6. Monitoring⁴¹

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 content of the monitoring plan provided by the applicant (EFSA, 2006b). The potential exposure to the environment of soybean 356043 would be through manure and faeces from animals fed with GM soybean or through accidental release into the environment of GM soybean seeds (e.g.; 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).

⁴¹ Technical dossier : section D11

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 plan proposed by the applicant includes: (1) the description of an approach involving operators (federations involved in soybean import and processing) reporting to the applicant 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.

The EFSA GMO Panel is of the opinion that the scope of the monitoring plan proposed by the applicant is in line with the intended uses of soybean 356043 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.

6.2. Conclusion

The scope of application EFSA-GMO-UK-2007-43 is for food and feed uses, import and processing of soybean 356043 and excludes cultivation. Considering the intended uses, the environmental risk assessment is concerned with indirect exposure mainly through manure and faeces from animals fed seeds produced by soybean 356043 and with the accidental release into the environment of viable seeds of soybean 356043 (e.g. during transportation and processing).

If case of accidental release into the environment of viable seeds of soybean 356043 (e.g. during transport and processing), there are no indications of an increased likelihood of establishment and spread of feral soybean 356043 plants, except in the presence of glyphosate and ALS-inhibiting 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. In the context of its intended uses, the theoretically possible transfer of the recombinant genes from soybean 356043 to gut or other environmental bacteria has not been identified to be a risk due to the lack of any selective advantage. 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 356043.

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 and the scientific opinion of the EFSA GMO Panel on post-market environmental monitoring (EFSA, 2006a,b).

The EFSA GMO Panel also recommends that appropriate management systems should be in place to restrict seeds of soybean 356043 entering cultivation as this would require specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2000.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was requested to carry out a scientific risk assessment of the soybean 356043 for food and feed uses, import and processing.

Appropriate molecular and bioinformatic analyses of the 356043 soybean insert and its flanking genomic regions have been undertaken. The expression of the genes introduced has been sufficiently analysed and the stability of the genetic modification has been demonstrated over several generations.

The molecular characterisation provided for the transformation event 356043 soybean is sufficient for the safety assessment. The GMO panel considers this to be an adequate analysis and the molecular characterisation does not indicate a safety concern.

No differences were identified between 356043 soybean and its conventional counterpart, except for the newly expressed proteins, for higher levels of the acetylated amino acids N-acetylaspartate (NAA) and N-acetylglutamate (NAG), and the odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid in seed from 356043 soybean. The levels of these acetylated amino acids and odd chain fatty acids fall outside the natural ranges observed for other commercial non-GM soybean varieties. The overall level of NAA and NAG (taken together) in soybean 356043 was found to be less than 0.15 % of the total amino acids. The total level of odd chain fatty acids amounts to less than 1% of total fatty acids. No statistically significant differences in total amino acid contents in seed were observed between the 356043 soybean and its conventional counterpart. Levels of major fatty acids in 356043 soybean seed were found to be comparable to those observed in the conventional counterpart.

No toxicity of the GAT4601 and the *Glycine max*-HRA proteins was observed in acute oral toxicity studies and repeated-dose (28 days) feeding studies using mice. The Panel is of the opinion that acute toxicity testing of the newly expressed proteins is of little additional value for the risk assessment of the repeated human and animal consumption of food and feed derived from GM plants. The studies on *in vitro* digestibility of the proteins showed that most of the proteins were degraded. In bioinformatics studies the proteins showed no homology to known toxic proteins and allergens.

The odd-chain fatty acids heptadecanoic, heptadecenoic and heptadecadienoic acid are normal constituents of plants and animals and have also been identified in human tissues. There is no information indicating that the intake of small amounts of these fatty acids via food or feed causes adverse effects. The EFSA GMO Panel is of the opinion that the estimated increases in intake levels of heptadecanoic, heptadecenoic and heptadecadienoic resulting from replacement of conventional soybean oil with oil from soybean 356043 do not raise safety concerns.

