VKM Report 2015: 14

Final health and environmental risk assessment of genetically modified soybean MON 87701

Scientific opinion on insect resistant, genetically modified soybean MON 87701 from Monsanto for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (Application EFSA/GMO/BE/2010/79)

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Scientific opinion on insect resistant, genetically modified soybean MON 87701 from Monsanto for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (Application EFSA/GMO/BE/2010/79)

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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

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.

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Abstract

Soybean MON 87701 expresses the *cry1Ac* gene from *Bacillus thuringiensis*. The encoded Cry1Ac protein confers resistance against specific lepidopteran pests. Updated bioinformatics analyses of the inserted DNA and flanking sequences in soybean MON 87701 have not indicated a potential production of harmful toxins and allergens or polypeptides caused by the genetic modification. Genomic stability of the functional insert and consistent expression of the *cry1Ac* gene, have been shown over several generations of soybean MON 87701. Data from several field trials performed in USA, Canada, Chile and Argentina during 2005-2006 show that soybean MON 87701 is compositionally, morphologically and agronomically equivalent to its conventional counterpart and other commercial soybean cultivars. Subchronic feeding studies with rats as well as nutritional assessment with broilers have not revealed relevant adverse effects of MON 87701. These studies indicate that MON 87701 is nutritionally equivalent to and as safe as conventional soybean cultivars. The Cry1Ac protein produced in soybean MON 87701 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 MON 87701 with the Cry1Ac protein:
- Is compositionally, morphologically and agronomically equivalent to its conventional counterpart and other commercial soybean cultivars
- Is unlikely to introduce a toxic or allergenic potential 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 genetically modified soybean MON 87701 (Unique Identifier MON-877Ø1-2) from Monsanto conferred resistance to specific lepidopteran insects is approved under Regulation (EC) No 1829/2003 for food and feed uses, import and processing since 10 February 2012 (Application EFSA/GMO/BE/2010/79, Commission Implementing Decision 2012/83/EU).

Soybean MON 87701 has previously been assessed for use as food and feed by the VKM GMO Panel (VKM, 2010a), as commissioned by the NFSA in connection with EFSA's public hearing of the application EFSA/GMO/BE/2010/79 in 2010. MON 87701 has also been evaluated by the VKM GMO Panel as a component of the stacked GM events MON 87701 × MON 89788 (EFSA/GMO/NL/2009/73) (VKM, 2010b).

The current food, feed and environmental risk assessment of soybean MON 87701 is based on information provided by the applicant in the application EFSA/GMO/BE/2010/79, relevant peer-reviewed scientific literature, and scientific opinions and comments from EFSA (EFSA, 2011d), VKM (VKM, 2010a) 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 MON 87701 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, 2011e), 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, 2011f).

The scientific risk assessment of soybean MON 87701 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.

Soybean MON 87701 expresses the *cry1Ac* gene which encodes for the Cry1Ac insecticidal crystal protein; δ-endotoxin derived from the bacterium *Bacillus thuringiensis*. MON 87701 was developed via *Agrobacterium*-mediated transformation of meristem tissues of conventional soybean A5547. This renders soybean MON 87701 protection against feeding damage caused by targeted lepidopteran pests including velvetbean caterpillar (*Anticarcia gemmatalis*), soybean looper (*Pseudoplusia includens*), soybean anxil borer (*Epinotia aporema*) and sunflower looper (*Rachiplusia nu*).

Molecular characterisation

The soybean MON 87701 contains a DNA fragment with one functional copy of the *cry1ac* gene integrated in the soybean MON 87701 genome. No other functional vector genes were found. Southern blot, ELISA and real-time PCR analyses revealed that the introduced gene is stably inherited and expressed over multiple generations. Bioinformatics comparison of the amino acid sequence of the newly expressed Cry1Ac protein did not reveal similarities to known allergenic or toxic proteins.

The VKM GMO Panel concludes that the molecular characterisation of soybean MON 87701 does not indicate a safety concern.

Comparative assessments

Field studies were carried out to assess the composition of forage, seed and processed fractions (meal, oil, protein isolate and lecithin), as well as agronomic and morphological characteristics of the GM soybean MON 87701 compared to the non-transgenic variety A5547 (control) and other conventional soybean cultivars. Few biologically significant differences were observed in the compositional data or in agronomic and morphological characteristics. With the exception of significantly higher vitamin E levels in MON 87701 compared to its comparator A5547, most of the 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 vitamin E levels were within the range of levels observed in the conventional soybean cultivars included in the trials. Thus the field studies investigating composition of soybean MON 87701 show no biologically relevant differences compared to conventional soybean cultivars.

Based on current knowledge and excluding the novel trait, the VKM GMO Panel concludes that soybean 87701 is compositionally, agronomically and morphologically equivalent to its conventional counterpart and other conventional soybean cultivars.

Food and feed risk assessment

Subchronic, toxicity studies with rats, a nutritional whole food study with broilers, and allergenicity assessment studies have been performed with soybean MON 87701. These studies have not revealed adverse effects or indicated any differences in the performance of animals fed soybean MON 87701 compared to conventional soybeans. Bioinformatics analysis revealed that the amino acid sequence of the Cry1Ac protein in soybean MON 87701 shares no sequence homology with 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 MON 87701 is nutritionally equivalent to and as safe as its conventional counterpart and other conventional soybean cultivars. It is unlikely that the Cry1Ac protein will introduce a toxic or allergenic potential in food or feed based on MON 87701 compared to conventional soybean cultivars.

Environmental assessment

Considering the intended uses of soybean MON 87701, 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 MON 87701.

With the exception of its insecticidal properties, soybean MON 87701 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 MON 87701. 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 MON 87701 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 MON 87701 with the Cry1Ac protein:
- Is compositionally, morphologically and agronomically equivalent to its conventional counterpart and other commercial soybean cultivars
- Is unlikely to introduce a toxic or allergenic potential 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*), MON 87701, EFSA/GMO/BE/2010/79, insecticidal properties, *Cry1Ac*, 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, insektresistente soyalinjen MON 87701 (unik kode MON-877Ø1-2) fra Monsanto ble godkjent til import, videreforedling og til bruk som mat og fôr under EU-forordning 1829/2003 10. februar 2012 (Kommisjonsbeslutning 2012/83/EU).

Soyalinjen MON 87701 ble første gang vurdert av VKMs faggruppe for GMO i 2010 (VKM, 2010a). Helserisikovurderingen ble utført på oppdrag av Mattilsynet i forbindelse med EFSAs offentlige høring av søknad EFSA/GMO/BE/2010/79 i november 2010. MON 87701 ble også vurdert av VKMs faggruppe for GMO som en komponent i krysningen MON 87701 × MON 89788 (EFSA/GMO/NL/2009/73) (VKM, 2010b).

Risikovurderingen av den genmodifiserte soyalinjen er basert på søkers dokumentasjon og uavhengige vitenskapelige publikasjoner, samt vitenskapelige vurderinger og kommentarer fra EFSA (EFSA, 2011d), VKM (VKM, 2010a) 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 (2010a) og EFSA (2011d) 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, 2011e; EFSA, 2011f) lagt til grunn for vurderingen.

Den vitenskapelige vurderingen omfatter transformeringsprosess og vektorkonstruksjon, karakterisering og nedarving av genkonstruksjonen, komparativ analyse av ernæringsmessig kvalitet, mineraler, kritiske toksiner, antinæringsstoffer, allergener og nye proteiner. Videre er agronomiske egenskaper, potensiale for utilsiktede effekter på fitness, genoverføring, og effekter på 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 MON 87701 uttrykker *cry1Ac*-genet fra *Bacillus thuringiensis* koder for Cry1Ac proteinet; δ-endotoxin. De transgene plantene blir derfor motstandsdyktig mot skadedyr (lepidopteran) som velvetbean caterpillar (*Anticarcia gemmatalis*), soybean looper (*Pseudoplusia includens*), soybean anxil borer (*Epinotia aporema*) and sunflower looper (*Rachiplusia nu*).

Molekylær karakterisering

MON 87701 har kun et funksjonelt *cry1Ac* gen 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 MON 87701. Southern blot, ELISA og real-time PCR viser at det introduserte genet er stabilt nedarvet og uttrykt over flere generasjoner, og i samsvar med de fenotypiske egenskapene til soya MON 87701.

VKMs faggruppe for GMO konkluderer med at den molekylære karakteriseringen ikke indikerer noen helserisiko ved soya MON 87701.

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 MON 87701, dens konvensjonelle motpart og andre konvensjonelle soyasorter ble sammenlignet. Tilgjengelig data viser at det med unntak av signifikant høyere vitamin E nivåer i MON 87701 samt noen andre små tilfeldige variasjoner i enkeltparametere, ikke foreligger biologisk relevante forskjeller mellom den genmodifiserte soyaen og konvensjonelle soyasorter.

Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO at soya MON 87701 er vesentlig lik dens konvensjonelle motpart, samt andre konvensjonelle sorter i forhold til næringsstoffsammensetning, og agronomiske og morfologiske egenskaper.

Helserisiko

Subkroniske toksikologistudier med rotter, ernæringsstudie med broilere og allergenisitetsstudier har blitt utført med soya MON 87701. Disse studiene har ikke vist negative effekter eller indikert forskjeller i ytelse hos dyr fôret med soya MON 87701 sammenlignet med konvensjonell soya. Med hjelp av bioinformatiske sammenligninger viser aminosyresekvensen av Cry1Ac proteinet 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 MON 87701 er ernæringsmessig lik og like trygg som dens konvensjonelle motpart og andre konvensjonelle sorter. Det er usannsynlig at Cry1Ac proteinet vil føre til toksiske eller allergiske reaksjoner fra mat og for som inneholder MON 87701 sammenlignet med konvensjonelle soyatyper.

Miljørisiko

Miljørisikovurderingen av soyalinje MON 87701 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 MON 87701 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 MON 87701 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 MON 87701 med Cry1Ac proteinet:
- Er 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

ADF	Acid detergent fibre; measure of fibre used for animal feed analysis. ADF measures most of the most indigestible structural components in plant cells (i.e. lignin, cellulose, silica and insoluble forms of nitrogen), but not hemicellulose.
Bt	*Bacillus thuringiensis*
bw	Body weight
Cp4epsps	The *5-enolpyruvylshikimate-3-phosphate synthase* gene from *Agrobacterium tumefaciens* strain CP4
CTP	Chloroplast transit peptide
DNA	Deoxyribonucleic acid
EC	European Commission
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	Environmental risk assessment
EU	European Union
FAO	Food and Agriculture Organisation
Fitness	Describes an individual's ability to reproduce successfully relative to that of other members of its population.
germplasm	Reproductive or vegetative propagating material of plants
GM	Genetically Modified
GMO	Genetically Modified Organism
GMP	Genetically Modified Plant
ILSI	International Life Sciences Institute
mRNA	Messenger RNA
MT/NFSA	Norwegian Food Safety Authority (Mattilsynet)

NDF	Neutral detergent fibre, measure of fibre used for animal feed analysis. NDF measures most of the structural components in plant cells (i.e. lignin, hemicellulose, cellulose, silica, tannins and cutins), but not pectin.
NTO	Non-target organism
OECD	Organisation for Economic Co-operation and Development
PCR	Polymerase chain reaction, a technique to amplify DNA by copying
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis. Technique to separate proteins according to their approximate size
Southern blot	Method used for transfer of electrophoresis-separated DNA fragments to a filter membrane and possible subsequent fragment detection by probe hybridisation
SPC	Soy protein concentrate
Western blot	Technique used to transfer proteins separated by gel electrophoresis by 3-D structure or denatured proteins by the length of the polypeptide to a membrane, where they might be identified by antibody labelling.

Background

On 17 May 2010, the European Food Safety Authority (EFSA) received from the Competent Authority of Belgium an application (Reference EFSA/GMO/BE/2010/79) for authorisation of the genetically modified insect resistant soybean MON 87701 (Unique Identifier MON-877Ø1-2), submitted by Monsanto 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/BE/2010/79 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 11 June 2010 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 November 2010 (VKM, 2010a). EFSA published its scientific opinion 26 July 2011 (EFSA, 2011d), and soybean MON 87701 was approved for food and feed uses, import and processing 10 February 2012 (Commission Implementing Decision 2012/83/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, 2011e), 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

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 current food, feed and environmental risk assessment of the genetically modified soybean MON 87701 is based on information provided by the applicant in the application EFSA/GMO/BE/2010/79, relevant peer-reviewed scientific literature, and scientific opinions from VKM (VKM, 2010a), EFSA (EFSA, 2011d) 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 MON 87701 is as safe as its conventional counterpart with respect to potential effects on human and animal health.

Genetically modified soybean MON 87701 (Unique Identifier MON-877Ø1-2) was developed to provide protection from specific lepidopteran insects via introduction of the modified *cry1Ac* gene sequence. Thus soybean MON 87701 produces the insecticidal, crystalline protein Cry1Ac. The DNA fragment containing the gene sequences for the trait was introduced into the meristem tissues of conventional soybean A5547 by *Agrobacterium tumefaciens* (renamed *Rhizobium radiobacter*) strain mediated transformation.

Cry1Ac is a δ-endotoxin produced by *Bacillus thuringiensis* during sporulation. When ingested by target insects, the endotoxin is activated by proteolytic cleavage, binds to the intestinal epithelium and forms pores in the cell membranes. This leads to cell lysis and eventually to death of the insect.

In soybean MON 87701, the introduced *cry1Ac* gene sequence is a codon-modified form of the coding sequence from *Bacillus thuringiensis* subsp. *kurstaki*. The introduced trait protects the plants from feeding damage caused by the lepidopteran insect species velvetbean caterpillar (*Anticarcia gemmatalis*), soybean looper (*Pseudoplusia includens*), soybean anxil borer (*Epinotia aporema*) and sunflower looper (*Rachiplusia nu*).

The genetic modification in soybean MON 87701 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 MON 87701 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, 2011e), 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, 2011f).

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, 2010a; Appendix I) and EFSA (EFSA, 2011d; Appendix II) assessed the molecular characterisation of the event MON-877Ø1-2 (MON 87701) 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

Initially, meristem tissue was transformed with two independent constructs: T-DNA I containing the *cry1* Ac gene and T-DNA II containing the *cp4epsps* gene, the latter used as a marker. The two T-DNAs were inserted at two independent loci within the plant genome. Following self-pollination, plants expressing *cry1Ac* only were selected for further development as MON 87701. MON 87701 therefore does not contain a functional *cp4epsps* marker gene.

Both the VKM and EFSA 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 MON 87701 genome. The data showed that a DNA fragment containing one functional copy of the *cry1Ac* gene only is present in the soybean MON 87701 genome. No other functional vector genes were detected. Bioinformatics analysis did not reveal disruptions of known endogenous soybean genes by the insertion of the *Cry1Ac* cassette that would raise a safety concern. Similarity searches with databases of known toxins and allergens did not indicate a potential for production of harmful proteins or polypeptides as a result of the genetic modification. Southern blot, ELISA and real-time PCR show that the introduced gene element was stably inherited and expressed over multiple generations in parallel with the observed phenotypic characteristics of soybean MON 87701. More recent literature concerning the molecular characterization of MON 87701 has not been identified.

2.1 Conclusions

Based on the above considerations, the VKM GMO panel concludes that the molecular characterisation of soybean MON 87701 does not indicate a safety concern.

3 Comparative assessments

Previously, the GMO panels of VKM (VKM, 2010a) and EFSA (EFSA, 2011d) assessed compositional and agronomic data provided by the applicant from various field trials conducted in North and South America in 2007-2008. 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 MON 87701 were compared to the non-transgenic variety A5547 (control), which is the conventional soybean variety that was originally modified to establish the MON 87701 event, in replicated field trials conducted in 2007 and 2007/2008 in USA and Argentina, respectively (Technical dossier; (Berman et al., 2009). In both, the trials were performed in five different sites and included MON 87701, its control counterpart A5547, and four other conventional soybean cultivars at each site. All were treated with pesticides according to conventional practice. A randomized block design was implemented with three replicates at each site. More recently, a peer-reviewed publication reports data from two field trials conducted in Brazil during the growing season 2007/2008 (Berman et al., 2010). Data obtained were compared to ranges for agronomic and compositional characteristics obtained from at least one of the four other commercial non-GM soybean cultivars planted at each site.

The above-mentioned field trials were conducted before more recent EFSA guidelines existed (EFSA, 2011e).

3.2 Compositional analysis

Monsanto Co. investigated the compositional equivalence of forage, seed and processed fractions (meal, oil, protein isolate and lecithin) of soybean MON 87701 to those of conventional soybean (Berman et al., 2009). To establish a range of natural variability for individual compositional components; seed, forage and processed fractions from conventional soybean cultivars on market were included in the study. Samples were analysed as follows:

1. Forage – Proximates (ash, fat, moisture and protein), carbohydrates and fatty acids
2. Seed – Proximates, carbohydrates, fibre, amino acids, fatty acids, antinutrients and vitamin E.
3. Meal (toasted and defatted) – Proximates, fibre, amino acids, and antinutrients
4. Oil (refined, bleached and deodorized) - Fatty acids and vitamin E
5. Protein isolate – Amino acids and moisture
6. Crude lecithin – Phosphatides

Although no significant differences were observed in forage parameters between MON 87701 and the conventional soybean control from any sites, some significant differences were observed in seed, meal, oil, protein isolate and crude lecithin. For example, carbohydrate by calculation, protein and nine amino acid levels, the fatty acid behenic acid (22:0), vitamin E, trypsin inhibitor, and daidzein levels in seed were significantly different between MON 87701 and its conventional counterpart at one or more sites from the US field trial. In Argentina, some statistically significant differences were observed for tryptophan, linolenic acid (18:3),

vitamin E and stachyose. With the exception of vitamin E, which was on average 19 and 23% higher in MON 87701 than its conventional counterpart at four of five sites in the US and all five sites in Argentina, respectively, most differences were small. However, with the exception of slightly lower carbohydrate levels observed at one site in the US, all values were within the range reported for the conventional cultivars included in the studies, as well as those reported in the ILSI crop composition database. Thus the differences were not considered biologically relevant from a food/feed safety or nutritional assessment view.

In addition, the composition of forage and seed from second-generation soybean MON 87701 was assessed in comparison to those of conventional soybean in multiple-replicated sites in two geographically and climatically distinct regions in Brazil during the 2007-2008 growing season (Berman et al., 2010). Fiber (ADF/NDF) and proximates were determined in forage and seed, while amino acids, fatty acids, antinutrients (lectin, phytic acid, raffinose, stachyose, isoflavones and trypsin inhibitor), and vitamin E were assessed in seed only. Based on hierarchical and principal component analyses performed on the compositional data, the authors conclude that natural variation due to location and/or germplasm contributes more to variation than the genetic modification. Notably, fatty acids and isoflavones were particularly variable.

VKM (VKM, 2010a) and EFSA (EFSA 2011b) concluded that soybean MON 87701 was compositionally equivalent to those of conventional soybean.

3.3 Agronomic traits and GM phenotype

Based on data collected from 16 field trials sites in the US in 2007 and eight field trial sites in Argentina during the 2007/2008 growing seasons, EFSA (EFSA, 2011d) and VKM (VKM, 2010a) concluded that agronomic traits and morphological parameters observed for soybean MON 87701, generally fell within the ranges observed for conventional cultivars. Any statistically significant differences observed (specifically for early stand count) were not considered to have biological relevance.

3.4 Conclusion

The VKM GMO Panel has considered the available data on compositional, agronomic and morphological characteristics and confirms that no biologically relevant differences were identified between soybean MON 87701, its corresponding counterpart and other conventional soybean cultivars. The few statistically significant differences observed 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 of the analytes.

Based on current knowledge and excluding the novel trait, the VKM GMO Panel concludes that soybean MON 87701 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, 2010a) and EFSA (EFSA, 2011d) evaluated food and feed safety assessments of soybean MON 87701 based on existing information, which was based on a 14-day acute toxicity study conducted with mice, a 42-day nutritional study with broilers, and two 90-day sub-chronic toxicity studies with rats. No observed adverse effect was reported. The VKM panel commented that it is recommended that NOAEL is set based on a 90-day repeated toxicity study, while acute toxicity studies are primarily for the determination of LD50. The safety assessments of soybean MON 87701 did not identify concerns regarding potential toxicity and allergenicity. To the best of our knowledge, no recent additional information or publication with regards to this section exists, thus the following assessment is a summary of previous evaluations, as well as information regarding product description and intended uses, which was not part of the previous VKM report (VKM, 2010a).

4.2 Product description and intended uses

Product description and intended uses were not considered in the previous VKM assessment (VKM, 2010a), but were considered in EFSA's evaluation (EFSA, 2011d) of soybean MON 87701. Therefore a summary, including considerations specific for Norwegian soybean use, are included below.

The genetic modification in soybean MON 87701 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 cultivars. 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.
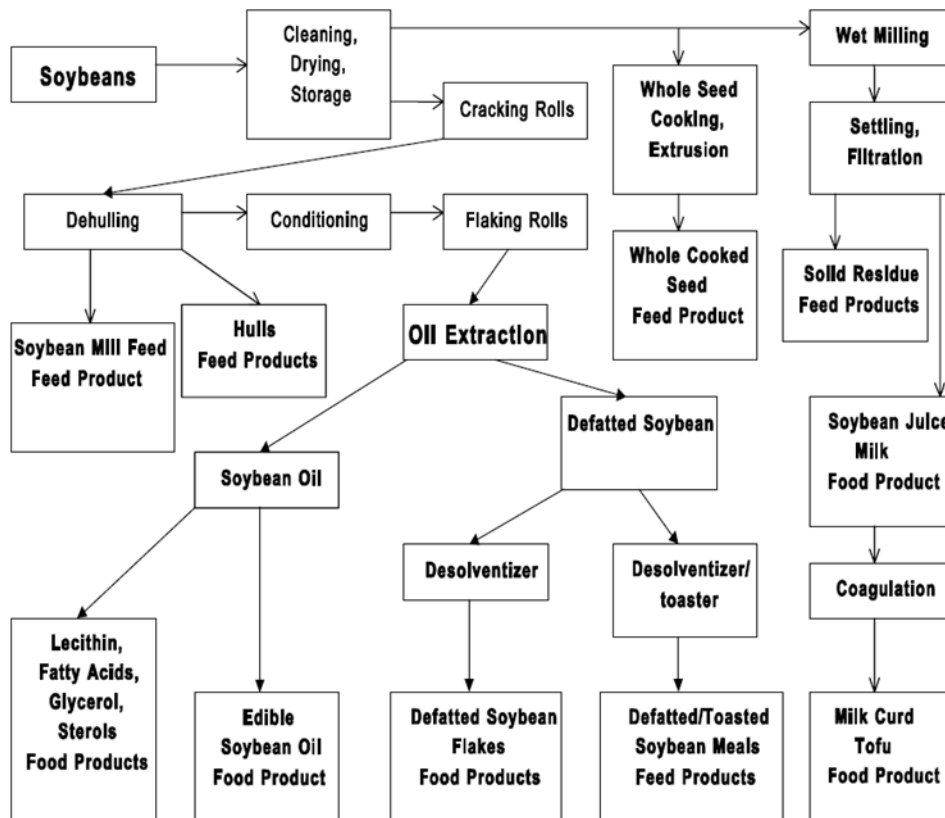
Figure 4.2-1. Processing of soybean, adapted from (OECD, 2012; Waggle and Kolar, 1979).

