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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

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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)

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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.

Acknowledgment

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has appointed the Panel on Genetically Modified Organisms (GMO) to answer the request from the Norwegian Food Safety Authority and the Norwegian Environment Agency. Project leaders from the VKM secretariat have been Anne Marie Bakke, Nana Asare, Ville Erling Sipinen and Merethe Aasmo Finne.

Dagrunn Engeset (VKM staff) and Inger Therese Lillegaard (VKM staff) are acknowledged for their valuable contribution to this scientific opinion [Chapter 4].

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

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 sulfonylureas. 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 IdentifierDP-356Ø43-5) 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 naturforvalting [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-356Ø43-5) 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). Helserisikovurderingen ble utført på oppdrag av Mattilsynet i forbindelse med EFSAs 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 EFSAs 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 EFSAs 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 sulfonylureaer 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-acetylerte 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-acetylerte 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

Miljørisikovurderingen 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 fôret 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 ferale 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-acetylerte 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
Bt	*Bacillus thuringiensis*
bw	Body weight
Cp4 epsps	The *5-enolpyruvylshikimate-3-phosphate synthase* gene from *Agrobacterium tumefaciens* 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	*Glycine max* –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-356Ø43-5) 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-356Ø43-5) 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-356Ø43-5 (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 actylated 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.

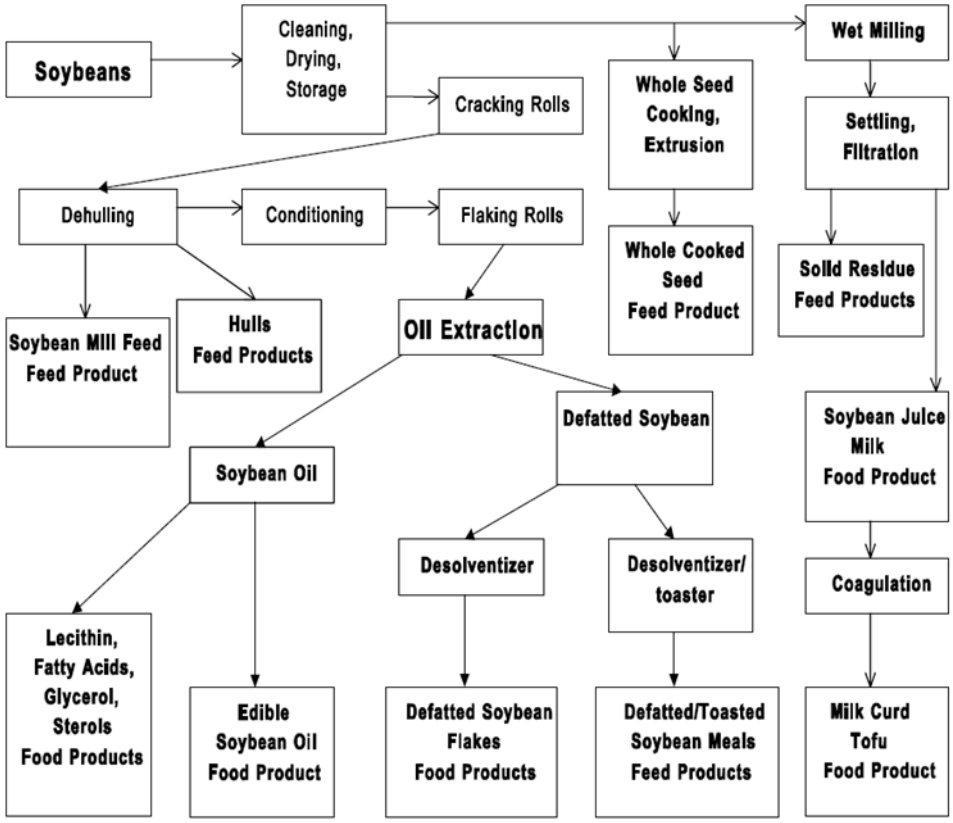

Figure 4.2-1. Processing of soybean, adapted from (OECD, 2012; Waggle and Kolar, 1979).

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 Crl: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 Crl: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-356Ø43-5 (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* nopalin synthase terminator (tNOS), *ctp2-cp4epsps*, the *bar* gene from *Streptomyces hygroscopicus* 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-356Ø43-5 (356043; *gat4601* and *gm-hra* genes), and maize grain from event DP-Ø9814Ø-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 FB_1, FB_2, FB_3 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.

9 References

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Appendix I

Appendix II

Appendix III