NAA and NAG are normal constituents in the mammalian metabolism. They are also present in conventional foodstuffs and thus consumed as part of a normal diet. The available scientific information indicates that under normal conditions NAA and NAG, like other N-acetylated amino acids, are deacetylated in the intestine to form the corresponding L-amino acids, which are further metabolised in the body. Regarding the exposure assessment the Panel has considered all available data but focused on data from EU countries for soybean consumers. Considering the outcome of a conservative intake assessment, the estimated increase in intake of NAA is more than 100 fold lower than the NOEL in the 90-day rat feeding study with NAA. Furthermore, in relation to the normal intake of L-aspartic acid and L-glutamic acid resulting from consumption of food protein, the estimated increases in the intake of NAA and NAG are considered low. Considering all the available information, the EFSA GMO Panel is of the opinion that the estimated increases in intake levels of NAA and NAG resulting from replacement of food products derived from conventional soybeans by the respective products derived from soybean 356043 do not raise safety concerns. The same conclusion applies to the use of feed materials derived from this genetically modified soybean.

Furthermore, a subchronic 92 day feeding study in rats using diets including meal and hulls derived from soybean 356043 provided no indications of adverse effects. Testing of extracts from soybeans 356043 with sera from patients allergic to soybean showed that the overall allergenicity of the whole plant had not been changed. A 42-day feeding study using broiler chickens demonstrated that soybean 356043 is nutritionally equivalent to its conventional counterpart and commercial non-GM soybean varieties included in this study. Therefore, the EFSA GMO Panel is of the opinion that soybean 356043 is as safe as its conventional counterpart with respect to potential effects on human and animal health in the context of its intended uses.

Considering the intended uses of soybean 356043, which exclude cultivation, there is no requirement for scientific assessment on possible environmental effects associated with the cultivation of this GM

soybean. In case of accidental release into the environment of viable seeds of soybean 356043 (e.g.; 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 glyphosate and ALS-inhibiting 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. In the context of its intended uses, the theoretically possible transfer of the recombinant genes from soybean 356043 to gut or other environmental bacteria has not been identified to be a risk due to the lack of any selective advantage. 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 356043. 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.

In conclusion, the EFSA GMO Panel considers that information available for soybean 356043 addresses the outstanding questions raised by the Member States and that the soybean 356043, as described in this application, 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.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the MS, dated 11 April 2007, concerning a request for placing on the market of 356043 in accordance with Regulation (EC) No 1829/2003.
2. Acknowledgement letter, dated 17 April 2007(Ref. SR/KL/shv(2007)2084365), from EFSA to the Competent Authority of the MS.
3. Letter from EFSA to applicant, dated 06 August 2007 (Ref.SR/KL/AC/shv(2007)2299681), requesting additional information under completeness check
4. Letter from applicant to EFSA, dated 11 September 2007, providing additional information under completeness check.
5. Letter from EFSA to applicant, dated 28 September 2007, delivering the 'Statement of Validity' for application EFSA-GMO-UK-2007-43 (Ref.SR/KL/shv(2007)2402780), Soybean 356043 submitted by Pioneer under Regulation (EC) No 1829/2003.
6. Letter from EFSA to applicant, dated 20 December 2007 (Ref.SR/KL/shv(2007)2589484), requesting additional information and stopping the clock.
7. Letter from applicant to EFSA, dated 12 February 2008, providing additional information
8. Letter from EFSA to applicant, dated 27 February 2008 (Ref.SR/KL/shv(2008)2720431), requesting additional information and maintaining the clock stopped.
9. Letter from applicant to EFSA, dated 15 April 2008, providing additional information.
10. Letter from EFSA to applicant, dated 22 July 2008 (Ref. PB/KL/md(2008)3185806), requesting additional information and maintaining the clock stopped.
11. Letter from applicant to EFSA, dated 8 September 2008, providing additional information.
12. Letter from EFSA to applicant, dated 8 September 2008 (Ref.PB/ZD/shv(2008)3279118), requesting additional information and maintaining the clock stopped.
13. Letter from applicant to EFSA, dated 15 October 2008, providing additional information