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 MON 87701 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 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.

According to EFSA (EFSA, 2011d), the applicant supplied data on the influence of temperature during processing of soybean MON 87701, concluding that 190°C for 15.5 min

reduced the quantity of immunodetectable Cry1Ac protein to levels below the detection limit and corresponding to a 94% reduction compared to the level detected in non-heat treated MON 87701.

4.4 Toxicological assessment of soybean MON 87701

4.4.1 Toxicological assessment of the expressed novel protein

4.4.1.1 Degradation in simulated digestive fluids

An *in vitro* pepsin digestion assay, which simulates gastric fluid, and subsequent SDS-PAGE colloidal blue gel staining and Western blot to identify peptide fragments, indicated that the Cry1Ac protein produced in *Escherichia coli* was degraded by at least 95% within 30 seconds of exposure to pepsin. Exposure to simulated gastric fluid for 2 min followed by exposure to simulated intestinal fluid (neutral pH) for 1 min resulted in complete disappearance of the peptide fragments, indicating complete digestion. Exposure of the Cry1Ac protein to intestinal fluid only for 5 min resulted in digestion below the limit of detection, but produced a trypsin-resistant core polypeptide of 55 kDa that was apparently stable throughout digestion. The biological relevance of this was not addressed by the applicant.

4.4.1.2 Acute toxicity testing

An acute oral toxicity study with CD-1 mice administered Cry1Ac protein as a single dose of 1460 and 1290 mg/kg bw in male and female mice, respectively, showed no adverse effects.

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, 2011e), and is therefore not taken into account in this risk assessment.

4.4.1.3 Toxicological assessment of new constituents other than proteins

No new constituent other than the Cry1Ac protein is expressed in soybean MON 87701 and no relevant changes in the composition of soybean MON 87701 were detected by the compositional analysis.

4.4.2 Toxicological assessment of the whole GM food/feed

Two independent 90-day sub-chronic toxicity studies with Sprague-Dawley rats were submitted by the applicant. The first study was performed with 5 groups of 12 animals/sex, fed up to 30% (w/w) of toasted and defatted soybean MON 87701, employing processed meal from conventional soybean A5547 (with comparable genetic background) as control. Diets were formulated according to specifications from Purina Mills International, Inc. Certified Rodent Lab diet #5002 (with the exception of 30% soybean meal employed, 15% is normally used) and presented ad libitum. Animals were observed twice daily for mortality and moribundity. Clinical examinations were performed twice daily. Detailed physical examinations as well as individual body weights and food consumption were assessed and recorded weekly. Clinical pathology evaluations (hematology, coagulation, urinalysis and

serum chemistry) and complete necropsies were conducted at completion of the study. Tissue microscopies were performed on selected tissues from all animals. No test substance-related deaths or clinical effects were evident at the end of the study. However, in the course of the study, mean weekly body weights, cumulative body weight gains and food consumption were noted to be slightly lower in the test group females compared to their respective control group, while males were not affected. A second study was performed to evaluate the biological significance of these findings.

The second 90-day study included both 15 and 30% (w/w) of processed meal from soybeans in the diets, employed 20 animals/sex/group, but lacked histopathological evaluations. No treatment related deaths or effects on clinical evaluations were noted. Contrary to the first study, mean weekly body weights, cumulative body weight gains and food consumption in the test group males were observed to be statistically significant higher than that of their respective control group, while females were not affected. Thus, the EFSA panel considered the observed differences in body weights unlikely to be related to intake of soybean MON 87701 processed meal. Although statistically significant differences were observed for some other parameters, these were generally within historical control ranges and showed no corresponding correlated changes in related parameters and thus considered incidental and not treatment related. It was concluded that administration of 30% processed meal of soybean MON 87701 to rats did not cause adverse effects.

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, 2011e).

4.4.3.1 Assessment of allergenicity of the newly expressed proteins

Cry proteins occur naturally in the *Bacillus thuringiensis* bacterium and is not known to be allergenic. The amino acid sequence of the Cry1Ac protein in soybean MON 87701 shares no sequence homology with known IgE-dependent allergens based on *in silico* analyses. The protein is heat-sensitive and acid-labile. Additionally, it is not glycosylated. Moreover, since Cry1Ac forms only 0.0013% of the total protein composition in seeds from soybean MON 87701, it was concluded that the newly expressed protein is unlikely to be allergenic based on the intended usage. Soybean MON 87701 is thus as safe as its conventional counterpart and other commercial cultivars.

4.4.3.2 Assessment of allergenicity of the whole GM plant

The applicant performed *in vitro* allergenicity studies with soybean extracts from MON 87701, its conventional counterpart and different commercial soybean cultivars, employing sera from 13 clinically documented IgE-dependent soy-allergic individuals and 5 non-allergic individuals. Similar protein/allergen profiles were observed, with no significant changes by ELISA. Additionally, 2D-electrophoresis followed by Western blot with human IgE antibodies

from allergic individuals demonstrated no relevant difference in the IgE-binding patterns, with the exception of serum from one rather high IgE-responder individual.

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 4.4.3.2) have been identified, no increased IgE-mediated allergenicity is anticipated for soybean MON 87701.

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 or other immune-mediated reactions. Adjuvanticity has not been routinely considered in the assessment of allergenicity of GMOs, but a possible immunogenicity and adjuvanticity of Cry proteins has been considered by EFSA and VKM (EFSA, 2009a; EFSA, 2010b; VKM, 2012). More recent publications support and contribute new data regarding the adjuvant properties of Cry1Ac protoxin in mice (see below).

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.

"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). The presence of enteritis will necessarily increase the intestinal permeability and thereby the likelihood for bystander sensitisation to occur.

Notably, development of food allergies involves the interplay of various factors such as genetic predisposition, the composition of the mucosa as well as infection status of the gastrointestinal tract, age, and the nutritional state of an individual (van Wijk and Knippels, 2007). No comprehensive immunology study has been performed with the GM product, with the exception of the *in vitro* allergenicity study detailed in section 4.4.3.2 (Technical dossier).

Although induction of IgE has not been shown for Cry1Ac, studies have shown that Cry1Ac possesses adjuvant effects. A study with mice revealed that Cry1Ac protoxin activated macrophages by up-regulation of CD86, CD80, MHCII and induction of proinflammatory cytokines IL-6, TNF-α and MCP-1 (Moreno-Fierros et al., 2013). Studies have also shown that Cry1Ac possesses adjuvant effects upon intranasal or intraperitoneal immunisations with pneumococcus antigen (Moreno-Fierros et al., 2003), amoeba lysate (Rojas-Hernández et al., 2004) as well as RB51 vaccine strain of *Brucella abortus* (González-González et al., 2015). Another immunology study mapped out systemic and immune reactivity of Cry1Ac employing mice and showed induction of IgM, IgG and secretory IgA after intra-gastric and intraperitoneal immunisations (Vazquez-Padron et al., 2000b). Additionally, the Cry1Ac protein has been shown to attach itself to the mucosa (Vazquez-Padron et al., 2000a), with the potential to elicit a strong IgG-response (Vazquez et al., 1999), which may be biologically relevant in the presence of food antigens or cross-priming against a bystander antigen (Brandtzaeg, 2010). Based on the more recent findings, the VKM panel is of the opinion that further clarification is needed on the role of Cry proteins as adjuvants. Additional information can be found in the VKM's assessment of adjuvant effects of Cry proteins (VKM, 2012).

4.5 Nutritional assessment of GM food and feed

Nutritional assessment of feed derived from soybean MON 87701 were supplied by the applicant and considered in the previous VKM (VKM, 2010a) and EFSA (EFSA, 2011d) assessments. Considerations specific for Norwegian soybean use are included below (see 4.5.1). The molecular characterisation of soybean MON 87701 exhibited no unintended effects. Comparative compositional and agronomical equivalence of soybean MON 87701 to conventional soybean cultivars has also been established (see 3.2 and 3.4). Nutritional equivalence of soybean MON 87701 is thus implied. This was considered confirmed by data from a 42-day nutritional equivalence study with broilers (detailed in section 4.5.2) fed soybean MON 87701, MON 87701 × MON 89788 (a stacked event, not included in the statistical analysis of MON 87701), a conventional control, and six commercial soybean cultivars, provided by the applicant.

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 MON 87701, the estimated average exposure of the European consumer to products of soybean MON 87701 would be approximately 3.4-3.7 g/person/ day (Technical dossier).

According to FAOSTAT databases (1961-2005), 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.

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. 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 MON 87701 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 approximately 375 000 tons (Skretting, 2013).

Assuming that 100% of the SPC was derived from soybean MON 87701, the estimated average exposure of Atlantic salmon (post smolt, 200 g) to products of soybean MON 87701 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

The applicant provided a 42-day nutritional assessment study with Cobb x Cobb 500 broilers. In brief, the study included soybean meals from soybean MON 87701, a conventional control, MON 87701 × MON 89788 (a stacked event, not included in the statistical analysis of MON 87701) and six other commercial non-GM cultivars. A randomized complete block design consisting of the nine feed treatments, with starter and grower/finisher diets (33% day 0-21 and 30% day 21-42, respectively) was employed. A total of 900 broilers (equal representation of each sex) were divided into 10 replicate groups for each experimental feeding group. Body weights, food consumption, carcass yields and mortality were assessed. During the course of the study, broiler mortality in 4 of 10 replicate groups fed MON 87701 was 8.3-25%, with a mean total mortality of 10% compared to means of 0.8-4.5% for other feeding groups fed conventional soybean cultivars. However, since mortalities observed in groups fed soybean event MON 87701 × MON 89788 were similar or lower than in groups fed the conventional soybean cultivars, the applicant attributed the cause of higher mortalities in some of the MON 87701-fed groups to incidental bacterial infection, dehydration, ascites and/or sudden death syndrome. Aside from mortality, no relevant differences in broiler performance, carcass yield or composition of meat was evident from the study.

EFSA (2011d) commented that although the differences in mortality in broilers fed MON 87701 may have been incidental, it was not considered "good practice" for studies devoted to nutritional wholesomeness and safety testing. EFSA (2011d) and VKM (2010a) concluded that the broiler feeding trial did not identify any relevant differences between MON 87701 and various commercial soybean varieties in broiler performance, carcass yield or meat composition. Furthermore, it confirmed the results of the comparative compositional analysis. Thus soybean MON 87701 was considered compositionally and, therefore, implicitly as nutritious as commercial soybean varieties.

4.6 Conclusion

Subchronic, toxicity studies in rats, a nutritional whole food study in broilers and allergenicity assessment studies have been performed with soybean MON 87701. These studies have not revealed adverse effects or indicated any differences in the performance of animals fed soybean MON 87701 compared to conventional soybeans. Bioinformatics analysis revealed that the amino acid sequence of the Cry1Ac protein in soybean MON 87701 shares no sequence homology with 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 MON 87701 is nutritionally equivalent to and as safe as its conventional counterpart and other conventional soybean cultivars. It is unlikely that the Cry1Ac protein will introduce a toxic or allergenic potential in food or feed based on MON 87701 compared to conventional soybean cultivars.

5 Environmental risk assessment

Since the last assessments of soybean MON 87701 conducted by the GMO panels of VKM (VKM, 2010a) and EFSA (EFSA, 2011d), 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/BE/2010/79, which excludes cultivation, the environmental risk assessment is concerned with the accidental release into the environment of viable soybean MON 87701 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 MON 87701 will increase the potential to establish feral populations. A series of field trials with soybean MON 87701 was conducted by the applicant at several locations in North and South America in 2007 and 2007-2008, growing seasons respectively, to compare the

agronomic performance and field characteristics of soybean MON 87701 with its comparators (see section 3). With the exception of insect resistance, the agronomic and phenotypic field trial data did not show major changes in plant characteristics indicating altered fitness, persistence and invasiveness of soybean MON 87701 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 MON 87701, or changes to its survivability (including overwintering), persistence or invasive capacity. Because the general characteristics of soybean MON 87701 are unchanged, the insect resistance 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 MON 87701 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 MON 87701. 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, 2009b; 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 horisontal 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 MON 87701 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 MON 87701 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 MON 87701 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 MON 87701 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 MON 87701 confers insect resistance only. Considering the intended uses of soybean MON 87701, which excludes cultivation, potential 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 MON 87701 confers insect resistance only. Considering the intended uses of soybean MON 87701, which excludes cultivation, potential 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 MON 87701, 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 MON 87701, 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 MON 87701.

Soybean MON 87701 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 MON 87701 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, 2011f). 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 MON 87701 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 MON 87701.

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 MON 87701 genome. The results show the presence of one fragment of the DNA insert containing one functional copy of the *cry1ac* gene 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 element was stably inherited and expressed over multiple generations in parallel with the observed phenotypic characteristics of soybean MON 87701.

Based on the above considerations, the VKM GMO panel concludes that the molecular characterisation of soybean MON 87701 does not indicate a safety concern.

Comparative assessments

The VKM GMO Panel has considered the available data on compositional, agronomic and morphological characteristics and confirms that no biologically relevant differences were identified between soybean MON 87701, its corresponding conventional counterpart and other conventional soybean cultivars. The few statistically significant differences observed 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 trait, the VKM GMO Panel concludes that soybean MON 87701 is compositionally, agronomically, and morphologically equivalent to its conventional counterpart and other conventional soybean cultivars.

Food and feed risk assessment

Subchronic, toxicity studies in rats, a nutritional whole food study in broilers and allergenicity assessment studies have been performed with soybean MON 87701. These studies have not revealed adverse effects or indicated any differences in the performance of animals fed soybean MON 87701 compared to conventional soybeans. Bioinformatics analysis revealed that the amino acid sequence of the Cry1Ac protein in soybean MON 87701 shares no sequence homology with 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 MON 87701 is nutritionally equivalent to and as safe as its conventional counterpart and other conventional soybean cultivars. It is unlikely that the Cry1Ac protein will introduce a toxic or allergenic potential in food or feed based on MON 87701 compared to conventional soybean cultivars.

Environmental assessment

Considering the intended uses of soybean MON 87701, 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 MON 87701.

Soybean MON 87701 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 MON 87701 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 MON 87701 with the Cry1Ac protein:
- Is compositionally, morphologically and agronomically equivalent to its conventional counterpart and other commercial soybean cultivars
- Is unlikely to introduce a toxic or allergenic potential 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 current knowledge. With added knowledge, VKM and its commissioning agencies could thereby provide greater certainty when communicating conclusions regarding the safety of the GM products.

Development of food allergies involves the interplay of various factors such as genetic predisposition, the composition of the mucosa as well as infection status of the gastrointestinal tract, age, and the nutritional state of an individual (van Wijk and Knippels, 2007).

Although there is limited knowledge with regards to Cry proteins as adjuvants, a recent study with mice revealed that *Bacillus thuringiensis* Cry1Ac protoxin activated macrophages by up-regulation of cell surface molecules and induction of proinflammatory cytokines (Moreno-Fierros et al., 2013). Previous studies have shown that Cry1Ac protein has immunogenic potential to elicit strong IgG-response (Vazquez et al., 1999) and the induction of IgG antibodies to food antigen and even crosspriming against a bystander antigen may be of biological significance (Brandtzaeg, 2010). Experimental studies both *in vitro* and *in vivo* have demonstrated that IgG antibodies that are not balanced by a mucosal IgA response can enhance the epithelial penetration of bystander proteins (Brandtzaeg, 2010). The Norwegian VKM GMO Panel still perceives the need for further clarification on the possible role of Cry proteins as adjuvants, especially in certain processed food and feed ingredients. The levels of Cry1Ac in these ingredients have not been reported and it can be expected that higher levels may be present in protein concentrates and isolates.

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Appendix I

Foreløpig helserisikovurdering av genmodifisert soya MON 87701 fra Monsanto Company (EFSA/GMO/BE/2010/79)

Uttalelse fra Faggruppe for genmodifiserte organismer i Vitenskapskomiteen for mattrygghet

Innspill til EFSAs GMO Extranet

Bidragsytere

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

Vurdert av

Faggruppe for genmodifiserte organismer:

Audun Nerland (leder), Åshild Andreassen, Per Brandtzæg, Askild Holck, Olavi Juntilla, Heidi Konestabo, Richard Meadow, Kåre M. Nielsen, Hilde-Gunn Opsahl Sorteberg, Rose Vikse

Koordinator(er) fra sekretariatet:

Arne Mikalsen og Merethe Aasmo Finne

EFSA/GMO/BE/2010/79 – MON 87701

Sammendrag

Helserisikovurderingen av den insektsresistente soyalinjen MON 87701 (EFSA/GMO/BE/2010/79) fra Monsanto er utført av Faggruppe for genmodifiserte organismer i Vitenskapskomiteen for mattrygghet. Mattilsynet ber Vitenskapskomiteen for mattrygghet om å vurdere soyalinje MON 87701 til bruk i næringsmidler og fôrvarer, men ikke til dyrking.

Risikovurderingen av den genmodifiserte soyaen er basert på dokumentasjon som er gjort tilgjengelig på EFSAs nettside GMO EFSA Extranet. I tillegg er det benyttet informasjon fra uavhengige vitenskapelige publikasjoner i vurderingen. MON 87701 er risikovurdert i henhold til tiltenkt bruk i EU/EØS-området, og i overensstemmelse med prinsippene som er nedfelt i EFSAs retningslinjer for risikovurdering av genmodifiserte planter og avledete næringsmidler og fôrvarer (EFSA 2006) og Organisation for Economic Co-operation and Development (OECD)s konsensusdokument for soya (OECD 2009).

Den vitenskapelige vurderingen omfatter transformeringsprosess, vektor, transgene konstrukt, komparativ analyse av ernæringsmessig kvalitet, mineraler, kritiske toksiner, metabolitter, antinæringsstoffer, allergener og nye proteiner. Videre er agronomiske egenskaper, potensiale for ikke tilsiktede effekter på fitness, samt mulig horisontal og vertikal genoverføring vurdert.

Soyalinjen MON 87701 er fremkommet ved *Agrobacterium*-mediert transformasjon av celler fra den kommersielle soyalinjen A5547. Den innsatte genkonstruksjonen i MON 87701 inneholder en enkeltkopi av et modifisert *cry1Ac*-gen fra bakterien *Bacillus thuringiensis* subsp. *kurstaki*. Genet koder for δ-endotoksinet Cry1Ac som gir plantene resistens mot enkelte skadegjørere i ordenen Lepidoptera, eksempelvis *Anticarcia gemmatalis* ("velvetbean caterpillar"), *Pseudoplusia includens* ("soybean looper"), *Epinotia aporema* ("soybean anxil borer") og *Rachiplusia nu* ("sunflower looper").

MON 87701 inneholder ingen markørgener for antibiotikaresistens.

Olje fra soyalinjen MON 87701 er primært tiltenkt brukt i næringsmiddelindustrien.

Komparative analyser
Analyser av ernæringsmessige komponenter er i hovedsak utført i tråd med OECDs konsensusdokument for soya (OECD 2009). Det er påvist statistisk signifikante forskjeller mellom soyalinje MON 87701 og kontroll i enkeltparametere. Forskjellene er imidlertid ikke konsistente over forsøksfelt, og verdiene for de analyserte komponentene ligger innenfor typiske verdier for andre soyasorter som er rapportert i litteraturen, og innenfor variasjonsområdene til kommersielle referansesorter som er inkludert i søkers dokumentasjon. Faggruppen konkluderer med at forskjellene som er påvist ikke har ernæringsmessig betydning.

Faggruppen påpeker at søker ikke har foretatt analyser av fosfatider i lecitin. Det understrekes at konsensusdokumentet i størst mulig grad skal følges når det legges fram dokumentasjon på nivåene av næringsstoffer, antinæringsstoffer og metabolitter.

Når det gjelder antinæringsstoffene, har søker slått sammen resultatene for de enkelte isoflavoner innenfor hver av isoflavongruppene. Det er stor variabilitet innenfor hver gruppe, og faggruppen etterlyser statistiske analyser for hvert enkelt forbindelse innenfor hver isoflavongruppe.

Feltforsøk over en vekstsesong viser signifikante forskjeller mellom MON 87701 og umodifisert kontroll for enkelte av de agronomiske karakterene som er evaluert. Gjennomsnittsverdiene for disse karakterene ligger imidlertid innenfor variasjonsområdene for referansesortene som var inkludert feltforsøkene.

3

Toksisitet og allergenisitet

Cry1Ac-proteinet, som uttrykkes som følge av genmodifiseringen, har ingen likheter med kjente allergener eller egenskaper som tilsier at de kan virke som allergener. Basert på testene som er omtalt i søkers dokumentasjon, dvs. at det i proteinet ikke er påvist aminosyresekvenser som er lik allergene proteiners epitoper, at proteinene brytes raskt ned av mage-tarmsaft, samt at konsentrasjonene av Cry1Ac-protein er svært lave (mindre enn 0,002 % av total proteinmengde), anser faggruppen det som lite trolig at proteinet medfører et signifikant større potensiale for utvikling av matallergi hos mennesker sammenlignet med umodifisert soya. Akutte 14 dagers fôringsstudier (oral sondefôring EPA-OPPTS (870.1100)) på mus med reinfremstilt Cry1Ac-protein, 90 dagers fôringsforsøk på rotter og 42 dagers fôringsforsøk med broilere viste ingen skadelige helseeffekter. Søker har ikke utført toksisitetsstudier på fisk med fôr som inneholder soya MON 87701.

Faggruppen påpeker at 90-dagers repeterte dosestudier bør benyttes ved fastsettelse av NOAEL. I disse sub-kroniske studiene er det brukt minst to doser av testfôret, samt kontrollfôr av umodifisert soya. Forsøksdyrene er eksponert over et så langt tidsrom at eventuelle uheldige helseeffekter ville blitt oppdaget. Rottene er også eksponert for høyere konsentrasjoner av Cry1Ac enn hva mennesker og dyr ville vært eksponert for i en naturlig ernæringsmessig situasjon.

På bakgrunn fra forsøk med Cry1Ac-protein som er dokumentert i denne søknaden, konkluder faggruppen med at det er lite sannsynlig at eksponering for Cry1Ac-protein i seg selv, og i de mengder som tilføres via fôr fra den genmodifisert soyaen, vil føre til helseskade hos dyr.

Nøkkelord

Soya, *Glycine max* (L.) Merr., genmodifisert soyalinje, MON 87701, EFSA/GMO/BE/2010/79, Cry1Ac, insektsresistens, helsemessig trygghet, helse, forordning 1829/2003/EF

4

Forkortelser og ordforklaringer

ADF	Acid detergent fiber, fiberfraksjon av ufordøyelig plantemateriale i fôr, vanligvis cellulosefiber dekket med lignin og silikat. Plantematerialet fordøyes med en syre-detergentløsning (ADF). Ufordøyd masse betegnes som ADF. Fôr med lavt ADF-innhold er mer fordøyelig og har større energiinnhold.
Allel	Et bestemt gen kan foreligge i ulike varianter (alleler). Allelene kan være dominante (bestemmende for fenotypen) eller recessive (vikende).
ARMG	Antibiotikaresistensmarkørgen
Backcross (BC)	Tilbakekryssing. Kryssing mellom en hybridlinje (avkom fra to genetisk ulike foreldre) og en av foreldrelinjene, alternativt en genetisk ekvivalent organisme. Strategi i planteforedling for å overføre primært kvalitative karakterer, for eksempel sjukdomsresistens, til elitelinjer av både kryssbefruktede og selvpollinerte arter. Gjentatte tilbakekryssinger reduserer det genetiske bidraget, som uønskede alleler fra den andre donorplanten. BC_1, BC_2 etc: betegnelse på 1. og 2. tilbakekryssingsgenerasjon, etc.
BLASTn	Algoritme som benyttes for homologisammenligning av nukleotidsekvenser.
BLASTP	Algoritme som benyttes for homologisammenligning av aminosyresekvenser i proteiner.
BLASTx	Algoritme som benyttes for oversetting fra kodende nukleotidsekvenser til aminosyresekvenser.
bp	Basepar
Codex	FAO/WHO-organ som etablerer globale handelsstandarder for mat.
CP4	*Agrobacterium* sp. stamme CP4
Cry	Krystallproteiner (δ-endotoksiner) fra *Bacillus thuringiensis*.
Cry1Ac	Et Cry1-klasse krystallprotein fra *Bacillus thuringiensis* subsp. *kurstaki*
DN	Direktoratet for naturforvaltning
DNA	Deoxyribonukleinsyre (DNA)
Dominant allel	Et allel som uttrykker samme fenotype, uavhengig av om allelene i genparet er like (homozygot) eller ulike (heterozygote).
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPSPS	5-enolpyruvylsikimat-3-fosfatsyntase
FAO	Food and Agriculture Organization, FNs organisasjon for ernæring og landbruk.
FIFRA	US EPA Federal Insecticide, Fungicide and Rodenticide Act. USAs føderale lov om insektdrepende midler, soppdrepende midler og midler mot skadedyr.
Fitness	Et individs relative evne til å føre sine gener/alleler videre til kommende generasjoner.
GLP	Good Laboratory Practices, retningslinjer for godt laboratoriearbeid.
GMO	Genmodifisert organisme
GMP	Genmodifisert plante
Locus	Spesifikk posisjon på kromosomet der et gen er lokalisert.
MALDITOF	Massespektrometrimetode for å måle molekylvekt til peptider.
Mendelsk nedarving	Lovmessig nedarvingsmønster ved ulike typer kryssinger.
MT	Mattilsynet
NDF	Neutral detergent fiber, dvs. fiberfraksjon som inneholder hemicellulose og ADF.
Northern blot	Teknikk for overføring av RNA til en membran for videre studier av overførte RNA-sekvenser.
Nær-isogen linje	Linjer eller sorter som er genetisk identiske, med unntak av ett lokus eller kromosom-segment.
OECD	Organisation for Economic Co-operation and Development
ORF	Open Reading Frame (åpen leseramme)
OSWP	Overseason whole plant

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PCR   Polymerase chain reaction. Polymerase kjedereaksjon. Metode for å syntetisere et stort antall kopier av en DNA-sekvens vha primere.

RNA   Ribonukleinsyre

SDS-PAGE   Natriumdodecylsulfat (SDS)-polyakrylamidelektroforese. Elektroforesemetode for separasjon av proteiner.

Southern blot Teknikk for overføring av DNA til en membran for videre studier av overførte DNA-sekvenser.

T-DNA   DNA fra Ti-plasmidet som er i jordbakterien *Agrobacterium tumefaciens*. Ti-plasmidet (Transfer-DNA) overføres fra bakterien, og settes inn i plantecellenes kjernegenom. T-DNAet som overføres avgrenses av V (venstre) og H (høyre) flankesekvenser, og begrenser derfor den delen av Ti-plasmidet som overføres og gjør at resten av vektoren ikke blir satt inn i plantekromosomene.

Utviklingsstadier hos soya:

<u>Vegetative stadier</u>
VE: oppspiring, synlige frøblad
V1: 1. nodium på hovedstengel med fullt utviklede blad
V(n): n'te nodium på hovedstengel med fullt utviklede blad

<u>Reproduktive stadier</u>
R1: begynnende blomstring (en åpen blomst ved et nodium)
R2: full blomstring
R3: begynnende belgdannelse
R4: fullt utviklede belger
R5: begynnende frødannelse
R6: fullt utviklede frø
R7: begynnende modning
R8: fysiologisk moden

USDA   United States Department of Agriculture

U.S. EPA   United States Environmental Protection Agency, USAs miljøvernmyndigheter

Western-blot Metode for overføring av proteiner til en membran som binder protein.

WHO   World Health Organisation. Verdens helseorganisasjon, organ under FN.

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Innholdsfortegnelse

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Bakgrunn

Faggruppe for genmodifiserte organismer i Vitenskapskomiteen for mattrygghet er bedt av Mattilsynet om å foreta en vurdering av helserisiko ved en eventuell godkjenning av den genmodifiserte soyalinjen MON 87701 (EFSA/GMO/BE/2010/79) for bruksområdene import, prosessering, næringsmidler og fôrvarer. MON 87701 er søkt omsatt i EU/EØS-området under forordning (EF) Nr. 1829/2003 om genmodifiserte næringsmidler og fôrvarer (artiklene 5,17,3 (1c) og 15(1c), og i overensstemmelse med direktiv 2001/18/EF, del C.

Søknaden ble fremmet og anbefalt av tyske myndigheter i mai 2010. Dokumentasjonen knyttet til søknaden ble lagt ut på EFSAs nettside GMO Extranet 11. juni 2010, med frist på 90-dager for innspill fra EU- og EØS/EFTA-landene. Norge har ikke tidligere uttalt seg om soyalinjen MON 87701.

MON 87701 er foreløpig ikke godkjent for kommersiell dyrking eller omsetning som mat og/eller fôr i noen land (CERA 2010; EFSA/GMO/BE/2010/79). I følge søker er soyalinjen primært tenkt dyrket i Brasil og Argentina. Søknader om godkjenning av MON 87701 for alle bruksområder er levert i USA, Brasil, Japan og Canada. I tillegg vil det bli søkt om godkjenning av MON 87701 til import og bruk som mat og fôr i Kina, Australia, New Zealand, Filippinene, Mexico, Malaysia, Taiwan, Sør-Korea og Indonesia.

Oppdrag fra Mattilsynet

Mattilsynet har i brev datert 12.5.2006 (ref. 2006/17817) gitt Vitenskapskomiteen for mattrygghet i oppdrag å foreta løpende risikovurderinger av genmodifiserte næringsmidler og fôrvarer som faller inn under EUs forordning 1829/2003/EF. VKM er bedt om å vurdere helseaspekter ved slike produkter, og på bakgrunn av vurderingene gi innspill til EFSAnet.

Søknad EFSA/GMO/BE/2010/79, genmodifisert soyalinje MON 87701, ble lagt ut på EFSAnet 11. juni 2010. Faggruppe for genmodifiserte organismer skal i tråd med oppdragbrev utarbeide helserisikovurdering av soyalinjen til import og industriell prosessering, samt til bruk som næringsmiddel og fôrvare. Søknaden omfatter ikke dyrking. Vurderingen skal utføres i henhold til tiltenkt bruk og i overensstemmelse med prinsippene som er nedfelt i EFSAs retningslinjer for risikovurdering av genmodifiserte planter og avledete næringsmidler og fôrvarer ("Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed" (EFSA 2006).

Produktet som ønskes vurdert
Genmodifisert soya, EFSA/GMO/BE/2010/79 (soya MON 87701).
Unik kode: MON-877Ø1-2
Status i EU: Søknad under 1829/2003/EF. EFSAs frist for innspill er 11.9.10.
Ønsket svarfrist til Mattilsynet er 8. september 2010.

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Risikovurdering

1 Innledning

Helserisikovurderingen av den genmodifiserte soyalinjen MON 87701 er basert på dokumentasjon som er gjort tilgjengelig på EFSAs nettside GMO Extranet. I tillegg er det benyttet uavhengige vitenskapelige publikasjoner med referee i vurderingen.

Faggruppen har vedtatt å benytte EFSAs retningslinjer for vurdering av genmodifiserte planter. Prinsippene som er lagt til grunn for vurderingen, er derfor hentet fra EFSAs dokument"Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed" (EFSA 2006). Ved vurdering av vesentlig likhet har faggruppen lagt vekt på OECDs konsensusdokument for soya (OECD 2009), som gir anbefalinger over hvilke parametere som bør undersøkes.

Det er kun medlemmene i Faggruppe for genmodifiserte organismer som har vurdert den genmodifiserte soyaen.

1.1   Beskrivelse av egenskap(er) og virkningsmekanismer

Soyalinjen MON 87701 er fremkommet ved *Agrobacterium*-mediert transformasjon av celler fra den kommersielle soyalinjen A5547. Den innsatte genkonstruksjonen i MON 87701 inneholder en enkeltkopi av et modifisert *cry1Ac*-gen fra bakterien *Bacillus thuringiensis* subsp. *kurstaki*. Genet koder for δ-endotoksinet Cry1Ac som gir plantene resistens mot enkelte skadegjørerere i ordenen *Lepidoptera*, eksempelvis *Anticarcia gemmatalis* ("velvetbean caterpillar"), *Pseudoplusia includens* ("soybean looper"), *Epinotia aporema* ("soybean anxil borer") og *Rachiplusia nu* ("sunflower looper").

2 Molekylær karakterisering

2.1 Transformasjonssystem og vektorkonstruksjon

MON 87701 er fremkommet ved *Agrobacterium*-mediert transformasjon av meristematisk vev fra den kommersielle soyalinjen A5547. Den binære vektoren PV-GMIR9, som inneholder to rekombinante DNA-fragmenter (T-DNA I og II), ble benyttet til transformasjonen. De rekombinante DNA-fragmentene inneholder henholdsvis en *cry1Ac*-ekspresjonskassett (T-DNA I) og en *cp4 epsps*-ekspresjonskassett (T-DNA II). Ved transformasjonen ble ekspresjonskassettene satt inn som to uavhengige loki. Transformanter ble selektert ved at de overlevde og vokste i nærvær av virkestoffet glyfosat. Påfølgende innavl av F$_1$-generasjonen førte til at T-DNA I (*cry1Ac*-kassetten) ble selektert fra T-DNA II (*cp4 epsps*-kassetten), og genotyper som inneholdt *cp4 epsps*-kassetten (T-DNA II) ble eliminert. MON 87701-plantene inneholder kun ett rekombinant DNA-fragment med *cry1Ac*-genkassetten (T-DNA I).

2.2 Karakterisering av geninnsettingen/genkonstruksjonen

Cry1Ac-ekspresjonskassetten inneholder to DNA-sekvenser fra vårskrinneblom (*Arabidopsis thaliana*). Vårskrinneblomsekvensene stammer fra genet *ribulose 1,5-difosfatkarboksylase* small subunit 1A (*RbcS4*-gen), promoter og ledersekvens P-*RbcS4*, samt N-terminal kloroplasttransittpeptid TS-*CTP*. TS-*CTP* -peptidet bidrar til å målrette uttrykket av Cry1Ac-proteinet til kloroplastene. Videre inneholder ekspresjonskassetten *cry1Ac*-sekvenser. T-*7S a',* en 3' ikke-translatert sekvens fra soya,

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avslutter transkripsjonen (se figur 1 og tabell 1 og 2). DNA-fragmentet inneholder ikke antibiotikaresistensgener.

Southern blot og sekvensanalyse er benyttet for å karakterisere det rekombinante DNA-fragmentet i planten. Molekylærbiologisk karakterisering viser at det er satt inn en enkelt kopi av DNA-fragmentet i soyaens genom. Gener og DNA-elementer i dette fragmentet er vist i figur 1 og tabell 1 og 2.
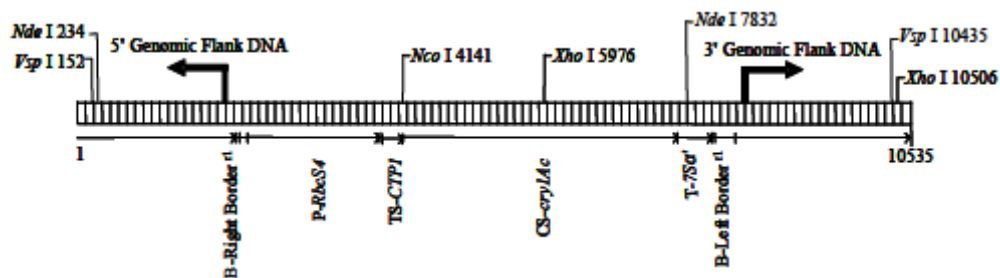
Figur 1. Rekombinant T-DNA I-fragment i soyaens genom.

Tabell 1.   Beskrivelse av de innsatte genene.

Cry1Ac-ekspresjonskassett	
P-RbcS4	Promoter, ledesekvens og 5′ ikke-translatert område fra *ribulose 1,5-difosfatkarboksylase* (*RbcS4*)-genet fra vårskrinneblom (*Arabidopsis thaliana*). Uttrykkes ikke i planten.
TS-CTP1	N-terminal kloroplasttransittpeptid (TS-*CTP*) fra *RbcS4*-genet. Overfører Cry1Ac til kloroplast.
CS-cry1Ac	Gen som koder for et syntetisk Cry1Ac-protein. Genet stammer fra *Bacillus thuringiensis*.
T-7S a′ 3'	DNA- sekvens fra soya som avslutter transkripsjonen (se tabell 2). Uttrykkes ikke i planten

Tabell 2.   **Størrelsesfordeling av gener og regulatoriske elementer i MON 87701**

Genetic element[1, 2]	Location in sequence[3]	Function and/or reference
Sequence flanking 5' end of the insert	1-2000	Soybean genomic DNA
B-Right Border [r1]	2001-2045	45 bp DNA region from the Right Border region remaining after integration (Depicker *et al.*, 1982)
IS	2046-2154	Sequence used in DNA cloning
P- *RbcS4*	2155-3877	Promoter, leader, and 5' non-translated region of the *Arabidopsis thaliana RbcS4* gene encoding ribulose 1,5-bisphosphate carboxylase small subunit 1A (Krebbers *et al.*, 1988)
TS-*CTP1*	3878-4141	Targeting sequence encoding the transit peptide of the *Arabidopsis RbcS* encoding small subunit 1A transit peptide, from *Arabidopsis thaliana*, present to direct the Cry1Ac protein to the chloroplast, (Krebbers *et al.*, 1988)
CS–Cry1Ac	4142-7678	Codon-modified coding sequence of the Cry1Ac protein of *Bacillus thuringiensis* (Fischhoff and Perlak, 1995)
IS	7679-7687	Sequences used in DNA cloning
T-*7S α'*	7688-8126	3' region of the *Sphas1* gene of *Glycine max* encoding the *7S α'* seed storage protein, ß-conglycinin, including 35 nucleotides of the carboxyl terminal ß-conglycinin coding region with the termination codon and the polyadenylation sequence (Schuler *et al.*, 1982). The element functions to terminate transcription and direct polyadenylation of the mRNA.
IS	8127-8162	Sequence used in DNA cloning
B-Left Border [r1]	8163-8426	264 bp DNA region from the Left Border region remaining after integration (Barker *et al.*, 1983)
Sequence flanking 3' end of the insert	8427-10535	Soybean genomic DNA

[1] Flanking sequences and intervening sequences (IS) are not regarded as functional genetic elements.

[2] P – Promoter; I – Intron; CS – Coding Sequence; T – 3' non-translated transcriptional termination sequence and polyadenylation signal sequences; B – Border.

[r1] Suscript in Left and Right Border indicates that the border sequences in MON 87701 are smaller than those in PV-GMIR9 plasmid due to the mechanism of *Agrobacterium* transformation.

[3] Numbering refers to the sequence in Arackal *et al.*(2009).

Molekylærbiologiske analyser viser at det rekombinante fragmentet i planten inneholder de samme gener som er på det tilsvarende rekombinante T-DNA I-fragmentet i plasmidet PV-GMIR9. Ett DNA-fragment på 6426 bp gjenfinnes som et enkelt lokus i soyaens genom.

Cry1Ac proteinet som uttrykkes i MON 87701 er undersøkt med Western-blot analyse og densitometri, SDS-PAGE og densitometri, proteolytisk peptidkartlegging med MALDI-TOF MS analyse av peptider, N-enden sekvensering ved Edman-degradering. For Cry1Ac er det foretatt undersøkt med insektslarver for se på insekticidvirkning. Det ble konkludert med at toksinet ikke var forskjellig fra *E. coli*-produsert toksin. Disse analysene viser at Cry1Ac-proteinet som produseres i soyaene er ekvivalent med Cry1Ac- proteinet som produseres i *E. coli*. Det ble ikke påvist glykoliseringsseter på proteinene.

Søker har sekvensert 2000 baser oppstrøms for 5'-enden og ca. 2100 baser nedstrøms for 3'-enden av innskuddet (tabell 2). I henhold til søker er flankesekvensene brukt ved søk i "Genbank non-redundant cDNA nucleotide database" (oppdatert 2. mai 2009), og "Genbank public non-redundant amino acid database" (oppdatert 28. april 2009). Søker har benyttet BLASTn og BLASTx algoritmer i sekvenssøkene. Søkene viser at flankesekvensene er soyasekvenser uten homologi til kodende eller regulatoriske sekvenser. DNA-analyser ved hjelp av Southern blot, DNA- sekvensanalyser, PCR og primere, som er spesifikke for sekvensene ved 5'-enden og 3'-enden for det transgene innskuddet, viser at ved innsettingen av plasmidets rekombinante T DNA I-fragment ble 32 bp fjernet og 16 bp satt inn i 5'-enden til det rekombinante DNAet. Søker har også vist at T-DNA II, "backbone"

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elementer og seleksjonsmarkørsekvenser fra plasmidet PV-GMIR9 ikke er tilstede i genomet til MON 87701. Videre er det vist at insertet er stabilt over fem generasjoner. Søker konkluderer ut fra sine sekvensanalyser med at innsettingen av det rekombinante DNA-fragmentet ikke har ødelagte kode- eller regulatoriske sekvenser i området rundt innskuddet.

2.3 Informasjon vedrørende uttrykk av innsatte gener og åpne leserammer (ORF)

I dokumentasjonen fra søker presenteres resultater fra to proteinekspresjonsstudier med soyalinjen MON 87701. Feltforsøkene, som ligger til grunn for studiene, ble gjennomført på henholdsvis fem lokaliteter i USA vekstsesongen 2007 og fem lokaliteter i Argentina 2007/2008 (tabell 3). Forsøkene ble lagt ut som fullstendig randomiserte blokkdesign med tre gjentak, og inkluderte foruten testlinjen MON 87701 (generasjon R_8 og R_9) en umodifisert kontroll med tilsvarende genetisk bakgrunn (A5547). I begge forsøksseriene ble det tatt prøver av bønne (frø), fôrfraksjon, røtter, samt blad på ulike tidspunkt gjennom vekstsesongen. I forsøkene i USA ble det også tatt tatt prøver av pollen. Ekspresjonen av Cry1Ac-protein ble målt ved hjelp av enzyme-linked immunosorbent assay (ELISA).

Med unntak for røtter, ble det detektert Cry1Ac-protein i alle undersøkte vev. I de nordamerikanske forsøkene varierte nivåene av Cry1Ac mellom 340 µg pr. g tørrvekt (t.v.) i blad på vekststadium V14-V16, til 2,3 µg pr. g råvekt i pollen (tabell 3). Resultatene fra de argentinske forsøkene viser høyere konsentrasjoner av det transgene proteinet i blad tidlig i vekstsesongen. I motsetning til resultatene fra USA, ble nivåene av Cry1Ac her redusert utover i vekstsesongen.

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Tabell 3. Nivå av Cry1Ac-protein i blad, hel plante, røtter, bønne og pollen fra MON87701 på ulike utviklingsstadier. Resultater fra feltforsøk i USA i 2007 og Argentina i 2007/2008.

Vevstype	Utviklingstrinn[1]	USA Gjennomsnitt (SD) Variasjonsområde (µg/g tørrvekt)	Argentina Gjennomsnitt (SD) Variasjonsområde (µg/g tørrvekt)
Blad	OSL-1 (V3-V4)	220 (70) 110-350	450 (77) 320-580
	OSL-2 (V6-V8)	260 (100) 130-500	290 (130) 150-580
	OSL-3 (V10-V12)	240 (110) 94-480	140 (52) 61-250
	OSL-4 (V14-V16)	340 (290) 78-960	180 (49) 78-250
Hel plante (fôr)	R6	29 (28) 8,2-95	70 (43) 10-150
Røtter R6	R6	LOD[2]	LOD
Bønne	R8	4,7 (0,79) 3,4-5,7	5,1 (0,82) 3,9-6,7
		(µg/g råvekt.)	
Pollen	R2	2,3 (0,58) 1,8-3,1	Ikke analysert

1 Utviklingstrinnene er beskrevet i oversikten over ordforklaringer/forkortelser
2 LOD: 0,347 µg/g råvekt

I henhold til søkers dokumentasjon er det gjort studier for å påvise endogene åpne leserammer i 5'- og 3' flankerende ende til det rekombinante DNA-fragmentet i soyaens genom. Det er søkt etter seks potensielle åpne leserammer, dvs. i 5'- og 3'-flankerende områder. Det ble ikke påvist endogene åpne leserammer ved 5'-enden og i 3'-enden. For de seks åpne leserammene i endene av insertet er det undersøkt for polypeptider som er lik eller større enn 8 aminosyrer. Det er påvist 5 mulige polypeptider som kan uttrykkes fra hver ende av insertet. Videre er det utført teoretiske analyser av de 5 mulige polypeptidene fra hver leseramme ved bruk av oppdatert versjoner av AD_2009 (allergendatabase), BLOSUM50 (identifiserer sekvenslikheter som omfatter gaps), PRT_2009 (GenBank protein database, utgave 169.0) og TOx_2009. Databasene viser ingen biologisk relevante strukturelle likheter til allergener, toksiner eller farmakologiske aktive proteiner. Resultatene fra disse teoretiske analysene viser at dersom noen av disse leserammene skulle bli transkribert, er det lite sannsynlig at dette vil resultere i polypeptid(er) som medfører potensielle toksiske, allergene eller har uheldige helsemessige konsekvenser.