14. Letter from applicant to EFSA, dated 6 November 2008, providing the timeline for submission of response.
15. Letter from applicant to EFSA, dated 27 April 2009, providing the timeline for submission of response.
16. Letter from applicant to EFSA, dated 6 October 2009, providing additional information
17. Letter from EFSA to applicant, dated 8 January 2010 (Ref.PB/KL/AC/lg(2009)4547393), requesting additional information and maintaining the clock stopped.
18. Letter from EFSA to applicant, dated 14 January 2010 (Ref. PB/KL/AC/lg(2010)4567551), requesting additional information and maintaining the clock stopped
19. Letter from applicant to EFSA, dated 19 February 2010, providing the timeline for submission of response.
20. Letter from applicant to EFSA, dated 5 March 2010, providing additional information
21. Letter from EFSA to applicant, dated 28 May 2010 (Ref.PB/KL/AC/mt(2010)4888857), requesting additional information and maintaining the clock stopped
22. Letter from applicant to EFSA, dated 12 July 2010, providing additional information
23. Letter from EFSA to applicant, dated 20 October 2010 (Ref.PB/KL/AC/mt(2010)5257028), requesting additional information and maintaining the clock stopped
24. Letter from applicant to EFSA, dated 7 December 2010, providing additional information
25. Letter from EFSA to applicant, dated 3 February 2011 (Ref. PB/KL/AC/mt(2011)5513291), re-starting the clock
26. Letter from applicant to EFSA, dated 11 March 2011, submitting spontaneously information
27. Letter from EFSA to applicant, dated 18 March 2011 (Ref. PB/KL/EvH/shv(2011)5631533), confirming receipt of information

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Appendix III



Soy products

By Dagrunn Engeset and Inger Therese Lillegaard

There are different soy-products on the market: milk replacement products (milk, sour cream, yoghurt, and cheeses), meat replacement products (soy granules to mix in water to make "minced meat", and ready made products like sausages, burgers, nuggets, and schnitzels), desserts (vanilla and chocolate puddings, ice creams, cheese cakes), soy flour, soy flakes, soy beans, soy fat/oils, and –sauce. There are also soy proteins in several diet bars and diet products, and in a few canned meat products. Many chocolates and biscuits contain soy lecithin.

In this project two different menus have been created; one full day week menu for a person with milk allergy and one full day week menu for a vegan (see below). We wanted to examine how much soy protein a person can get, realistically, by replacing meat and milk products with soy-products.

Reason for the choice of menus

The milk allergy menu

Milk allergy or intolerance is relatively common diseases. Persons with such diseases will have to look for alternatives to milk and milk products, and soy products will be a natural choice for many of them. There are other milk replacement products on the market, but in this scenario we envision a person who prefers soy over other products. This menu is also relevant for persons who for various reasons do not want to use milk products and therefore replaces them with soy products.

The vegan menu

A vegan does not eat any products of animal origin; meat, fish, milk, and egg. In this scenario we envision a vegan who has previously eaten normal food and wish to replace meat products with meat replacement products like soy sausages and-burgers in addition to replacing milk products. In both menus all milk products are replaced with soy products: soy milk substitute milk for drinking, milk in waffles, milk in porridge and on breakfast cereals, in smoothies, and in cheese sauces.

Coffee milk is substituted with soy cream in coffee or tea. Cheeses are replaced by different soy cheeses and/or tofu on bread, and in dishes like lasagne and pizza. Tofu is also used in cheese cake, smoothies, and in salads.

Soy yoghurt, ice cream, cream, and sour cream replace ordinary yoghurt, ice cream, cream, and sour cream. In the vegan menu meat products are replaced by meat substitutes of soy and of tofu in wraps and in lasagne.

The menus are made with an estimated energy requirement of 10MJ/day. We assume that in pure soy products (e.g. soy milk) all the protein come from soy. In mixed products the amount of soy protein is estimated based on how much soy was stated in the table of content printed on the food label.

7 days vegan menu, high preference for soy products

(Envision a person who has previously eaten meat and is looking for meat substitutes like soy burgers and sausages)

Monday:

Breakfast: Cereals with nuts and soy milk, orange juice, coffee/tea with soy cream
Lunch: course bread with soy cheese, cucumber and tomato, bell pepper, peanut butter, soy milk, coffee/tea with soy cream
Snack: banana, walnuts
Dinner: soy burger, burger bread, tomato, lettuce, pickles, raw onion, soy cheese, soy chocolate dessert, water
Supper: mixed salad with tofu, vinaigrette dressing and pita bread, tea

Tuesday:

Breakfast: cereals with nuts and soy milk, orange juice, coffee with soy cream (like Monday)
Lunch: tofu wrap (tortilla with tofu + vegetables), soy milk, coffee with soy cream
Snack: apple, soy ice cream
Dinner: Steamed vegetables with cheese sauce (made of soy milk and soy cheese), water, soy yoghurt with nuts and raisins
Supper: oat porridge with raisins and soy milk

Wednesday:

Breakfast: Soy smoothie (tofu, soy milk, banana, strawberries)
Lunch: tofu wrap, soy milk, coffee (like Tuesday)
Snack: soy yoghurt
Dinner: Soy sausages, mixed salad with tofu, rice, water, vanilla soy dessert
Supper: course bread with peanut butter, soy cheese and vegetables, soy milk and coffee (like lunch Monday)