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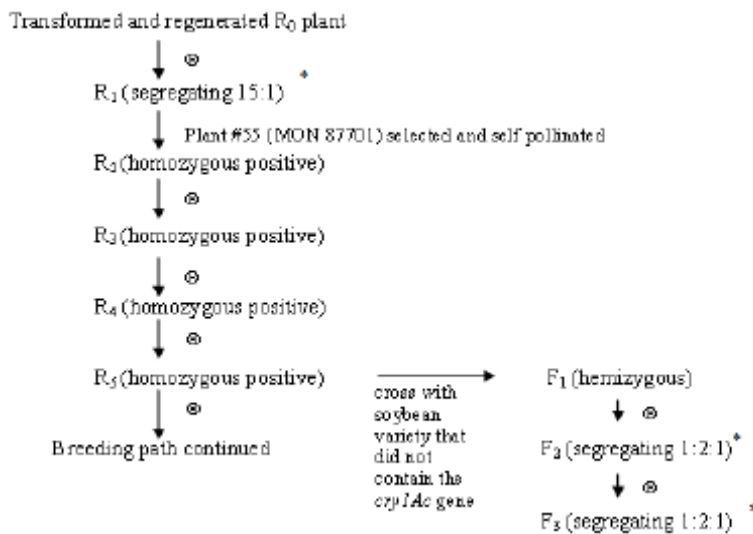
Figur 2. **Kryssingsskjema for generering av spaltingsdata fra MON 87701.**

2.4 Nedarving og stabilitet av innsatt DNA

I henhold til dokumentasjonen fra søker er genetisk stabilitet undersøkt ved analyse av genomisk DNA fra fem ulike generasjoner (R_4, R_5, R_6, R_8, R_9). Resultatene fra Southern blot-analysene viser at det rekombinante DNA-innskuddet er integrert i genomet og nedarves stabilt over generasjoner. Fenotypisk stabilitet er vist ved spaltingsdata fra generasjonene R_1, F_2 og F_3 (se figur 2). Segregasjons-analysene (chi-kvadrat-test) viser forventet spaltingstall for insektsresistens, og det konkluderes med at rekombinante DNA-fragmentet følger mønsteret for mendelsk nedarving av et enkelt, dominant lokus (forvenetet hemizygot nedarving).

2.5 Vurdering basert på tilgjengelig datagrunnlag

Den transgene soyalinjen MON 87701 har fått tilført et *cry1Ac*-gen. I henhold til søkers informasjon vedrørende integreringsplass og flankesekvenser til de integrerte transgenene, samt analyser vha Southern blot er det grunn til å tro at transgenet sitter i et lokus i genomet. Det konkluderes med at nedarvingen av *cry1Ac*-genet i soyalinjen MON 87701 følger mønsteret for mendelsk nedarving av et dominant locus, og at fusjonsproteiner ikke uttrykkes i MON 87701.

Faggruppen har ikke identifisert noe risiko knyttet til den molekylærbiologiske karakteriseringen av det rekombinante innskuddet i MON 87701. Faggruppen finner informasjonen tilstrekkelig for vurdering av soyalinjen.

Kommentarer fra faggruppen:
På side 169 fotnote 40 i det tekniske dossieret har Monsanto presentert gjennomsnittsresultater for Cry1Ac til 4,9 % per tørrvekt i MON87701 bønner. Det riktige er 4,9 mikrogram/g tørrvekt.

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3   Komparative analyser

3.1   Valg av komparator og forsøksdesign

I henhold til søkers dokumentasjon er den transgene soyalinjen MON 87701 testet i en serie feltforsøk over en vekstsesong i sentrale dyrkingsområder for soya i USA og Argentina.

Feltforsøkene for komparative analyser av ernæringsmessige karakterer ble utført på henholdsvis fem lokaliteter i USA i 2007 og fem lokaliteter i Argentina i 2007/2008. Den konvensjonelle soyalinjen A5547, med tilsvarende genetisk bakgrunn som testlinjen men som ikke uttrykker Cry1Ac-protein, ble benyttet som umodifisert kontroll. I tillegg var det inkludert henholdsvis 24 og 20 umodifiserte, kommersielle soyalinjer som referansesorter i forsøkene (fire sorter på hvert forsøkssted). Testlinje, komparator og referansesorter ble plantet i fullstendig randomiserte blokkdesign med tre gjentak på hver lokalitet. Søker viser til at dyrkingsregimet var i henhold til vanlig praksis i den enkelte region der forsøkene var lokaliserte. Det ble tatt ut prøver fra tre gjentak per lokalitet for analyser av ernæringsmessig viktige komponenter både hos test- og kontrollinjen, mens prøver fra ett gjentak ble lagt til grunn for analyser av referansesortene.

Statistiske analyser
I Nordisk ministerråds rapport "Safety Assessment of Novel Food Plants: Chemical Analytical Approaches to the Determination of Substantial Equivalence" (TemaNord 1998), anbefales det at tilstrekkelig antall prøver må analyseres for å få adekvat sensitivitet for statistisk analyse. Spredning i enkeltparametre skal være sammenlignbare for genetisk modifisert plante og umodifisert plante**.** I rapporten er det anbefalt at spredningen i enkeltverdier bør ligge innenfor ± 20 %. Faggruppe for genmodifiserte organismer benytter denne anbefalingen som grunnlag for vurdering av forsøksresultatene.

3.2   Analyser av ernæringsmessige komponenter

Hovedkomponenter i soyabønne og fôr
Med unntak for analyser av fosfatider i lecitin, er valg av analyseparametere gjort i henhold til OECDs konsensusdokument for soya (OECD 2009). Det er foretatt en rekke analyser av hovedkomponenter i fôr og bønne, samt belg, olje, mel, rostet mel, proteinisolat og lecitin. Resultatene av analyser av hovedkomponenter i bønne og fôr er sammenlignet med analytiske data for soya fra databasen ILSI Crop Composition (ILSI 2008).

Det ble totalt analysert for 64 ulike komponenter, 57 i bønne og syv i fôr. Konsentrasjonen av ni av fettsyrene var imidlertid lavere enn påvisningsgrensene, noe som medførte at disse ble ekskludert fra de statistiske analysene.

Fôrfraksjon
Fôrfraksjonen ble analysert med hensyn på innhold av aske, fett, protein, kalorier, total fiber, vann, ADF (acid detergent fiber), NDF (neutral detergent fiber) og karbohydrater. Statistiske analyser over asteder viste ingen signifikante forskjeller mellom MON 87701 og den nær-isogene linjen for noen av de analyserte komponentene (tabell 4).

Tabell 4.   **Resultater fra analyser av fôrfraksjon fra testlinjen MON 87701 og kontrollinjen A5547. Fra feltforsøk i Argentina 2007/2008 og USA 2007.**

Komponenter analysert (g/100 g tørrvekt)	Argentina 2007/2008 Gj.snitt [Variasjonsområde]		USA 2007 Gj.snitt [Variasjonsområde]	
	Kontroll A5547	**MON 87701**	**Kontroll A5547**	**MON 87701**
Vann (% råvekt)	69,78 (62,76-72,79)	69,10 (59,81-73,17)	73,41 (69,4-78,1)	72,86 (70,1-76,8)
Aske	6,09 (5,56-6,53)	6,02 (5,45-6,84)	6,32 (5,10-8,13)	5,84 (5,05-7,46)
Fett	4,91 (3,73-7,22)	5,01 (3,45-6,99)	5,65 (4,23–7,23)	5,30 (3,60-6,82)
Protein	18,31 (14,93-20,38)	18,78 (15,87-21,34)	17,07 (14,20–23,3)	17,39 (13,56-20,034)
Karbohydrater	70,70 (66,44-73,87)	70,18 (65,73-74,36)	70,97 (63,68-74,26)	71,04 (68,29-76,80)
ADF	34,70 (29,71-46,90)	34,87 (30,51-40,14)	36,53 (27,42-42,06)	37,17 (30,04-58,25)
NDF	41,46 (35,55-50,64)	39,53 (32,80-44,60)	45,57 (34,24-64,19)	47,16 (37,02-55,99)

Soyabønne
Når det gjelder soyabønne er dett foretatt analyser av følgende komponenter: protein, fett, aske, karbohydrater, ADF, NDF, total fiber, råfiber, vann, karbohydrater, aminosyrer (18), fettsyrer (C14-C22), fosfor, jern, kalium, kalsium, magnesium, totalmengde vitamin E, isoflavoner (daidzein, glycitein, genistein), oligosakkaridene raffinose og stakyose, sekundære metabolitter og anti-næringsstoffene (lektiner, trypsinhemmer, og fytinsyre).

Hovedkomponenter i soyabønne
Følgende hovedkomponenter i soyabønne er analysert: vann, protein, fett, aske, ADF, NDF, råfiber og karbohydrater (tabell 5). Analysene ble utførte under god laboratoriepraksis (GLP) Det ble påvist signifikante forskjeller mellom MON 87701 og isogen kontroll for protein og karbohydrat ved analyse over steder i USA (p< 0,05). Tilsvarende forskjeller ble ikke vist i de argentinske feltforsøkene.

Tabell 5.   **Resultater fra analyser av hovedkomponenter og fiber i bønner fra testlinjen MON 87701 og kontrollinjen A5547. Fra feltforsøk i Argentina 2007/2008 og USA 2007.**

Komponenter analysert (g/100 g tørrvekt)	Argentina 2007/2008 Gj.snitt [Variasjonsområde]		USA 2007 Gj.snitt [Variasjonsområde]	
	Kontroll A5547	**MON 87701**	**Kontroll A5547**	**MON 87701**
Vann (% råvekt)	10,04 (8,70 - 11,63)	10,05 (9,53 – 11,25)	6,84 (5,44 - 8,74)	7,52 (5,86 - 10,70)
Aske	4,92 (4,46 - 5,25)	4,86 (4,57 - 5,24)	5,14 (4,70 - 5,88)	5,20 (4,70 - 5,90)
Fett	18,41 (17,59-19,28)	18,49 (18,05-19,35)	20,12 (17,24 - 22,55)	20,29 (17,33 - 23,08)
Protein	37,89 (36,35-39,62)	38,05 (36,05 - 39,20)	37,80 (32,29–41,87)	39,27 (36,49-42,23)
Karbohydrater	38,78 (36,73 - 40,36)	38,62 (36,73 – 39,92)	36,44 (29,88 - 43,48)	34,22 (21,58 - 39,61)
ADF	14,88 (12,61 - 19,45)	15,06 (13,30-20,51)	15,62 (14,00 - 19,02)	15,58 (13,53 - 17,05)
NDF	16,23 (15,00 - 17,53)	16,12 (14,22 – 19,09)	17,28 (15,02 - 22,45)	17,33 (15,06 - 21,80)

Fettsyresammensetning i soyabønne
Fettsyresammensetningen i soyabønne er målt i henhold til OECDs konsensusdokument for soya (OECD 2009). OECD anbefaler at følgende fettsyrer analyseres: palmitinsyre (C16:0), stearinsyre (C18:0), oljesyre (C18:1), linoljesyre (C18:2), linolensyre (C18:3) og arakidonsyre (C20:0). I henhold til søkers dokumentasjon er det analysert for innhold av totalt 14 fettsyrer i den transgene linjen MON 87701 (tabell 6). Innholdet av de enkelte fettsyrene ble sammenlignet både innen og over lokaliteter. Statistiske analyser over alle lokaliteter i USA viser signifikante forskjeller mellom testlinje og kontroll for fettsyren behensyre (p=0,022). Tilsvarende viser resultater fra forsøkene i Argentina signifikante forskjeller for linolensyre (p≤0,001). Resultatene viser imidlertid at forskjellene er små og innenfor toleranseintervallene som ble målt med grunnlag i referansesortene som inngikk i studien. Verdiene ligger også innenfor typiske verdier som er rapportert i litteraturen.

Aminosyrer i soyabønne
I henhold til dokumentasjonen er innholdet av både essensielle og ikke-essensielle aminosyrer analysert (tabell 7). Analysene er gjort i henhold til OECDs konsensusdokument for soya (OECD 2009). Statistiske analyser over steder i USA viser signifikante forskjeller mellom testlinje og kontroll for alanin (p=0,027), glycin (p=0,007), histidin (p<0,001), isoleucin (p=0,031), leucin (p=0,046), lysin (p=0,012), serin (p=0,004), treonin (p=0,024) og valin (p=0,04). Videre ble det påvist signifikante forskjeller for aminosyren tryptofan (p=0,001) i prøver fra de argentinske feltene. Verdiene for alle aminosyrene ligger imidlertid innenfor typiske verdier som er rapportert i litteraturen og toleranseintervallene for referansesortene som inngikk i studien.

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Tabell 6.   **Resultater fra analyser av fettsyrer i bønner fra testlinjen MON 87701 og kontrollinjen A5547. Fra feltforsøk i Argentina 2007/2008 og USA 2007.**

Komponenter analysert (% av totale fettsyrer)	Argentina 2007/2008 Gj.snitt (S.E.) [Variasjonsområde]		USA 2007 Gj.snitt (S.E.) [Variasjonsområde]	
	Kontroll A5547	**MON 87701**	**Kontroll A5547**	**MON 87701**
14:0 Myristinsyre p^1=0,203; p^2=0,769	0,084 (0,0017) [0,075 - 0,096]	0,086 (0,0017) [0,075 - 0,096]	0,094 (0,0031) [0,083 - 0,11]	0,093 (0,0031) [0,082 - 0,10]
16:0 Palmitinsyre p^1=0,570; p^2=0,359	11,43 (0,12) [10,80 - 12,04]	11,49 (0,12) [10,80 - 12,04]	11,88 (0,12) [11,50 - 12,13]	11,80 (0,12) [11,32 - 12,30]
16:1 Palmitolsyre p^1=0,468; p^2=0,372	0,087 (0,0033) [0,070 - 0,10]	0,084 (0,0033) [0,063 - 0,10]	0,095 (0,0033) [0,078 - 0,11]	0,092 (0,0033) [0,073 - 0,11]
17:0 Heptadekansyre p^1=0,309 p^2=0,553	0,11 (0,0025) [0,096 - 0,11]	0,11 (0,0025) [0,095 - 0,12]	0,093 (0,0021) [0,082 - 0,099]	0,094 (0,0021) [0,084 - 0,10]
18:0 Stearinsyre p^1=0,948; p^2=0,328	4,98 (0,11) [4,59 - 5,63]	4,98 (0,11) [4,76 - 5,35]	4,70 (0,22) [4,03 - 5,36]	4,59 (0,22) [3,97 - 5,36]
18:1 Oljesyre p^1=0,513; p^2=0,486	18,73 (0,32) [17,69 - 19,99]	18,64 (0,32) [17,84 - 19,93]	22,71 (1,28) [20,34 - 28,78]	22,35 (1,28) [19,21 - 26,64]
18:2 Linolsyre p^1=0,062; p^2=0,320	54,51 (0,33) [53,20 - 55,53	54,17 (0,33) [53,20 - 54,80]	51,76 (0,95) [47,18 - 54,07]	52,16 (0,95) [49,32 - 54,63]
18:3 Linolensyre p^1=<0,001; p^2=0,276	8,97 (0,20) [8,32 - 9,90]	9,34 (0,20) [8,58 - 9,91]	7,11 (0,45) [5,34 - 8,26]	7,24 (0,45) [5,55 - 8,41]
20:0 Arkidonsyre p^1=0,913; p^2=0,836	0,46 (0,012) [0,42 - 0,55]	0,46 (0,012) [0,42 - 0,52]	0,51 (0,025) [0,41 - 0,57]	0,51 (0,025) [0,41 - 0,58]
20:1 Gadolinsyre p^1=0,983; p^2=0,683	0,15 (0,0040) [0,13 - 0,19]	0,15 (0,0040) [0,13 - 0,17]	0,23 (0,012) [0,18 - 0,28]	0,24 (0,012) [0,19 - 0,28]
20:2 Eikosadiensyre p^1=0,870; p^2=0,585	0,046 (0,0040) [0,020 - 0,077]	0,042 (0,0040) [0,019 - 0,062]	0,042 (0,0030) [0,020 - 0,047]	0,040 (0,0030) [0,020 - 0,054]
22:0 Behensyre p^1=0,367; p^2=0,022	0,44 (0,0095) [0,39 - 0,49]	0,45 (0,0095) [0,40 - 0,50]	0,54 (0,028) [0,45 - 0,65]	0,56 (0,028) [0,46 - 0,65]
10:0 Kaprinsyre p^2=0,607	ikke analysert	ikke analysert	0,21 (0,014) [0,16 - 0,26]	0,20 (0,014) [0,14 - 0,25]
17:1 Heptadekensyre p^2=0,981	ikke analysert	ikke analysert	0,041 (0,0032) [0,019 - 0,047]	0,041 (0,0031) [0,023 - 0,048]

p^1= feltforsøk i 2007/2008; p^2=feltforsøk i 2007

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Tabell 7. **Resultater fra analyser av aminosyrer i bønner fra testlinjen MON 87701 og kontrollinjen A5547. Fra feltforsøk i Argentina 2007/2008 og USA 2007.**

Komponenter analysert (% av totale fettsyrer)	Argentina 2007/2008 Gj.snitt (S.E.) [Variasjonsområde]		USA 2007 Gj.snitt (S.E.) [Variasjonsområde]	
	Kontroll A5547	MON 87701	Kontroll A5547	MON 87701
Alanin p^1=0,636 p^2=0, 027	1,62 (0,021) [1,43 - 1,75]	1,63 (0,021) [1,55 - 1,78]	1,69 (0,029) [1,59 - 1,82]	1.72 (0,029) [1.66 - 1.84]
Arginin p^1=0,571 p^2=0,138	2,67 (0,062) [2,34 - 2,97]	2,70 (0,062) [2,41 - 3,03]	2,58 (0,069) [2,37 - 2,89]	2.68 (0,069) [2.36 - 3.00]
Asperginsyre p^1=0,700p^2=0,339	4,75 (0,095) [4,01 - 5,18]	4,79 (0,095) [4,22 - 5,62]	4,85 (0,10) [4,46 - 5,34]	4.90 (0,10) [4.61 - 5.26]
Cystin p^1=0,190 p^2=0,718	0,57 (0,018) [0,46 - 0,69]	0,54 (0,018) [0,41 - 0,70]	0,61(0,0051) [0,56 - 0,69]	0.62 (0,0051) [0.57 - 0.67]
Glutaminsyre p^1=0,521 p^2=0,177	7,19 (0,16) [5,49 - 7,82]	7,31 (0,16) [6,35 - 8,41]	7,53 (0,15) [6,89 - 8,26]	7.65 (0,15) [7.25 - 8.21]
Glycin p^1=0,804 p^2=0,007	1,63 (0,034) [1,42 - 1,77]	1,64 (0,034) [1,49 - 1,79]	1,70 (0,026) [1,64 - 1,85]	1.75 (0,026) [1.63 - 1.89]
Histidin p^1=0,818 p^2<0,001	1,04 (0,021) 0,90 - 1,19]	1,04 (0,021) [0,92 - 1,17]	1,08 (0,015) [1,03 - 1,15]	1.12 (0,015) [1.05 - 1.18]
Isoleucin p^1=0,586 p^2=0,031	1,69 (0,030) [1,41 - 1,84]	1,71 (0,030) [1,49 - 1,84]	1,76 (0,037) [1,64 - 1,96]	1.81 (0,037) [1.68 - 1.99]
Leucin p^1=0,551 p^2=0,046	2,81 (0,043) [2,41 - 3,01]	2,84(0,043) [2,61 - 3,06]	2,94 (0,066) [2,73 - 3,29]	3.04 (0,066) [2.82 - 3.36]
Lysin p^1=0,509 p^2=0,012	2,49 (0,045) [2,22 - 2,75]	2,53 (0,045) [2,28 – 2,85]	2,62 (0,060) [2,42 - 2,91]	2.74 (0,060) [2.48 - 2.99]
Metionin p^1=0,118 p^2=0,754	0,50 (0,014) [0,42 - 0,59]	0,47 (0,014) [0,39 - 0,55]	0,53 (0,012) [0,47 - 0,59]	0.53 (0,012) [0.48 - 0.58]
Fenylalanin p^1=0,478 p^2=0,073	1,90 (0,038) [1,63 - 2,10]	1,93(0,038) [1,75 - 2,17]	2,04 (0,056) [1,91 - 2,38]	2.15 (0,056) [1.91 - 2.48]
Prolin p^1=0,849 p^2=0,082	1,93 (0,029) [1,71 - 2,06]	1,94 (0,029) [1,80 - 2,12]	1,96 (0,035) [1,85 - 2,12]	2.01 (0,035) [1.86 - 2.19]
Serin p^1=0,533 p^2=0,004	1,89(0,033) [1,51 - 2,06]	1,91 (0,033) [1,74 - 2,07]	1,96 (0,032) [1,87 - 2,13]	2.03 (0,032) [1.90 - 2.19]
Threonin p^1=0,601 p^2=0,1024	1,47 (0,024) [1,23 - 1,60]	1,48 (0,024) [1,35 - 1,59]	1,55 (0,020) [1,49 - 1,68]	1.60 (0,020) [1.50 - 1.72]
Tryptophan p^1=0,001 p^2=0,102	0,49 (0,0058) [0,41 - 0,51]	0,52 (0,0058) [0,49 - 0,56]	0,50 (0,0068) [0,46 - 0,53]	0.51 (0,0068) [0.47 - 0.54]
Tyrosin	1,01 (0,033)	0,99 (0,033)	1,10 (0,034)	1.13 (0,034)

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p^1=0,686 p^2=0,213	[0,74 - 1,22]	[0,85 - 1,26]	[0,98 - 1,22]	[0.96 - 1.13]
Valin p^1=0,622 p^2=0,040	1,81 (0,04) [1,50 - 1,94]	1,83 (0,040) [1,59 - 2,00]	1,86 (0,032) [1,76 - 2,04]	1.92 (0,032) [1.80 - 2.07]

p^1= feltforsøk i 2007/2008; p^2=feltforsøk i 2007

Vitaminer
OECDs konsensusdokument har ikke satt opp vitaminer som komponenter det skal måles for i soya (OECD 2009). Konsensusdokumentet inkluderer imidlertid en oversikt der vitaminene folinsyre, vitamin B1, vitamin B2, vitamin E og vitamin K inngår. I OECD-dokumentet blir det fremhevet at soyabønne er en god kilde til vitaminene folinsyre og vitamin K, og at soyaolje en god kilde til vitamin K. Data vedrørende disse vitaminene kommer fra de internasjonale databasene ILSI, USDA Nutrient database og Stuttgart. Dokumentasjonen knyttet til foreliggende søknad inneholder kun analyser av vitamin E. Det ble påvist signifikante forskjeller mellom testlinje og den nær-isogene kontrollen ved analyse over steder i USA og Argentina for dette vitaminet (p<0.001) (tabell 8). Verdiene ligger imidlertid innenfor typiske verdier som er rapportert i litteraturen, samt toleranseintervallene for referansesortene som inngikk i studien.

Tabell 8   **Resultater fra analyser av vitamin E i prøver fra testlinjen MON 87701 og kontrollinjen A5547. Fra feltforsøk i Argentina 2007/2008 og USA 2007.**

Komponenter analysert (g/100 g tørrvekt)	Argentina 2007/2008 Gj.snitt [Variasjonsområde]		USA 2007 Gj.snitt [Variasjonsområde]	
	Kontroll A5547	MON 87701	Kontroll A5547	MON 87701
Vitamin E	3,42 [2,87-4,11]	4,40 [3,61 – 5,29]	6,24 [4,88- 7,94]	7,69 [6,36 – 9,62]

Mineraler
OECDs konsensusdokument har ikke satt opp mineraler som komponenter det skal måles for i soya (OECD 2009). Konsensusdokument inneholder imidlertid en oversikt der mineralene fosfat, jern, kalium, kalsium, kobber, mangan, magnesium, natrium og sink inngår. I OECD- dokumentet blir det fremhevet at soyabønne er en god kilde til mineralene jern, kalsium, kalium og magnesium. Data vedrørende disse mineralene kommer fra de internasjonale databasene ILSI, USDA Nutrient database, NRC (US National Research Council) og Stuttgart. Søkers dokumentasjon inneholder ingen analyser av mineraler.
Søkers dokumentasjon inneholder ingen analyser av mineraler.

Isoflavoner (fytoøstrogener)
Valget av analyseparametere er gjort i henhold til OECDs konsensusdokument for soya (OECD 2009). I henhold til søkers dokumentasjon er det målt for følgende isoflavoner: daidzein, glycetein, og genistein. Det er funnet statistisk signifikante forskjeller for daidzein (p=0,04) over alle lokaliteter i USA, men ikke i Argentina.. Søker har kun beregnet totalmengdene for isoflavongruppene daidzeiner, genisteiner og glyciteiner over steder (tabell 9).

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Tabell 9.   **Resultater fra statistiske analyser av ulike isoflavoner i prøver fra testlinjen MON 87701 og kontrollinjen A5547. Fra feltforsøk i Argentina 2007/2008 og USA 2007.**

Komponenter analysert (g/100 g tørrvekt)	Argentina 2007/2008 Gj.snitt [Variasjonsområde]		USA 2007 Gj.snitt [Variasjonsområde]	
	Kontroll A5547	**MON 87701**	**Kontroll A5547**	**MON 87701**
Daidzein	934,74 [826,96 - 1095,41]	960,33 [930,62 - 1090,89]	604,88 [198,95 - 830,65]	667,54 [188,96 - 983,26]
Genistein	858,71 [757,45 - 976,36]	886,75 [778,99 - 960,43]	594,58 [244,95 - 760,87]	655,57 [214,73 - 863,84]
Glycitein	184,78 [136,52 - 217,42]	200,02 [143,11 - 252,60]	156,93 [61,28 - 227,25]	164,87 [61,08 - 228,79]

Oligosakkarider og antinæringsstoffer
Valget av analyseparametere er gjort i henhold til OECDs konsensusdokument for soya (OECD 2009) (tabell 10). Variansanalyse over steder viser signifikante forskjeller mellom MON 87701 og kontroll for komponentene stakyose og trypsinhemmer både i USA og Argentina. Verdiene ligger innenfor typiske verdier som er rapportert i litteraturen.

Tabell 10.   **Resultater fra analyser av oligosakkarider og antinæringsstoffer fra testlinjen MON 87701 og kontrollinjen A5547. Fra feltforsøk i Argentina 2007/2008 og USA 2007.**

Komponenter analysert	Argentina 2007/2008 Gj.snitt (SD) [Variasjonsområde]		USA 2007 Gj.snitt (SD) [Variasjonsområde]	
	Kontroll A5547	**MON 87701**	**Kontroll A5547**	**MON 87701**
Lektin (H.U./mg råvekt)	3,46 (0,43) [1,60 - 8,39]	2,70 (0,42) [1,48 - 5,03]	0,72 (0,19) [0,28 - 1,28]	0,96 (0,19) [0,062 - 2,01]
Fytinsyre (% tørrvekt)	1,55 (0,12) [1,02 - 2,10]	1,46 (0,12) [0,99 - 1,92]	1,97 (0,12) [1,31 - 2,66]	1,85 (0,12) [1,39 - 2,29]
Raffinose (% tørrvekt)	1,15 (0,039) [0,98 - 1,29]	1,14 (0,039) [0,95 - 1,35]	1,34 (0,19) [0,43 - 1,85]	1,33 (0,19) [0,49 - 1,70]
Stakyose (% tørrvekt)	4,02 (0,11) [3,14 - 4,38]	3,82 (0,11) [3,35 - 4,21]	4,93 (0,63) [2,27 - 6,65]	4,59 (0,63) [1,83 - 6,42]
Trypsinhemmer (TIU/mg t.v.)	27,21 (1,42) [23,45 - 30,96]	27,77 (1,41) [18,89 - 33,26]	28,57 (1,24) [22,49 - 34,20]	26,06 (1,23) [21,65 - 32,53]

Fosfolipider
I OECDs konsensusdokument for soya er det foreslått at fosfatider, som er en sekkebetegnelse på fosfolipider, bør måles i lecitin. Konsensusdokumentet spesifiserer imidlertid ikke hvilke fosfolipider som bør analyseres. Søker har ikke analysert for innhold av fosfolipider i soyabønner og i lecitin.

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Prosesserte produkter
Soyabønne blir prosessert til en rekke ulike formål, hovedsakelig fôr, olje og mel (OECD 2009) (vedlegg 1). Avfettet rostet mel blir hovedsakelig benyttet til fôr, mens avfettet mel blir brukt som mat. De ulike proteinfraksjonene fra avfettet soyamel blir brukt i forskjellige matvarer for mennesker. Soyaolje brukes primært i næringsmidler, som matolje og i salatdressinger.

Analyser av soyaolje
I dokumentasjonen fra Monsanto er det ikke presentert data fra analyser av soyaolje. I henhold til OECDs konsensusdokument (OECD, 2009) er soyaolje en god kilde til vitamin K.

Kommentarer fra FG3-medlemmer:
Grønne bladgrønnsaker, vegetabilske oljer og plantemargarin er gode kilder for vitamin K. Et fermentert soyaprodukt er en god kilde for vitamin K_2, som er formen som produseres av bakterier. Vitamin K_2 produseres også av bakterier i menneskets tarm. Litteraturstudier utført av EFSA viser at gjennomsnittinntaket hos voksne i europeiske land og USA varierer fra 60 til 250 mikrogram/dag (EFSA 2008). Anbefalt inntak for både barn og voksne er 1 mikrogram/kg kroppsvekt/dag (EFSA 2008).

Etter faggruppens oppfatning er analyser av vitamin K i soyaolje ikke påkrevet. Dette fordi vitamin K-mangel er svært sjelden eller ikke eksisterende hos voksne.

Analyser av allergener fra soya.
I OECDs konsensusdokument for soya er ett av kapitlene viet allergener i soya. Soyabønner inneholder ca. 16 proteiner som binder IgE (L'Hocine & Boye 2007), og disse betraktes som potensielle allergener. Både uraffinert og raffinert soyaolje er vist å inneholde allergene proteiner (Ramazzoti *et al.* 2008).

OECDs konsensusdokument gir ingen anbefalinger med hensyn på analyser av mulige allergene proteiner i soya.

3.3   Agronomiske egenskaper

Registreringer av agronomiske karakterer ble foretatt i feltforsøk i USA og Argentina i 2007 og 2007/2008. De nordamerikanske forsøkene ble etablert på 16 ulike lokaliteter i sentrale dyrkingsområder for soya i USA. Den konvensjonelle soyalinjen A5547, med tilsvarende genetisk bakgrunn som testlinjen men som ikke uttrykker Cry1Ac-protein, ble benyttet som umodifisert kontroll i forsøkene. I tillegg var det inkludert fire kommersielle soyasorter som referansesorter på hvert av forsøksfeltene, til sammen 25 ulike sorter. Testlinje, komparator og referansesorter ble plantet i randomiserte forsøksdesign med tre gjentak per lokalitet. Hver forsøksrute bestod av åtte planterekker, der registreringer av fenotypiske karakterer ble foretatt på to av rekkene. I henhold til søker var dyrkingsregimet for øvrig i tråd med vanlig praksis i den enkelte region der forsøkene var lokaliserte.

De argentinske feltforsøkene ble lagt ut på åtte ulike lokaliteter i områder der en forventer at den kommersielle produksjonen av soyalinjen MON 87701 i hovedsak vil foregå. Forsøksdesign og – opplegg er i hovedsak tilsvarende de nordamerikanske feltforsøkene.

I dokumentasjonen fra søker er det presentert data fra registreringer av fenotypiske og agronomiske karakterer, samt samspill med en rekke miljøfaktorer. Fenotypiske karakterer som ble vurdert inkluderte kvantitative karakterer som vitalitet hos frøplanten, plantetetthet, plantehøyde, legde, tidlighet (målt som antall dager til 50 % blomstring), frøstørrelse og frøavling. I tillegg ble det gjort observasjoner av ulike karakterer knyttet til resistens mot ulike biotiske (sjukdommer, skadedyr) og abiotiske stressfaktorer. I henhold til søkers dokumentasjon er det foretatt statistiske analyser over

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lokaliteter for hver av de fenotypiske karakterene. Det er også kjørt statistiske analyser innen hvert av forsøksstedene. Det er ikke foretatt statistiske sammenligninger mellom testlinje og referansesorter, men det er beregnet referanseområde for hver karakter basert på minimums- og maksimumsverdier for de kommersielle sortene.

Resultatene fra de nordamerikanske forsøkene viste ingen signifikante forskjeller mellom testlinje MON 87701 og konvensjonell kontroll for noen av de undersøkte karakterene ved analyse over forsøkssteder (tabell 11). Statistiske analyser innen lokaliteter viste signifikante forskjeller mellom MON 87701 og kontroll for 20 av 140 sammenligninger (p<0,05) (data ikke vist).

Resultatene fra variansanalysen over forsøksfelt i Argentina viser signifikante forskjeller mellom MON 87701 og nær-isogen kontroll for tre av de observerte variablene (p<0,05) (tabell 12). Plantetetthet tidlig i vekstsesongen og vanninnhold i frø ved høsting var signifikant lavere hos den transgene soyalinjen sammenlignet med umodifisert kontroll. Tilsvarende ble det dokumentert signifikant høyere testvekt hos testlinjen sammenlignet med kontroll. Forskjellene i plantetetthet på vekststadium V2-V4 hadde imidlertid ingen effekt på avling eller plantetetthet ved høsting. Gjennomsnittsverdiene for disse karakterene ligger også innenfor variasjonsområdene for referansesortene som var inkludert feltforsøkene. For de øvrige agronomiske karakterene ble det ikke påvist forskjeller mellom MON 87701 og kontroll.

I henhold til søker ble seks karakterer knyttet til abiotisk stress og sjukdomsresistens evaluert på hver av de åtte forsøkslokalitetene fire ganger i løpet av vekstsesongen. Valg av skadegjørere og øvrige parametere ble valgt ut fra relevans i den enkelte region i den aktuelle dyrkingsperioden. Dokumentasjonen inkluderer ikke resultater fra disse observasjonene, og det er ikke utført statistisk analyse av dette datamaterialet. I henhold til søker ble det, med unntak av mottagelighet for mosaikkvirus ved en lokalitet en gang i løpet av vekstsesongen, ikke observert forskjeller mellom testlinje og kontroll for noen av karakterene som ble observert.

3.4   Vurdering basert på tilgjengelig datagrunnlag

Analyser av ernæringsmessige komponenter er i hovedsak utført i tråd med OECDs konsensusdokument for soya (OECD 2009). Det er påvist statistisk signifikante forskjeller mellom soya MON 87701 og kontroll i enkeltparametere. Forskjellene er imidlertid ikke konsistente over forsøksfelt, og verdiene for de analyserte komponentene ligger innenfor typiske verdier for andre soyasorter som er rapportert i litteraturen, og innenfor variasjonsområdene til kommersielle referansesorter som er inkludert i søkers dokumentasjon. Faggruppen konkluderer med at forskjellene som er påviste ikke har ernæringsmessig betydning.

Faggruppen påpeker at søker ikke har foretatt analyser av fosfatider i lecitin. Det understrekes at konsensusdokumentet i størst mulig grad skal følges når det legges fram dokumentasjon på nivåene av næringsstoffer, antinæringsstoffer og metabolitter.

Når det gjelder antinæringsstoffene, har søker slått sammen resultatene for de enkelte isoflavoner innenfor hver av isoflavongruppene. Det er stor variabilitet innenfor hver gruppe, og faggruppen etterlyser statistiske analyser for hvert enkelt forbindelse innenfor hver isoflavongruppe.

Feltforsøk over en vekstsesong viser signifikante forskjeller mellom MON 87701 og umodifisert kontroll for enkelte av de agronomiske karakterene som er evaluert. Gjennomsnittsverdiene for disse karakterene ligger imidlertid innenfor variasjonsområdene for referansesortene som var inkludert feltforsøkene. Søker viser til at forskjellene som er påviste er uten biologisk relevans, og konkluderer med morfologisk og agronomisk ekvivalens mellom den transgene linjen MON 87701 og nær-isogen kontroll.

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Tabell 11. **Resultater fra variansanalyse over steder for fenotypiske og agronomiske karakterer for testlinjen MON 87701, nær-isogen kontroll A5547, samt kommersielle referansesorter. Fra feltforsøk i USA vekstsesongen 2007.**

Fenotypiske karakterer	MON 87701		Kontroll A5547		Test-kontroll	Variasjonsområde referansesorter inkl. RR-sorter[2]		Variasjonsområde konvensjonelle referansesorter[3]	
	Gj. snitt	S.E.	Gj. snitt	S.E.	p-verdi[1]	Min.	Max.	Min.	Max.
Plantetetthet (V2-V4) (antall pl. i 2 av planterk.)	230,7	7,90	233,1	6,19	0,8679	135,9	298,0	135,9	274,9
Frøplantevitalitet (V2-V4) (1-9)	3,7	0,20	3,8	0,20	0,6353	2,3	5,8	2,7	5,8
Tidlighet (R1-R2) (antall dager til 50 % blomstring)	206,5	1,69	206,7	1,67	0,3920	197,6	219,7	200,2	219,7
Plantehøyde (R8) (cm)	81	0,61	78	0,52	0,1075	49,3	102,4	49,3	102,4
Legde (R8) (0-9)	2,0	0,31	1,8	0,24	0,5639	0,0	7,3	0,0	7,3
Tap av knopper (R8) (0-9)	0,6	0,15	0,4	0,09	0,2039	0,0	2,0	0,0	2,0
Plantetetthet (R8)	206,2	8,09	211,7	7,01	0,5621	111,5	284,8	111,5	257,3
Vanninnhold frø (høsting) (%)	13,1	0,40	12,7	0,36	0,2567	10,0	14,7	10,0	14,7
100-frøvekt (g)	16,8	0,36	16,5	0,34	0,4909	13,2	20,6	13,2	20,6
Testvekt (kg/L)	0,71	0,75	0,71	0,83	0,2276	0,65	0,78	0,65	0,78
Avling (kg/ha)	3063	3,14	3164	2,79	0,2320	1174	4595	1174	4595

[1] *: p<0,05
[2] Minimums- og maksimumsverdier for 23 kommersielt tilgjengelige referansesorter, inkludert Roundup Ready (RR)-soya 40-3-2.
[3] Minimums- og maksimumsverdier for 20 konvensjonelle, kommersielle referansesorter.

Tabell 12. **Resultater fra variansanalyse over steder for fenotypiske og agronomiske karakterer for testlinjen MON 87701, nær-isogen kontroll A5547, samt kommersielle referansesorter. Fra feltforsøk i Argentina vekstsesongen 2007/2008.**

Fenotypiske karakterer	MON 87701		Kontroll A5547		Test-kontroll	Variasjonsområde konvensjonelle referansesorter[3]	
	Gj. snitt	S.E.	Gj. snitt	S.E.	p-verdi[1]	Min.	Max.
Plantetetthet (V2-V4) (antall pl. i 2 av planterk.)	96,9*[1]	5,42	105,9	5,50	0,0468	103,8	204,0
Frøplantevitalitet (V2-V4) (1-9)	2,2	0,37	2,1	0,35	0,8288	1,0	6,0
Tidlighet (R1-R2) (antall dager til 50 % blomstring)	48,2	0,17	48,2	0,25	0,8125	39,0	52,3
Plantehøyde (R8) (cm)	99,8	3,65	95,3	3,39	0,1805	73,9	124,2
Legde (R8) (0-9)	3,9	0,29	3,3	0,27	0,0990	0,5	6,7
Tap av knopper (R8) (0-9)	0,2	0,08	0,2	0,08	0,9541	0,0	0,7
Plantetetthet (R8)	90,5	5,38	97,1	5,20	0,1200	86,0	190,3
Vanninnhold frø (høsting) (%)	11,0*	0,21	11,6	0,18	0,0020	9,4	13,2
100-frøvekt (g)	15,8	0,21	16,0	0,23	0,6108	10,9	18,5
Testvekt (kg/L)	171,3*	0,95	169,2	1,14	0,0144	157,0	181,4
Avling (kg/ha)	2,7	0,13	2,5	0,14	0,0523	1,3	3,7

[1] *: $p < 0,05$

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4 Dokumentasjon av toksisitet, allergenisitet og næringsverdi

4.1 Toksisitet

Akutt oral toksisitetsstudie på mus ved eksponering av renfremstilt Cry1A-c protein
Monsanto har utført en akutt-toksisk studie på mus (studie **CRO-2007-325**) med oral eksponering av Cry1Ac-protein produsert av bakterien *E. coli*. Forsøket er utført i henhold til god laboratoriepraksis (GLP) (EU-direktiv 88/320/EC) og akutt oral toksisitetsretningslinjene fra U.S. EPA og OECD (U.S. EPA Health Effects Test Guidelines, OPPTS 870.1100; Acute Oral Toxicity (August 1998), OECD Guideline for Testing of Chemicals; Method No. 420: Acute Oral Toxicity-Fixed Dose Method; July 17, 1992). Hunn- og hannmus fra stamme Crl:CD-1 ble eksponert for 1290 mg Cry1Ac-protein/kg kroppsvekt. Som kontroll ble det benyttet bovint serumalbumin (BSA) (1280 mg/kg kroppsvekt). Samtlige forsøksdyr ble observert for kliniske tegn på forgiftning over en periode på 15 dager. Observasjonene ble foretatt to ganger daglig på arbeidsdager og en gang daglig i helger.

Følgende parametre ble vurdert: "Unormal oppførsel ved håndtering", pels, skinn, holdning (posture), spyttavsondring, respirasjon, aktivitet/våkeperiode (arousal level), kramper, skjelving, unormale bevegelser, unormalt ganglag ("gait abnormals"), tåreflyt, palpebral closure, exophthalmus, vurdering av avføring og urin, samt pupillestørrelse. Ved avslutning av forsøket ble alle dyrene avlivet, og bukhule, toraks, samt en rekke organer og vev ble undersøkt makroskopisk. Det ble ikke påvist økt mortalitet eller toksiske effekter på dyrene som ble eksponert for Cry1Ac. Det ble imidlertid påvist signifikant redusert kroppsvekt hos hannmus, men ikke hos hunnmus. Monsanto har foretatt en ny studie på hannmus med 1460 mg Cry1Ac/kg kroppsvekt. Denne studien viste ingen redusert kroppsvekt hos dyr fôret med transgent protein sammenlignet med kontrollgruppen. NOAEL for hann- og hunnmus ble satt til henholdsvis 1460 og 1290 mg Cry1Ac-protein/kg kroppsvekt. For BSA ble NOAEL satt til 1280 mg/kg kroppsvekt.

Kommentarer fra arbeidsgruppe mat & fôr:
"Acute oral toxicity"-studier (OECD 401 eller 423) er ikke anbefalte studier for beregning av NOAEL, siden disse studiene utføres i løpet av 14 dager. Fra 14 dagers akuttstudier bestemmes LD50, ikke NOAEL. I henhold til OECD retningslinje 401 og 423 "Acute oral toxicity", skal det benyttes tre doser pr kjønn der den høyeste dosen er 2000 mg substans/kg kroppsvekt.

I studien CRO-2007-325 er det oppgitt en EC50, som heller burde ha vært benyttet av søker. Faggruppen påpeker at NOAEL skal vurderes ut fra en 90 dagers repetert dosestudie. I disse sub-kroniske studiene er det benyttet to doser av henholdsvis testfôr og umodifisert kontrollfôr. Faggruppen påpeker at i 90-dagersstudien er forsøksdyrene eksponert over et så langt tidsrom at eventuelle uheldige helseeffekter ville blitt oppdaget. Rottene er også tilført høyere konsentrasjoner av Cry1Ac enn hva mennesker (ca. 5 ganger), og dyr ville vært eksponert for i en naturlig ernæringsmessig situasjon.

Fôringsforsøk på broiler
Søknaden inneholder dokumentasjon fra ett 42-dagers fôringsforsøk på Cobb x Cobb 800 broilere. Det ble benyttet totalt 900 dyr. Forsøksdyrene ble fordelt på 9 grupper med 100 dyr per gruppe, halvparten av hvert kjønn (studie **MSL0021800**). Soyabønner som ble benyttet i dette forsøket kommer fra feltforsøk utført i Arkansas, Illinois og Nebraska vekstsesongen 2007. Dyrene ble fôret med prosessert mel fra den transgene linjen MON 87701, isogen kontrollinje A5547 og 6 umodifiserte kommersielle sorter. Soyamelet ble undersøkt for mykotoksiner og pesticider, uten at det ble påvist mykotoksiner, organofosfater, organonitrogener, organoklorider eller metylkarbamater. I tidlig vekstfase (0-21 dager) var andelen soyamel i fôret ca. 33 %, i mellomvekstfasen og avslutningsfasen (21-42 dager) ca. 30 %. Fôringsforsøket ble utført i 2009. Følgende parameter ble undersøkt: mortalitet, vektøkning og

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fôreffektivitet. Skrott, bryst, lår, leggmuskel, vinger, og abdominalt fett ble målt både som gjennomsnittlig vekt og som % av kroppsvekt til avkjølte, avlivede broilere. I henhold til søker ble det ikke påvist statistisk signifikante forskjeller mellom testlinjen MON 87701, kontrollinjen A5547 og de seks umodifiserte referansesortene.