Thursday:

Breakfast: cereals with nuts and soy milk, orange juice, coffee with soy milk
Lunch: bread lunch like Monday
Snack: Soy smoothie (like breakfast Wednesday)
Dinner: Vegetable soup, course rye bread with milk free margarine, water
Supper: bread with peanut butter, soy cheese, bell pepper, coffee with soy cream, orange juice

Friday:

Breakfast: bread breakfast (like Thursday supper)
Lunch: mixed salad with tofu (like Monday supper)
Snack: Soy waffle with jam and soy sour cream (waffles of soy milk, peanut butter, soy oil, buck wheat, corn starch, corn flour), soy chocolate milk (hot) with whipped cream (soy whipping spray cream)
Dinner: Spinach and tofu lasagne (lasagne plates, spinach, tofu, soy milk, soy cheese, tomato sauce) with mixed salad and white bread, wine and water
Supper: fruit salad

Saturday:

Breakfast: Soy smoothie (as previous)
Lunch: Soy waffle (like Friday snack)
Snack: Milk chocolate without milk, cashew nuts, raspberries

Dinner: Vegetarian bean casserole, pita bread, wine, water, soy chocolate dessert
Supper: Vegan pizza (marguerita with soy cheese), beer, potato chips

Sunday:

Breakfast: soy sausages, chapatti, onion, pickles, tomato juice, tea

Lunch: tofu wrap (like lunch Tuesday)

Snack: fruit salad

Dinner: Vegan meatballs (chickpeas, tofu, water, rolled oats, wheat flour) in tomato sauce, spaghetti, mixed salad, soda, soy chocolate dessert

Supper: vegan cheesecake with raspberries (cheese cream topping: soy cream cheese, tofu, sugar, lemon), coffee

7 day menu, milk allergy - replaces milk products with soy products.

Monday:

Breakfast: Oat porridge (like vegan)

Lunch: Bread with salami and soy cheese, tomato/cucumber/bell pepper, orange juice, coffee

Snack: Banana, walnuts

Dinner: Sausages without milk, mashed potatoes with soy milk, mixed salad, water

Supper: Coarse bread, boiled egg, pickled herring, milk free margarine, mayonnaise, soy milk

Tuesday:

Breakfast: Bread breakfast (like Monday lunch)

Lunch: Bread lunch (like Monday supper)

Snack: Smoothie (like vegan)

Dinner: Vegetable soup (like vegan Thursday)

Supper: omelette with bread, soy milk, tea

Wednesday:

Breakfast: Weetabix with soy milk

Lunch: Bread lunch (like Monday supper)

Snack: Banana and nuts

Dinner: Meat balls, mushy peas, potatoes, carrots, sauce, lingonberry jam, water

Supper: Oat porridge (like vegan)

Thursday:

Breakfast: Smoothie (soy milk, strawberries, banana, apple juice)

Lunch: Bread lunch (like Monday supper)

Snack: Soy yoghurt with nuts, grapes

Dinner: Fish gratin made with soy milk, carrots, bacon, water, soy chocolate dessert

Supper: oat porridge (like vegan)

Friday:

Breakfast: Corn flakes with soy milk, coffee, orange juice

Lunch: Tomato soup with macaroni (without milk), white bread, water

Snack: Milk chocolate without milk, cashew nuts, raspberries

Dinner: Lasagne (cheese sauce of soy milk and soy cheese), mixed salad, pita bread, wine, water, soy ice cream

Supper: Pizza with soy cheese, beer, potato chips

Saturday:

Breakfast: Egg and bacon, bread, orange juice, coffee

Lunch: Mixed salad with chicken and tofu, pita bread, water

Snack: Smoothie (like Thursday breakfast)

Dinner: Rice porridge made with soy milk, mutton ham, lemonade

Supper: Taco with soy sour cream and soy cheese, beer

Sunday:

Breakfast: Omelette with soy cheese, bread, cucumber/bell pepper, orange juice, tea

Lunch: waffle with soy milk (ordinary waffle with egg where soy milk replaces milk), jam, soy sour cream, coffee with soy cream and sugar

Snack: Milk free milk chocolate, nuts, fruit

Dinner: Salmon with potato, soy sour cream, cucumber, carrots, water, fruit salad

Supper: Vegan cheesecake with raspberries, coffee