Fôringsforsøk på rotter -90 dager
Monsanto har utført to 13 ukers (90 dagers) toksisitetstester på gnagere (rotter) med fôr som inneholder mel fra MON 87701 og A5547 (**studie WIL-50352,2009 og WIL-50362, 2009**).

1. Studie WIL-50352, 2009

Fôringsforsøket inkluderte hann- og hunnrotter, total 5 grupper à 12 rotter/kjønn. Forsøksdyrene ble fôret med standard rottefôr (PMI certified Rodent LabDiet #5002) tilsatt 30 % soyamel. Standard rottefôr inneholder normalt 15 % soyamel. LabDiet of Purina Mills Inc. omformulerte standardfôret slik at det ble tilpasset et innhold med 30 % soya. Dette fôret ble tilsatt henholdsvis soya MON 87701, en umodifisert kontrollsort (A5547) og tre kommersielle umodifiserte referansesorter (Anand, UA4805,Ozark). Fôringsforsøket er utført i henhold til GLP (U.S. EPA FIFRA 40 CFR part 160), samt OECDs retningslinjer nummer 408 subkroniske tester på dyr (Guidelines for Testing of Chemicals, Health Effects Test Guidelines, Section 408), U.S. EPA, OPPTS 870.3100 90-Day Oral Toxicity in Rodents, Health Effects Test Guidelines (1998) og Commission Directive 2001/59/EC, Part B.26, Methods for the Determination of Toxicity (2001).

Alle forsøksdyrene ble observert to ganger daglig for døde eller døende individer. Kroppsvekt og fôrinntak ble målt på alle dyrene en gang per uke. Gjennomsnittlig inntak av MON 87701 for henholdsvis hanndyr og hunndyr var 22,0 g/kg kroppsvekt/dag og 25,5 g/kg kroppsvekt/dag. Det ble utført detaljerte kliniske undersøkelser, målinger av fôrinntak, makroskopiske og mikroskopiske undersøkelser av organene, samt klinisk patologisk undersøkelser av urin og blod fra samtlige dyr i hver gruppe ved avliving. Det ble ikke påvist vesentlige endringer i de undersøkte parametrene. I forhold til hunnrotter i kontrollgruppen, ble det påvist signifikante lavere kroppevekt hos hunnrotter (4,7 % - 9,6 %) fôret med MON 87701. De statistiske forskjellene ble påvist i uke 6 og ukene 9 til 13. Det ble imidlertid ikke påvist signifikante forskjeller i kroppsvekt mellom kontroll- og hannrotter i testgruppen. I henhold til søker ble det ikke påvist andre testrelaterte kliniske endringer.

2. Studie WIL-50362, 2009

Fôringsforsøket inkluderte både hann- og hunnrotter, totalt 4 grupper à 20 rotter/kjønn. Standard rottefôr inneholder normalt 15 % soyamel. LabDiet of Purina Mills Inc. omformulerte standardfôret slik at det ble tilpasset et innhold med 15 % og 30 % soya. Dette fôret ble tilsatt henholdsvis soya MON 87701 og en umodifisert kontrollsort (A5547).

Studien inkluderte to grupper eksponert for fôr med MON 87701, hhv 15 og 30 % modifisert soya, og to kontrollgrupper eksponert for fôr tilsatt 15 % og 30 % umodifisert kontroll A5547. Det ble ikke brukt kommersielle, umodifiserte referansesorter i dette fôringsforsøket. Fôringsforsøket er utført i henhold til GLP (U.S. EPA FIFRA 40 CFR part 160), samt OECDs retningslinjer nummer 408 subkroniske tester på dyr (Guidelines for Testing of Chemicals, Health Effects Test Guidelines, Section 408), U.S. EPA, OPPTS 870.3100 90-Day Oral Toxicity in Rodents, Health Effects Test Guidelines (1998) og Commission Directive 2001/59/EC, Part B.26, Methods for the Determination of Toxicity (2001).

Alle forsøksdyrene ble observert to ganger daglig for døde eller døende individer. Kroppsvekt og fôrinntak ble målt på alle dyrene en gang per uke. Gjennomsnittlig inntak av MON 87701 var 10,8- og 21,1 g/kg kroppsvekt/dag for hanndyr, og 12,0- og 24,3 g/kg kroppsvekt/dag for hunndyr. Det ble utført detaljerte kliniske undersøkelser, målinger av fôrinntak, makroskopiske og mikroskopiske undersøkelser av organene, samt klinisk patologisk undersøkelser av urin og blod fra alle dyr i hver gruppe ved avliving. Det ble ikke påvist noen vesentlige endringer i de undersøkte parametrene. I

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forhold til hunnrotter, både kontroll og testrotter, ble det påvist noe høyere kroppsvekt hos hannrotter (både kontroll og testrotter). I henhold til søker ble det ikke påvist testrelaterte kliniske endringer.

Kommentarer fra arbeidsgruppen:
Arbeidsgruppen påpeker at NOAEL burde vurderes ut fra 90-dagers repeterte dosestudier. I disse sub-kroniske studiene er det benyttet to doser av henholdsvis testfôr og umodifisert kontrollfôr. Forsøksdyrene er eksponert over et så langt tidsrom at eventuelle uheldige helseeffekter ville blitt oppdaget. Rottene er også eksponert for høyere konsentrasjoner av Cry1Ac enn hva mennesker (>4 og >7 ganger for henholdsvis barn og voksne, 97,5 persentil (tabell 12)), og dyr ville vært eksponert for i en naturlig ernæringsmessig situasjon.

4.2   Allergenisitet

Cry1Ac-protein
Med enkelte unntak, er proteiner som er matallergener generelt varme- og syrestabile. Proteinene er stabile både overfor mage- og tarmsafter, og er ofte hovedprotein-komponenter i matvaren. Typiske mengder er fra 1 til 80 % av proteininnholdet. Cry1Ac-proteinet som er benyttet i undersøkelsene for allergenisitet er produsert av bakterien *E. coli*. Proteinet er modifisert slik at det er identisk med proteinet som er i MON 87701. Cry1Ac er testet i simulert mage (SGF)- og tarmsaft (SIF). Mengde Cry1Ac i SGF- og SIF-analysene var henholdsvis 176 μg og 280 μg. Nedbrytning av bakterielt produsert Cry1Ac i SGF ved pH 2 er hurtig, og Cry1Ac protein ble degraderer fullstendig innen 30 sekunder. Påvisningsgrensen med fargestoffet Brilliant Blue G på SDS-PAGE gel er 0,0025 μg. Cry1Ac-proteinet ble fragmentert innen 5 minutter i SIF (pH 7,5). Den trypsinresistente delen av proteinet var stabilt i mere enn 24 timer. Påvisningen av Cry1Ac og fragmenter fra proteinet er utført med Western-blot ved bruk av antistoff mot proteinet. Søker hevder med grunnlag i disse testene at proteinet også brytes raskt ned i menneskets mage- og tarmkanal.

Søkers dokumentasjon inkluderer en undersøkelse av glykosylering av Cry1Ac-proteinet. Cry1Ac-protein ble renfremstilt fra 1 kg frø (bønne) fra MON 87701-planter. Analyse av eventuelle bundne sukkermolekyler på Cry1Ac-protein ble foretatt med en metode fra firmaet Molecular Probe. Det ble ikke påvist sukkermolekyler på Cry1Ac- proteinet.

På bakgrunn av at det ikke ble funnet sekvenshomologi til allergene proteiner, glykosyleringsseter på Cry1Ac-proteinet, samt at mengden av Cry1Ac-protein i bønner fra MON 87701 er målt til ca. 0,0013 % av totalt proteininnhold, konkluderes det med at det er lite sannsynlig at Cry1Ac- proteinet vil utgjøre et allergent potensiale for mennesker.

Bt-proteiner
Flesteparten av *Bacillus thuringiensis* (*Bt*)-plantevernmidler inneholder krystalltoksiner (protoksin) og levende sporer fra *Bt*-bakterien (EHC 1999). Laboratoriestudier med pattedyr indikerer ingen potensielle allergiske reaksjoner mot *Bacillus thuringiensis* eller dets komponenter, innbefattet delta (δ)-endotoksinet i krystallproteinet (EHC 1999). Til tross for vel 50 års bruk av plantevernmidler med *Bacillus thuringiensis* var. *kurstaki* er det med ett unntak, ingen bekreftede rapporter over øyeblikkelige eller forsinkede allergiske reaksjoner. Dette til tross for betydelig human oral-, dermal- og inhalasjonseksponering (EHC 1999). Helseundersøkelse av en liten gruppe gårdsarbeidere (48 arbeidere) som brukte *Bt*-plantevernmidler viste ved hudtesting en signifikant reaksjon mot *Bt*-sporeekstrakter i forhold til lav- og medium *Bt*-eksponeringsgrupper (Bernstein *et al*. 1999). Lav- og medium-eksponeringsgruppene var ikke i direkte kontakt med *Bt*-plantvernmidler. Positiv hudtest mot *Bt* pro-δ-endotksiner ble også påvist hos to arbeidere i gruppen som benyttet *Bt*-plantevernmidler (Bernstein *et al*. 1999).

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4.3   Vurdering basert på tilgjengelig datagrunnlag

Cry1Ac-proteinet, som uttrykkes som følge av genmodifiseringen, har ingen likheter med kjente allergener eller egenskaper som tilsier at de kan virke som allergener. Basert på testene som er omtalt i søkers dokumentasjon, dvs. at det i proteinet ikke er påvist aminosyresekvenser som er lik allergene proteiners epitoper, at proteinet brytes raskt ned av mage-tarmsaft, samt at konsentrasjonen av Cry1Ac-protein er svært lav (mindre enn 0,002 %), anser faggruppen det som lite trolig at proteinet medfører et signifikant større potensiale for utvikling av matallergi hos mennesker sammenlignet med umodifisert soya. Akutte fôringsstudier (oral sondefôring) på mus med reinfremstilt Cry1Ac-protein, 42 – og 90 dagers fôringsforsøk med henholdsvis broilere og rotter viste ingen skadelige helseeffekter.

5   Vurdering av søkers dokumentasjon, kunnskapshull

Adjuvans (fremming av immunreaksjon mot andre stoffer)
Det har ikke vært utført immunologiske studier med de transgene produktene. Det er vist at Cry1Ac-proteinet binder seg til musetarmoverflaten (Vazquez-Padron *et al*. 2000a) og induserer immunologiske reaksjoner mot seg selv og mot proteiner gitt samtidig (Vazquez *et al*. 1999). Immunologisk kartlegging av systemisk og mukosal immunreaksjon på Cry1Ac har videre påvist at mus lager både systemisk IgM, IgG og sekretorisk IgA etter intraperitonal og intragastrisk immunisering (Vazques-Padron *et al*. 2000b). I en annen studie er det vist at Cry1Ac hadde utpreget mukosal adjuvanseffekt ved å potensere IgM-, IgG- og IgA-responsen mot hepatittvirusantigen og bovint serumalbumin som ble gitt med sondefôring samtidig med Cry1Ac (Vazquez *et al*. 1999). Produksjonen av IgE-antistoff, som er knyttet til allergisk reaksjon, ble ikke målt. Også i tidligere studier (Prasad & Shetna 1975) er det påvist adjuvanseffekt av krystallprotein fra *Bacillus thuringiensis*. Adjuvanseffekten av Cry1Ac er bekreftet ved intranasal og intraperitoneal immunisering i to senere publikasjoner med henholdsvis pneumokokk-antigen (Moreno-Fierros *et al*. 2003) og amøbe-lysat (Rojas-Hernández *et al*. 2004). Adjuvanseffekten av Cry1Ac ble funnet å være like sterk som adjuvanseffekten av koleratoksin (Vazques-Padron *et al*. 1999), som er et mye brukt slimhinneadjuvans i eksperimentelle studier av vaksinasjon og av allergi, og som regnes for å være det sterkeste slimhinneadjuvans vi kjenner.

I en senere studie av Guimaraes *et al*. 2008 (Guimaraes *et al.* 2008) undersøkte man adjuvanseffekter av Cry1Ab i forbindelse med allergisk sensibilisering overfor peanøttekstrakt (PE). Koleratoksin (CT), som er en kjent Th2 adjuvant, ble benyttet for sammenligning. I disse forsøkene ble det ikke indusert spesifikk IgE ved bruk av Cry1Ab som adjuvant, mens induksjon av spesifikk IgE ble påvist ved bruk av CT som adjuvant. Imidlertid viste den samme undersøkelsen at når PE ble gitt sammen med Cry1Ab så medførte dette at når musene på et senere tidspunkt ble provosert med PE så økte produksjon av leukotriener (CTC4 og CTE4) i bronkiene umiddelbart. I tillegg ble det 36 timer etter provokasjonen målt økt produksjon av Th2- og Th17 cytokiner og økt influks av neutrofiler og eosinofiler. Det ble dermed konkludert med at Cry1Ab har en adjuvant effekt i forhold til allergi.

Det gjennomsnittlige inntaket av tørket soyabønne er beregnet av Monsanto Company (tabell 13). Det daglige inntaket av disse matvarene i Europa er beregnet som g/person/dag, mens 97,5 persentilen for høyt inntak på verdensbasis er beregnet som g/kg kroppsvekt/dag.

Tabell 13.   Estimater over inntak av soya fra WHO GEMS/Food Programme.

| | DAGLIG INNTAK (g/person/dag) | | | HØYT INNTAK (g/kg kroppsvekt/dag) | |
| | Gjennomsnittlig inntak per capita i EU | | | Høyest 97,5 percentil | |
Råvare	Cluster B (Sør-Europa)	Cluster E (Sentral-Europa)	Cluster F (Nord-Europa)	Generell befolkning	Barn ≤ 6 år
Soyabønne (tørket)	36,4	35,3	39,2	3,03	5,55

Kilde: Monsanto Company, søknad EFSA/GMO/BE/2010/79.

Spesielle målgrupper, som barn, har et større inntak av soya enn det beregnede gjennomsnittlige inntaket på verdensbasis. Det estimerte inntaket for store porsjoner, 97,5 persentil, for barn under 6 år er beregnet til 5,55 g/kg kroppsvekt/dag, mens inntaket for den generell befolkningen er beregnet til 3,03 g/kg kroppsvekt/dag (tabell 13). Teoretiske beregninger fra søker viser at dersom hele soyainntaket (97,5 persentilen) kommer fra MON 87701 vil dette kunne medføre et inntak for den generelle befolkningen på 18,4 µg/kg kroppsvekt/dag og for barn som er lik eller yngre enn 6 år på 33,7 µg/kg kroppsvekt/dag. Den totale inntaksmengden av Cry1Ac for voksent individ som veier 60 kg og et barn på 6 års som veier 21 kg, vil bli henholdsvis ca. 1100 - og 710 µg/dag. For de estimerte inntaksmengdene av Cry-protein som er presentert her er det ikke tatt hensyn til eventuelle nedbrytning av proteinet ved prosessering. De estimerte inntaksmengdene av Cry-proteinet antas å representere den høyest tenkelige mengden. Tilsvarende konsentrasjoner av Cry1Ac som er vist å gi mukosal adjuvanseffekt ved sondefôring av mus er fra 0,1 µg til 100 µg per mus (Vazquez *et al*. 1999).

Adjuvansdosene som benyttes for immunisering av mus og mennesker i andre sammenhenger (ved injeksjoner) er ofte av samme størrelsesorden, det vil si at om lag samme dose (ca. 10 µg) brukes til mus og menneske. Det er tvilsomt om denne sammenligningen kan overføres til tarmimmunisering siden den effektive konsentrasjonen av Cry-protein/tarmareal vil bli langt lavere hos menneske enn mus. Teoretisk sett kan konsentrasjonen av Cry1Ac i soya føre til økt utvikling av allergi mot matvarer spist sammen med soyaen, foruten mot soya i seg selv. Man ville vente at adjuvanseffekten kom til syne først og fremst som økt forekomst av allergi mot de matvarene der matallergi fra før er vanligst. IgE ble ikke målt i de refererte studiene av adjuvanseffekt av Cry1Ac-proteinet.

Et realistisk inntak av Cry-protein vil være vesentlig lavere enn de mengdene som er angitt ovenfor. Soya er en bulkvare hvor flere typer soya fra mange åkre samles i felles siloer før videre prosessering. Man vil således aldri spise 100 % MON 87701. Stort sett spiser vi prosessert soyaprodukter hvor, i mange tilfeller, Cry-proteinet er helt eller delvis degradert eller er fjernet. Søker oppgir at Cry1Ac - proteinet brytes raskt ned i magesaft. Eksponering av tarmepitel for Cry1Ac-proteinet forventes dermed å være marginal.

Cry proteiner er ikke varmestabile. I maisgrøt er det for eksempel vist at konsentrasjonen av Cry1Ab ble redusert med 90 % etter 3 minutters oppvarming ved 75 °C. Proteinet kunne ikke påvises etter steking av tortillas ved 190 °C i 10 s (de Luis *et al.* 2009). Dette innebærer igjen at eksponering av tarmepitel for Cry proteiner vil være marginal hvis maten er kokt eller stekt.

Induksjon av IgE er ikke vist for Cry1Ac Det finnes lite litteratur på området omkring betydning av adjuvanter for induksjon av IgE-mediert allergisk respons. Den foreliggende litteratur tyder i flere tilfeller på at betydningen er liten. I en musemodell for allergiutvikling mot lupin ga bruk av koleratoksin (CT) økt immunrespons for andre klasser av immunglobuliner, men ingen IgE respons.

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Forfatterne antyder at IgE-respons er mer avhengig av indre egenskaper ved allergenene, og ikke relatert til CT-adjuvans (Foss *et al.* 2006). I en lignende rottemodell viste CT også kun en begrenset effekt på utvikling av peanøttallergi (de Jonge *et al.* 2007). De Jonge *et al.* viste også at det var krevende å klare å indusere allergi i rottene. Rotter som gikk på streng diett i 3 generasjoner ga IgE respons, mens rotter som gikk på allergenfri diett i én generasjon ga ikke IgE respons etter indusering. Disse forsøkene indikerer en begrenset betydning av adjuvans for utvikling av IgE-mediert allergi. Utvikling av matallergi skyldes et komplekst samspill av faktorer som genetisk predisposisjon, alder ved introduksjon av allergenet, amming, sammensetning av ernæring, sammensetning av tarmfloraen og infeksjonsstatus i mage-tarmsystemet (van Wijk & Knippels 2007).

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6 Innspill til EFSA GMO Extranet søknad EFSA/GMO/BE/2010/79

General comments

There seems to be a misprinting on page 169 in the Technical Dossier where it is stated: "The mean levels of Cry1Ac protein in harvest seed is 4.9%".According to Table 7 and Table 8 the mean Cry1Ac levels are 4.7 µg/g dw and 5.1 µg/g dw, respectively. The mean Cry1Ac level should therefore be 4.9 µg/g dw, not 4.9%, since 4.9% is 49 mg/g dw.

D.07.08

Toxicology

In the acute toxicity studies in mice, the applicant has used a single dose of Cry1Ac-protein, i.e. 1290 mg/kg body weight. According to the OECD guideline 401 "Acute oral toxicity", a limit test at one dose level of at least 2000 mg/kg bodyweight should be carried out. In the OECD guideline 423 it is recommended to use 3 doses.

"Acute oral toxicity" studies (OECD 401 or 423) is not recommended studies for evaluation of NOAEL since these studies are only performed for 14 days and will not be able to show long term effects of the substance. According to toxicological practice NOAEL is the lowest dose where there is no adverse effect, i.e. at least one higher dose has to give an adverse effect. The Norwegian GMO Panel is of the opinion that the applicant should use neither NOAEL nor MOE (margin of exposure) when assessing potential health risk from dietary exposure of Cry1Ac derived from MON 87701.

D 7.09

Allergenicity:

According to the applicant the epitope test shows that Cry1Ac protein does not share structurally and immunologically relevant amino acid sequence similarities with known allergens, and that the Cry-protein has no similarities to IgE epitopes of allergenetic proteins. However, this Cry-protein has immunogenic potential to elicit strong IgG-response (Vazquez et al.1999) and the induction of IgG antibodies to food antigen and even crosspriming against a bystander antigen may be of biological significance (Brandtzaeg, 2010). Experimental studies both *in vitro* and *in vivo* have demonstrated that IgG antibodies that are not balanced by a mucosal IgA response can enhance the epithelial penetration of bystander proteins (Brandzaeg, 2010).

Due to remaining uncertainty that Cry1Ac may enhance systemic and mucosal immune responses to co-administered antigens, the Norwegian GMO Panel still sees the need for further clarification on the possible role of Cry proteins as adjuvants.

Brandtzaeg, P. (2010) Food allergy: separating the science from the mythology. Nat. Rev. Gastroenterol. Hepatol. 7, 380–400; doi:10.1038/nrgastro.2010.80

Vazquez RI. Moreno-Fierros L. Neri-Bazan L. De La Riva GA. Lopez-Revilla R., 1999. *Bacillus thuringensis* Cry1Ac protoxin is a potent systemic and mucosal adjuvant. Scand J Immunol., 49: 578-84.

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Vurdering basert på tilgjengelig datagrunnlag

Analyser av ernæringsmessige komponenter er i hovedsak utført i tråd med OECDs konsensusdokument for soya (OECD 2009). Det er påvist statistisk signifikante forskjeller mellom soyahybrid MON 87701 og kontroll i enkeltparametere. Forskjellene er imidlertid ikke konsistente over forsøksfelt, og verdiene for de analyserte komponentene ligger innenfor typiske verdier for andre soyasorter som er rapportert i litteraturen, og innenfor variasjonsområdene til kommersielle referansesorter som er inkludert i søkers dokumentasjon. Faggruppen konkluderer med at forskjellene som er påvist ikke har ernæringsmessig betydning.

Faggruppen påpeker at søker ikke har foretatt analyser av fosfatider i lecitin. Det understrekes at konsensusdokumentet i størst mulig grad skal følges når det legges fram dokumentasjon på nivåene av næringsstoffer, antinæringsstoffer og metabolitter.

Når det gjelder antinæringsstoffene, har søker slått sammen resultatene for de enkelte isoflavoner innenfor hver av isoflavongruppene. Det er stor variabilitet innenfor hver gruppe, og faggruppen etterlyser statistiske analyser for hvert enkelt forbindelse innenfor hver isoflavongruppe.

Cry1Ac-proteinet som uttrykkes som følge av genmodifiseringen, har ingen likheter med kjente allergener eller egenskaper som tilsier at proteinet kan virke som allergen. Basert på testene som er omtalt i søkers dokumentasjon, dvs. at det i proteinet ikke er påvist aminosyresekvenser som er lik allergene proteiners epitoper, at proteinet brytes raskt ned av mage-tarmsaft, samt at konsentrasjonen av Cry1Ac- protein er svært lave (mindre enn 0,002 % av total proteinmengde), anser faggruppen det som lite trolig at proteinet medfører et signifikant større potensiale for utvikling av matallergi hos mennesker sammenlignet med umodifisert soya.

Akutte 14 dagers fôringsstudier (oral sondefôring EPA-OPPTS (870.1100)) på mus med reinfremstilt Cry1Ac-protein, 90 dagers fôringsforsøk på rotter og 42 dagers fôringsforsøk med broilere viste ingen skadelige helseeffekter. Søker har ikke utført toksisitetsstudier på fisk med fôr som inneholder soya MON 87701.

Faggruppen påpeker at 90-dagers repeterte dosestudier bør benyttes ved fastsettelse av NOAEL. I disse sub-kroniske studiene er det brukt minst to doser av testfôret, samt kontrollfôr av umodifisert soya. Forsøksdyrene er eksponert over et så langt tidsrom at eventuelle uheldige helseeffekter ville blitt oppdaget. Rottene er også eksponert for høyere konsentrasjoner av Cry1Ac enn hva mennesker og dyr ville vært eksponert for i en naturlig ernæringsmessig situasjon.

På bakgrunn fra forsøk med Cry1Ac-protein som er dokumentert i denne søknaden, konkluder faggruppen med at det er lite sannsynlig at eksponering for Cry1Ac-protein i seg selv, og i de mengder som tilføres via fôr fra den genmodifisert soyaen, vil føre til helseskade hos dyr.

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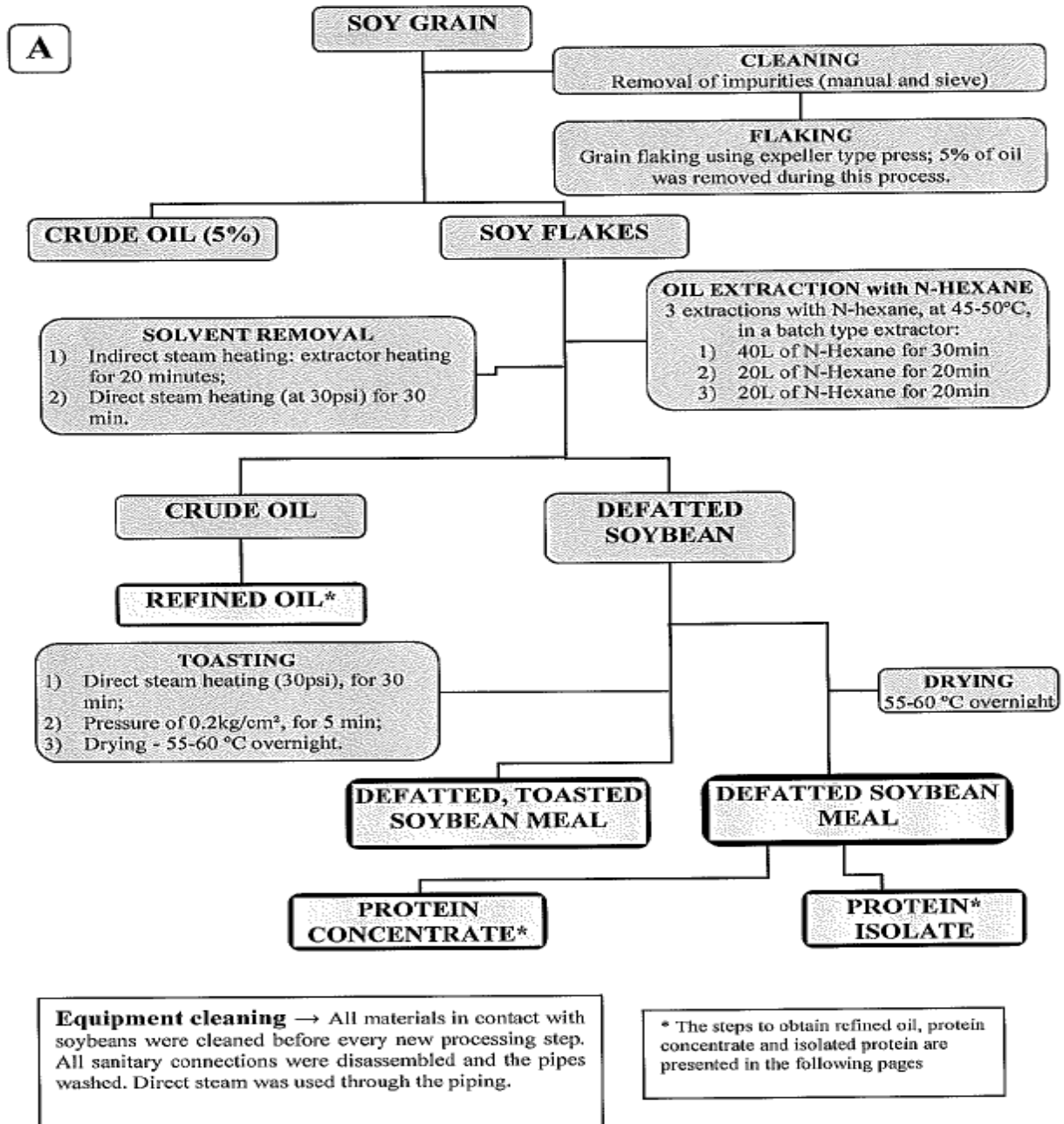
Vedlegg 1

Prosessering av soyabønne.
Ved prosessering av soyabønne dannes det en rekke produkter, som olje, proteinisolat, proteinkonsentrat, avfette rostet mel, avfettet mel, fôrprodukter m.m., se oversikt hentet fra OECDs soyadokument. Søker har lagt ved oversikter over produksjon av de forskjellige fraksjonene som ble produsert hos ITAL, se oversikt merket henholdsvis figur A, B og C.
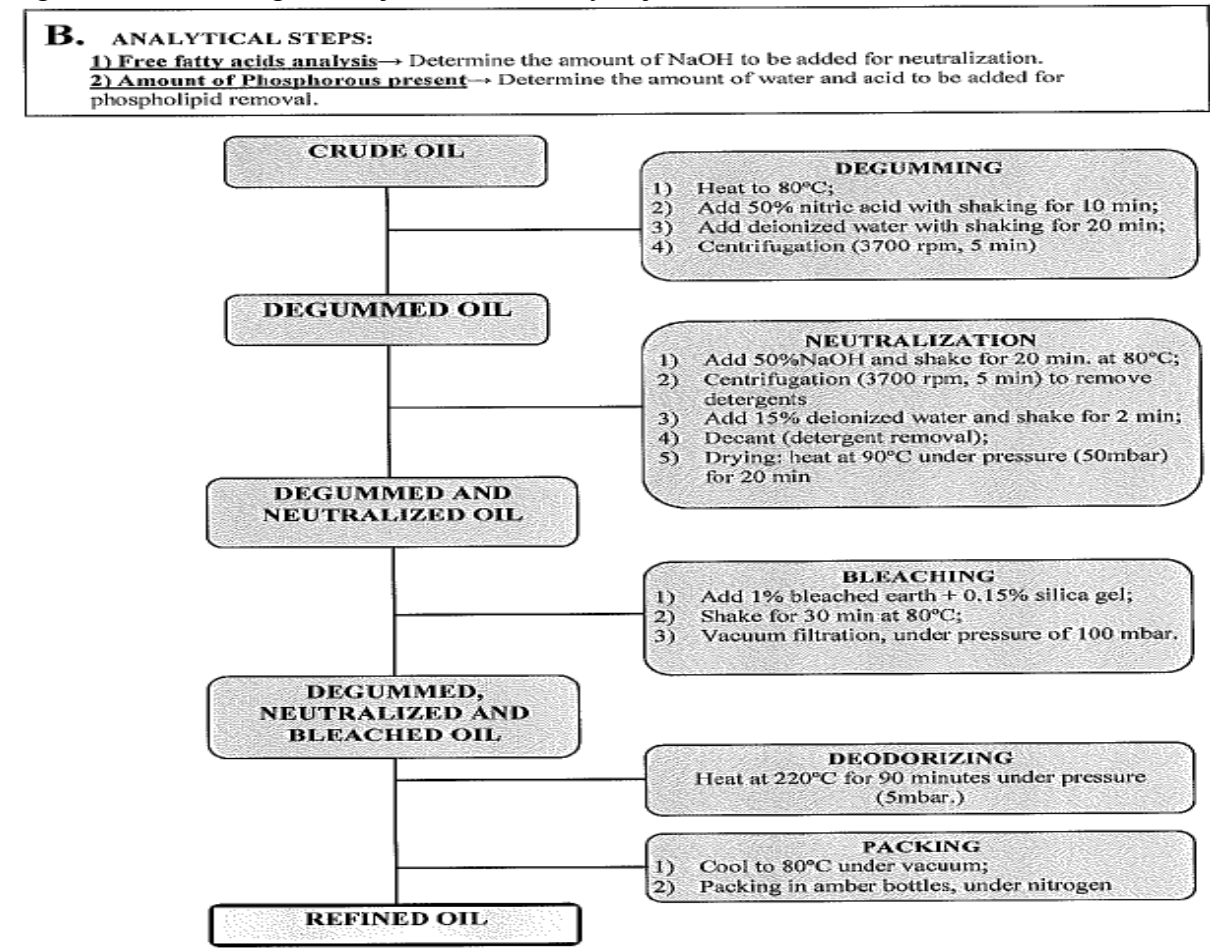
WHOLE SOYBEAN PROCESSING
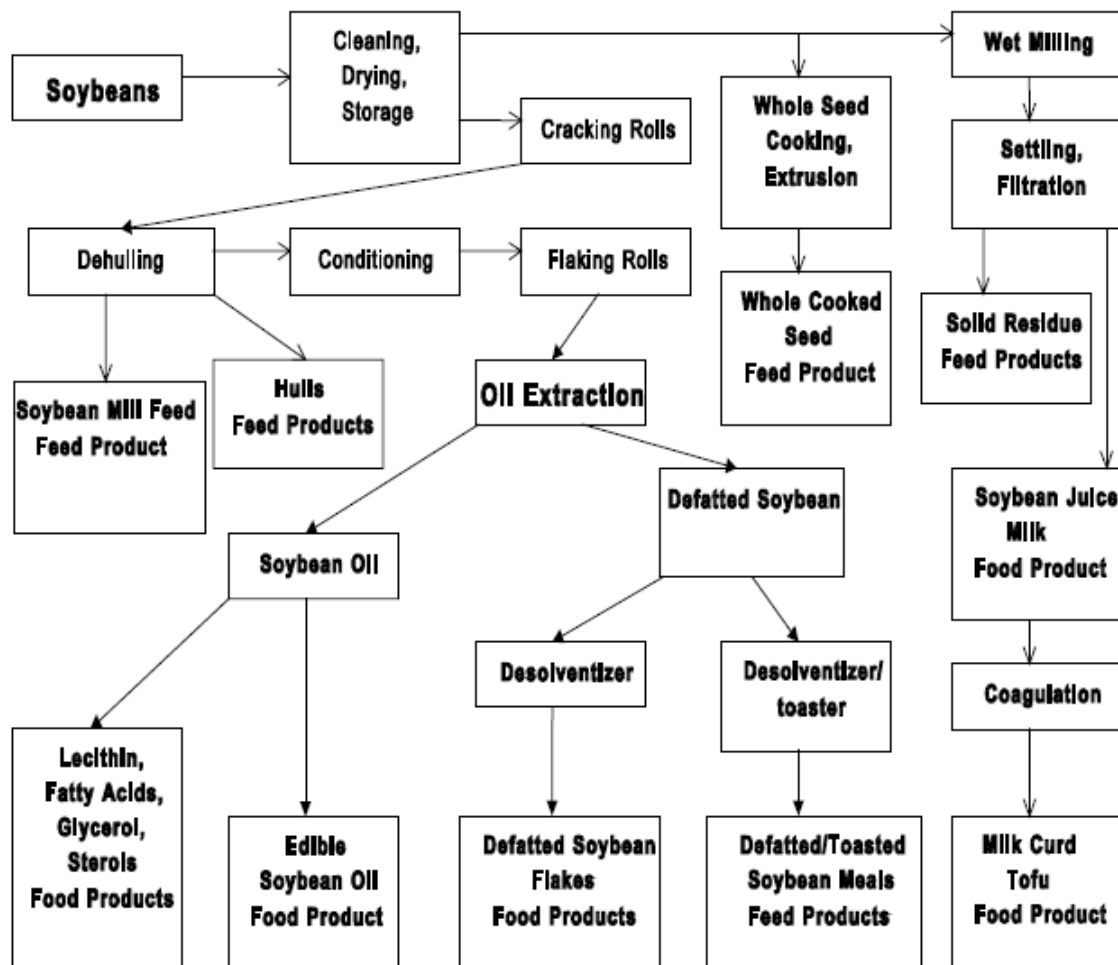
Søkers skjematisk fremstilling av prosessering av soyabønne (figur A), soyaolje (figur B) og proteinisolat og proteinkonsentrat (figur C).

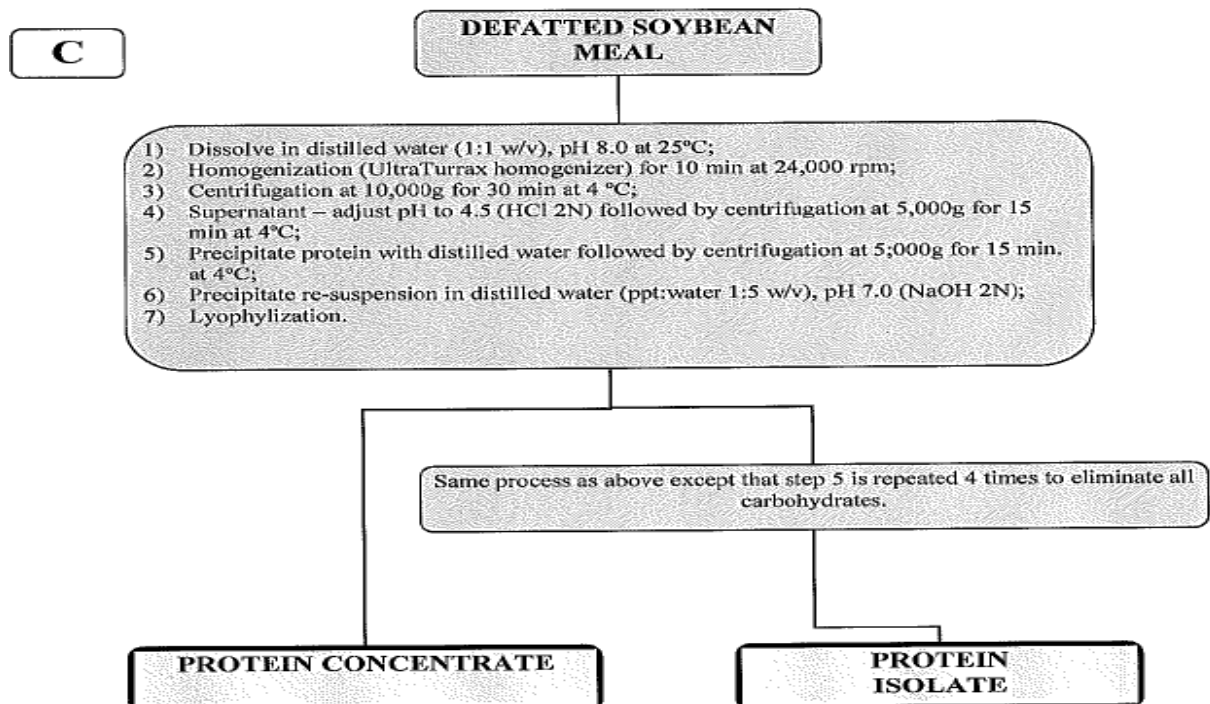Figur A. Prosessering av soyabønne

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Figur B: Prosessering av råolje til raffinert soyaolje

B. ANALYTICAL STEPS:
1) Free fatty acids analysis→ Determine the amount of NaOH to be added for neutralization.
2) Amount of Phosphorous present→ Determine the amount of water and acid to be added for phospholipid removal.

CRUDE OIL

DEGUMMING
1) Heat to 80°C;
2) Add 50% nitric acid with shaking for 10 min;
3) Add deionized water with shaking for 20 min;
4) Centrifugation (3700 rpm, 5 min)

DEGUMMED OIL

NEUTRALIZATION
1) Add 50%NaOH and shake for 20 min. at 80°C;
2) Centrifugation (3700 rpm, 5 min) to remove detergents
3) Add 15% deionized water and shake for 2 min;
4) Decant (detergent removal);
5) Drying: heat at 90°C under pressure (50mbar) for 20 min

DEGUMMED AND NEUTRALIZED OIL

BLEACHING
1) Add 1% bleached earth + 0.15% silica gel;
2) Shake for 30 min at 80°C;
3) Vacuum filtration, under pressure of 100 mbar.

DEGUMMED, NEUTRALIZED AND BLEACHED OIL

DEODORIZING
Heat at 220°C for 90 minutes under pressure (5mbar.)

PACKING
1) Cool to 80°C under vacuum;
2) Packing in amber bottles, under nitrogen

REFINED OIL

Figur C: Prosessering av avfettet soyamel til proteinisolat og proteinkonsentrat.

C

DEFATTED SOYBEAN MEAL

1) Dissolve in distilled water (1:1 w/v), pH 8.0 at 25°C;
2) Homogenization (UltraTurrax homogenizer) for 10 min at 24,000 rpm;
3) Centrifugation at 10,000g for 30 min at 4 °C;
4) Supernatant – adjust pH to 4.5 (HCl 2N) followed by centrifugation at 5,000g for 15 min at 4°C;
5) Precipitate protein with distilled water followed by centrifugation at 5;000g for 15 min. at 4°C;
6) Precipitate re-suspension in distilled water (ppt:water 1:5 w/v), pH 7.0 (NaOH 2N);
7) Lyophylization.

Same process as above except that step 5 is repeated 4 times to eliminate all carbohydrates.

PROTEIN CONCENTRATE

PROTEIN ISOLATE

EFSA/GMO/BE/2010/79 – MON 87701

Appendix II

SCIENTIFIC OPINION

Scientific Opinion on application (EFSA-GMO-BE-2010-79) for the placing on the market of insect resistant genetically modified soybean MON 87701 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This scientific opinion is an evaluation of a risk assessment of the genetically modified, insect resistant, soybean MON 87701 for food and feed uses, import and processing. Soybean MON 87701 was developed through *Agrobacterium*-mediated transformation. It contains a single insert consisting of a *cry1Ac* expression cassette, encoding the Cry1Ac protein that confers resistance against specific lepidopteran insects. The stability of the insert was confirmed over multiple generations. Bioinformatic analyses of the insert and its flanking regions, and levels of newly expressed protein did not raise safety concerns. Comparative analyses of compositional, phenotypic and agronomic characteristics indicated that soybean MON 87701 is not different from its conventional counterpart (A5547) and equivalent to commercial soybean varieties, except for having an increased vitamin E content (still within normal ranges) and expressing the Cry1Ac protein. The safety assessment of the Cry1Ac protein and soybean MON 87701 identified no concerns regarding potential toxicity and allergenicity. A feeding study on broiler chickens confirmed that defatted soybean MON 87701 meal is as nutritious as conventional defatted soybean meal. There are no indications of an increased likelihood of establishment and spread of feral soybean plants. Considering its intended use as food and feed, environmental risks associated of an unlikely but theoretically possible horizontal gene transfer from soybean MON 87701 to bacteria have not been identified. Potential interactions of soybean MON 87701 with the biotic and abiotic environment were not considered due to the low level of exposure. The monitoring plan and reporting intervals are in line with the intended uses of soybean MON 87701. The EFSA GMO Panel considers that the soybean MON 87701, as described in this application, is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment in the context of its intended uses.

KEY WORDS

GMO, soybean (*Glycine max*), MON 87701, insect resistant, Cry1Ac, human and animal health, import and processing, Regulation (EC) No 1829/2003

SUMMARY

Following the submission of an application (EFSA-GMO-BE-2010-79) under Regulation (EC) No 1829/2003 from Monsanto, the Panel on Genetically Modified Organisms of the European Food Safety Authority (EFSA GMO Panel) was asked to deliver a scientific opinion on the safety of insect resistant genetically modified (GM) soybean MON 87701 (Unique Identifier MON-877Ø1-2) for food and feed uses, import and processing.

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-BE-2010-79, additional information supplied by the applicant, scientific comments submitted by the Member States, and relevant scientific publications. The scope of application EFSA-GMO-BE-2010-79 is for food and feed uses, import and processing of soybean MON 87701 within the European Union as any non-GM soybean but excludes cultivation in the EU. The EFSA GMO Panel evaluated soybean MON 87701 with reference to the intended uses and appropriate principles described in its Guidance Document of the Scientific Panel on Genetically Modified Organisms for the Risk Assessment of Genetically Modified Plants and Derived Food and Feed (EFSA, 2006a). The scientific evaluation of the risk assessment included molecular characterisation of the inserted DNA and expression of the corresponding proteins. An evaluation of the comparative analysis of composition, phenotypic and agronomic characteristics was undertaken, and the safety of the new proteins and the whole food/feed was evaluated with respect to potential toxicity, allergenicity and nutritional wholesomeness. An evaluation of the environmental impacts and the post-market environmental monitoring plan were undertaken.

Soybean MON 87701 was transformed using *Agrobacterium tumefaciens*. Soybean MON 87701 expresses the *cry1Ac* gene leading to the production of the Cry1Ac insecticidal crystal protein (δ-endotoxin). The Cry1Ac protein provides protection from feeding damage caused by specific lepidopteran pests in the soybean.

The molecular characterisation data establish that the genetically modified soybean MON 87701 contains one copy of an intact *cry1Ac* expression cassette. No other parts of the plasmid used for transformation are present in the transformed plant. Results of the bioinformatic analysis of the 5' and 3' flanking sequences and ORFs spanning the newly created DNA junctions did not indicate any safety concern. The stability of the inserted DNA was confirmed over several generations and a Mendelian inheritance pattern was demonstrated.

The EFSA GMO Panel compared the composition, phenotype and agronomic characteristics of soybean MON 87701 and its conventional counterpart (A5547), assessed all statistical differences identified, and came to the conclusion that soybean MON 87701 is compositionally not different from its conventional counterpart except for having an increased vitamin E content (still within the normal range of soybeans) and expressing the Cry1Ac protein. Except for expressing the Cry1Ac protein, soybean MON 87701 is also compositionally and agronomically equivalent to commercial soybean varieties. The risk assessment of the newly expressed protein and the whole crop included an analysis of data from analytical and bioinformatics studies, as well as in vitro and in vivo studies. The EFSA GMO Panel concluded that the soybean MON 87701 is as safe as its conventional counterpart and that the overall allergenicity of the whole plant is not changed.

The application EFSA-GMO-BE-2010-79 concerns food and feed uses, import, and processing. Therefore, there is no requirement for scientific information on possible environmental effects associated with the cultivation of soybean MON 87701. There are no indications of an increased likelihood of establishment and spread of feral soybean plants in case of accidental release into the environment of viable soybean MON 87701 grains during transportation and processing for food and feed uses. Taking into account the scope of the application, both the rare occurrence of feral soybean plants and the low levels of exposure through other routes indicate that the risk to target and non-

target organisms is extremely low. The unlikely but theoretically possible transfer of the recombinant gene from soybean MON 87701 to environmental bacteria does not raise concern due to the lack of a selective advantage in the context of its intended uses. The scope of the post-market environmental monitoring plan provided by the applicant is in line with the intended uses of soybean MON 87701. Furthermore, the EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

In conclusion, the EFSA GMO Panel considers that the information available for soybean MON 87701 addresses the scientific issues indicated by the Guidance document of the EFSA GMO Panel and the scientific comments raised by the Member States, and that the soybean MON 87701 is as safe as its conventional counterpart with respect to potential effects on human and animal health or the environment in the context of its intended uses.

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BACKGROUND

On 17 May 2010, the European Food Safety Authority received from the Belgian Competent Authority an application (Reference EFSA-GMO-BE-2010-79) for authorisation of genetically modified (GM) soybean MON 87701 (Unique Identifier MON-877Ø1-2) submitted by Monsanto within the framework of Regulation (EC) No 1829/2003 on GM food and feed[4]. After receiving the application EFSA-GMO-BE-2010-79 and in accordance with Articles 5(2)(b) and 17(2)b of Regulation (EC) No 1829/2003, EFSA informed the Member States and the European Commission, and made the summary of the application publicly available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 11 June 2010, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the European Commission and consulted nominated risk assessment bodies of the Member States, including the national Competent Authorities within the meaning of Directive 2001/18/EC[5], following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member States had three months after the date of receipt of the valid application (until 11 September 2010) within which to make their opinion known.

The Scientific Panel on Genetically Modified Organisms of EFSA (EFSA GMO Panel) carried out an evaluation of the scientific risk assessment of the GM soybean MON 87701 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. The EFSA GMO Panel carried out the safety evaluation in accordance with the appropriate principles described in the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006a). In addition, the scientific comments of the Member States, the additional information provided by the applicant, and relevant scientific publications were taken into consideration.

On 21 June 2010, 15 November 2010, and 04 March 2011, the EFSA GMO Panel requested additional information from the applicant. The applicant provided the requested information on 01 July 2010, 03 January 2011, and 15 March 2011.

In giving its opinion on soybean MON 87701 to the European Commission, the Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of six months from the acknowledgement of the valid application. As additional information was requested by the EFSA GMO Panel, the time-limit of 6 months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

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

TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific assessment of soybean MON 87701 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation

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[4] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. Official Journal of the European Communities, L268, 1-23.
[5] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. Official Journal of the European Communities, L106, 1-38.

(EC) No 1829/2003. Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of GMOs or food/feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environment and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)e of Regulation (EC) No 1829/2003.

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

ASSESSMENT

1.   Introduction

The GM soybean MON 87701 (Unique Identifier MON-877Ø1-2) was evaluated with reference to its intended uses, taking account of the appropriate principles described in the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006a). The evaluation of the risk assessment presented here is based on the information provided in the application, as well as additional information from the applicant, scientific comments submitted by the Member States and relevant scientific publications.

2.   Issues raised by Member States

The issues raised by the Member States are addressed in Annex G of the EFSA overall opinion[6] and have been considered in this scientific opinion.

3.   Molecular characterisation

3.1.   Evaluation of relevant scientific data

3.1.1.   Transformation process and vector constructs[7]

Meristem tissue excised from embryos of germinated seeds of conventional soybean A5547 was transformed with the binary plasmid PV-GMIR9 using *Agrobacterium tumefaciens* (renamed *Rhizobium radiobacter*) strain ABI. The plasmid PV-GMIR9 contained two T-DNAs. T-DNA I contained the *cry1Ac* expression cassette that provides insecticidal activity against specific lepidopteran insects. T-DNA II contained the CP4 *epsps* cassette conferring tolerance to glyphosate, which in this case served as the selectable marker for transformation. The two-T-DNA system utilised here enabled the cassettes encoding the trait of interest and the selectable marker to be inserted at two independent genetic loci within the genome of the plant. Transformants were selected with glyphosate and shoot formation was induced without callus phase. After self-pollination of the transformed R_0 plant, an R_1 plant (designated as MON 87701) that contained a single T-DNA I but did not contain T-DNA II was selected for further development.

The two T-DNA cassettes present in plasmid PV-GMIR9 consisted of the following elements between their respective right and left border regions:

T-DNA I (*cry1Ac* expression cassette): (1) promoter and 5' non-translated region of the *Arabidopsis thaliana rbcS4* gene (*rbcS4* gene encodes ribulose 1,5-bisphosphate carboxylase (Rubisco) small subunit 1A) to provide expression in the photosynthetic tissues; (2) sequence encoding the transit peptide of *rbcS4* gene to target the protein to the chloroplast; (3) modified coding sequence of the *cry1Ac* gene of *Bacillus thuringiensis* to confer resistance to specific lepidopteran insects; (4) 3' region of soybean *sphas1* gene (*sphas1* gene encodes β-conglycinin, a 7S α' seed storage protein), including 35 nucleotides of the carboxy-terminus of β-conglycinin coding region, termination codon and polyadenylation sequence. The *cry1Ac* gene of *B. thuringiensis* was modified by site-directed mutagenesis to increase its expression in the plant. The amino acid sequence of the processed protein in the plant is nearly identical (>99 %) to that of *B. thuringiensis*, with seven amino acid differences

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which falls within the normal variation among Cry1Ac proteins. In addition there are four additional amino acids in the N-terminus derived from the chloroplast transit peptide.

T-DNA II (CP4 *epsps* expression cassette): (1) FMV promoter from Figwort Mosaic Virus 35S RNA gene, which drives transcription in most plant cells; 5' non-translated leader sequence from the *Arabidopsis shkG* gene (*shkG* gene encodes EPSPS) to enhance expression; (2) sequence encoding the transit peptide of *shkG* gene to target the protein to the chloroplast; (3) modified coding sequence of the *aroA* gene from *A. tumefaciens* strain CP4 encoding the EPSPS protein to confer tolerance to glyphosate during the selection of transformants; (4) 3' non-translated transcriptional termination sequence and polyadenylation signal sequence from pea *rbcS2* gene *E9* (*rbcS2* gene encodes Rubisco small subunit).

Additional functional elements in the plasmid vector outside of the T-DNAs, and thus not expected to be transferred to the soybean genome, were: (1) *oriV* origin of replication to maintain the plasmid in *Agrobacterium*; (2) *ori-pBR322* origin of replication to maintain the plasmid in *Escherichia coli*; (3) *rop* repressor of primer (ROP) protein to maintain plasmid copy number in *E. coli*; (4) *aad*A bacterial selectable marker (promoter and coding regions) to confer spectinomycin/streptomycin resistance.

3.1.2.   Transgene constructs in the genetically modified plant[8]

Molecular analyses indicated that the GM soybean MON 87701 contains a single insert with one copy of the intact *cry1Ac* expression cassette. No elements from the T-DNA II or vector backbone were detected. Southern analyses of genomic DNA from soybean MON 87701 and its non-GM counterpart A5547 were performed using appropriate combinations of restriction endonucleases and eleven overlapping probes that cover the whole plasmid. The probes corresponding to the different elements of T-DNA I showed the expected hybridisation signals, whereas no signal was observed for any of the probes corresponding to the vector backbone of PV-GMIR9, including T-DNA II. Some probes detected endogenous soybean sequences as part of T-DNA I was originally isolated from soybean.

The nucleotide sequence of the insert as well as both 5' and 3' flanking regions were determined from soybean MON 87701. This confirmed the conclusions drawn from the Southern analyses. Comparison to the parental soybean A5547 indicated that in soybean MON 87701, a 32 bp DNA segment of endogenous DNA had been deleted and 14 bp have been introduced immediately 5' of the insertion site.

To determine the possible disruption of known endogenous soybean genes by the insertion in soybean MON 87701, bioinformatic analyses were carried out on the genomic sequences flanking the insert (c.a. 1.5 kb on each side of the insert). In addition, the possible presence of coding sequences in the soybean genome flanking the insert was analysed. BLASTN searches were performed against EST (Expressed Sequence Tag) database and non-redundant nucleotide database and BLASTX search against non-redundant amino acid database. The results did not indicate the interruption of a soybean coding sequence(s) with known function in the MON 87701 event.

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

3.1.3.1. Expression of the introduced gene

Cry1Ac levels were analysed by enzyme-linked immunosorbent assay (ELISA) from a number of plant parts including root, leaf, seed and forage, from replicated field trials across five locations in the

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[8] Technical dossier/section D2
[9] Technical dossier/section D3

US (2007) and five locations in Argentina (2007-2008). Considering the scope of the application, the Cry1Ac protein levels in seeds are considered most relevant. In 2007 US growing season the mean level was 4.7 µg/g dry weight (dw) and range 3.4–5.7 µg/g dw. In 2007-2008 Argentina growing season the mean level was 5.1 µg/g dw and range 3.9-6.7 µg/g dw.

3.1.3.2. Putative cryptic open reading frames in soybean MON 87701

Bioinformatic analyses were performed on hypothetical polypeptides encoded by the 5' and 3' junctions between the insert and soybean genomic DNA as well as on the open reading frames of the entire insert. The purpose was to predict the expression of intended or unintended novel (poly)peptides with toxic or allergenic properties or other adverse biological activity. DNA sequences were translated in all six reading frames. Each translated sequence was compared to protein databases, including allergen sequence database, toxin sequence database and a database containing sequences of all known proteins. The FASTA analyses included both overall sequence alignments as well as searches for short identical stretches of at least eight contiguous amino acids against the allergen database. No alignment met or exceeded the Codex Alimentarius (2009) threshold for potential allergenicity, and no relevant similarities to known toxic proteins other than Bt proteins (Cry1Ac) were found.

3.1.4. Inheritance and stability of the inserted DNA[10]

Genetic stability of the inserted DNA was studied by Southern analysis from five generations, all of them produced by self-pollination. The restriction enzyme/probe combinations used were sufficient to conclude that all the generations tested retained only a single copy which was stably inherited in subsequent generations.

Stability was also demonstrated by testing the presence of the Cry1Ac protein (by ELISA or lateral flow strips) or the *cry1Ac* gene (real-time PCR) over several generations produced by self-pollination. Furthermore, plants of F_2 and F_3 generations, derived from soybean MON 87701 (R_5 generation) back-crossed with a conventional soybean variety, were analysed by event-specific real-time PCR for the presence of the *cry1Ac* gene and the results were subjected to segregation analysis. In total nearly 2000 plants were tested. The results confirmed that the *cry1Ac* gene was stably inherited and followed a Mendelian segregation pattern.

3.2. Conclusion

The molecular characterisation data establish that the GM soybean MON 87701 contains one copy of an intact *cry1Ac* expression cassette. No other parts of the plasmid used for transformation are present in the transformed plant. Results of the bioinformatic analysis of the 5' and 3' flanking sequences and ORFs spanning the newly created DNA junctions did not indicate any safety concern. The stability of the inserted DNA was confirmed over several generations and a Mendelian inheritance pattern was demonstrated.

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[10] Technical dossier/section D5

4. Comparative analysis

4.1. Evaluation of relevant scientific data

4.1.1. Choice of comparator and production of material for the compositional assessment[11]

The application EFSA-GMO-BE-2010-79 for food and feed use, import and processing of soybean MON 87701 within the European Union presented compositional data of seed and forage material of soybean MON 87701 collected in field trials in the USA in 2007 and in Argentina in the season 2007/2008. The results of these studies have recently been published (Berman et al., 2009). These field trials compared the composition of soybean MON 87701 with a soybean conventional counterpart having a comparable genetic background. The conventional counterpart was the non-transgenic Asgrow variety A5547, which was the commercial soybean variety originally used when the soybean was transformed to establish the MON 87701 event.[12]

In both years/seasons the field trials were performed at five different sites, all of which are representative for soybean cultivation areas in the USA and Argentina, respectively. Each field trial included soybean MON 87701, the conventional counterpart (A5547) and four different commercial non-GM soybean varieties per field trial site, all being treated with pesticides according to conventional practice. Overall, 20 commercial soybean varieties were used as reference lines aimed at providing data on the natural variation in composition of this food and feed plant. The reference lines were characterized by event-specific PCR analysis for the presence or absence of MON 87701. Samples of one of the replicates of MON 87701 from one of the trial sites and of one of the replicates of A5547 from another site were found to be contaminated with GM material and were excluded from the study. At each trial site, soybean MON 87701, the conventional counterpart and the reference lines were planted following a randomized complete block design with three replicates at each site. Whereas all replicates of soybean MON 87701 and its conventional counterpart were chemically analysed for selected soybean constituents, only one of the replicates of the reference lines was analysed for these constituents.

4.1.2. Compositional analysis[13]

Soybean seeds were harvested and analysed for proximates (protein, fat, ash, and moisture), carbohydrates by calculation, fiber fractions (acid detergent fiber (ADF) and neutral detergent fiber (NDF)), amino acids, fatty acids, vitamin E, anti-nutrients (i.e. phytic acid, trypsin inhibitor, lectins, stachyose and raffinose) and other secondary metabolites (isoflavones). Forage was analysed for proximates, carbohydrates by calculation, and fibre fractions (ADF, NDF). In total, 64 different compounds were analysed, 57 in seeds and seven in forage, essentially those recommended by OECD (2001). The data on each compound were statistically analysed for potential differences in levels between soybean MON 87701 and its conventional counterpart within-site and across-sites (sites of the trial combined). Nine of the fatty acids analysed in material from the field trials in the USA and 11 in the material from Argentina were rare and often found at levels below the limit of quantification; when this occurred in more than 50 % of the samples, the analyte in question was excluded from the statistical analysis. When the value for a given compound was statistically different between soybean MON 87701 and its conventional counterpart, such value was compared to those occurring in the commercial soybean varieties included in the study, as well as to the ranges in the level of the compound in soybean published in the scientific literature and the ILSI crop composition database (Ridley et al., 2004).

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[11] Technical dossier/section D7.2
[12] Technical dossier/section C1
[13] Technical dossier/section D7.1

When the compositional data for seed samples from the field trials in the USA were statistically evaluated across sites, statistically significant differences between soybean MON 87701 and its conventional counterpart were found for 15 analytes: the proximates protein and carbohydrates; the amino acids alanine, glycine, histidine, isoleucine, leucine, lysine, serine, threonine, and valine; the fatty acid 22:0 behenic acid; vitamin E, trypsin inhibitor, and daidzein. For forage, none of the analysed compounds showed significant differences between soybean MON 87701 and its conventional counterpart. The evaluation of the compositional data for seed samples per site revealed that, out of the 15 analytes found to be significantly different, five were significantly different at one individual field trial sites, three at two sites, and one at four sites. The statistically significant differences found were usually small. Apparently, the increase in the nine amino acids identified reflected the increased protein content of the seed. Some inconsistent changes in daidzein levels were noted. The only case where the difference was appreciable was vitamin E (23.2 % increase); the vitamin E level was significantly higher in soybean MON 87701 than in its conventional counterpart at four of the five field trial sites (17-37 %). In all cases except one, the level of vitamin E and all other measured compounds were within the range defined by the commercial reference varieties included in the study and reported by the ILSI crop composition database (Ridley et al., 2004) or the USDA-ISO (2006) isoflavone database. The exception was a single calculated carbohydrate value for soybean MON 87701, found to be slightly below the range defined by the commercial soybean varieties, which did not raise concern for the EFSA GMO Panel. Also, the 17 additional statistically significant differences (not significant in the overall analysis) identified in the per location analysis of other soybean constituents were small and within the range defined by the commercial reference varieties included in the field trials.

The statistical evaluation of compositional data of seed samples across sites of Argentinean field trials, revealed a statistically significant difference between soybean MON 87701 and its conventional counterpart for four analytes: the amino acid tryptophan, the fatty acid 18:3 linolenic, vitamin E, and stachyose. For forage, none of the analysed parameters differed significantly between soybean MON 87701 and its conventional counterpart. The evaluation per site revealed that, of these four constituents, tryptophan was significantly different at two individual field trial sites, linolenic acid at three sites, vitamin E at all five sites, and stachyose at none of the five sites. The statistically significant differences found between soybean MON 87701 and its conventional counterpart were usually small, and in all cases the levels registered were within the range defined by the commercial reference varieties included in the study and reported by the ILSI crop composition database or the USDA-ISO isoflavone database. Also, the nine additional statistically significant differences identified in the per location analysis of other soybean constituents were small, and all but one (moisture content in seeds of the control material) were within the range defined by the 20 soybean reference varieties.

Thus, only the vitamin E level was significantly different between seeds of soybean MON 87701 and its conventional counterpart when statistically analysed across field trial sites both in 2007 (7.69 vs 6.24 mg/100g dry weight) and the season 2007/2008 (4.40 vs 3.42 mg/100g d.w.). Analysis per site revealed increased vitamin E levels at nine of the 10 field trial sites studied. The EFSA GMO Panel concludes that the vitamin E level is increased (on average around 25 %) in soybean MON 87701 but that the level still is within the range of values commonly observed in conventional commercial soybean varieties, as defined by the reference lines, and by the ILSI crop composition database.

Berman et al. (2010) recently published compositional data on the soybean event MON 87701 grown at two field trial sites in Southern Brazil and at two field trial sites in Northern Brazil in 2007/2008. Whereas the MON 87701 event occurred in the A5547 genetic background in field trials performed in Southern Brazil, it occurred in the Monsoy 8329 background in the northern field trials. These studies confirmed the compositional information obtained from the field trials in the USA and Argentina. Hierarchical cluster analysis and principal component analysis of the Brazilian data further showed that location (site and region) and/or germplasm (genetic background) effects contributed more to the compositional differences between soybean MON 87701 and its conventional counterparts than the genetic modification.

The EFSA GMO Panel considered the total set of compositional data supplied and the statistically significant differences between soybean MON 87701 and its conventional counterpart in the light of the field trial design, measured biological variation and the level of the studied compounds in commercial soybean varieties, and concludes that soybean MON 87701 is compositionally not different from its conventional counterpart soybean A5547 except for having an increased vitamin E content (still within the normal range of soybeans) and expressing the Cry1Ac protein. Except for expressing the Cry1Ac protein, soybean MON 87701 is compositionally equivalent to commercial soybean varieties.

4.1.3.   Agronomic traits and GM phenotype[14]

Based on data collected at 16 field trial sites in the USA in 2007 and 8 field trial sites in Argentina during the season 2007/2008, the applicant performed a comparative assessment of the phenotypic and agronomic characteristics of soybean MON 87701 and its conventional counterpart (A5547). A randomized complete block design was used at each field trial site. These field trials also included several commercial soybean varieties (four per site) used as reference material to estimate the range in baseline values for the studied phenotypic and agronomic parameters in commercial soybean varieties. All materials were grown under normal agronomic conditions for the geographical region; all maintenance chemicals were commercially registered products and were applied at recommended rates. The phenotypic and agronomic characteristics evaluated were early stand count, seedling vigour, plant growth stages, days to 50 % flowering, flower colour, plant pubescence, plant height, lodging, pod shattering, final stand count, seed moisture, 100 seed weight, test weight (g/250 ml), and yield. Seed dormancy and germination, and pollen characteristics were also considered.

In the field trials performed in the USA, no significant differences were detected between soybean MON 87701 and the conventional counterpart regarding the phenotypic and agronomic parameters investigated. In the field trials performed in Argentina early stand count (96.9 vs 105.9 plants in defined rows) and seed moisture were reduced, and test weight increased. Differences observed for early stand count, seed moisture and test weight were observed in two, five and three out of eight sites respectively. Whereas seed moisture and the test weight were within the range of values defined by the reference soybean varieties, the early stand count for soybean MON 87701 (96.9 plants) were slightly below the range for the reference varieties (103.8-204.0 plants). The applicant suggested that the lower early stand count could be due to different climatic conditions during production of the seeds used for the present field trials, as differences in field emergence due to climatic factor have been observed for soybean varieties with reduced raffinose content (Meis et al., 2003). The reference seeds used in the present study were produced under commercial production practices in a temperate climate in the USA while the MON 87701 seeds were produced in a subtropical environment in Puerto Rico. The EFSA GMO Panel found the explanation acceptable. As indicated above, the reduced early stand count did not influence yield and final stand count.

Specific studies were performed on pollen morphology and viability. There was no difference in percent viable pollen, pollen diameter and pollen morphology between soybean MON 87701 and the conventional counterpart. Seed germination and dormancy characteristics were evaluated on soybean seeds obtained from one field trial site. Whereas no hard seeds were detected in soybean MON 87701 at a temperature of 20° C, 0.5 % of the A5547 seeds were hard at this temperature. As this difference in seed hardness was within the range of soybean reference varieties and no difference was detected at other temperatures, the EFSA GMO Panel did not find this observation as indicating relevant alterations in germination characteristics.

The reference material included in some of the field trials to provide estimates of the natural variation in studied phenotypic and agronomic characteristics was a mixture of GM and non-GM soybean varieties. Therefore, the EFSA GMO Panel asked the applicant to provide ranges in the levels of

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[14] Technical dossier/section D4

studied parameters based on only the non-GM reference varieties. Relevant ranges were provided and showed that the original conclusions were confirmed by the new analysis.[15] The EFSA GMO Panel concludes that the field studies on agronomic performance and phenotypic characteristics identified no difference between soybean MON 87701 and its conventional counterpart that are likely to be biologically relevant. Levels in parameters that showed a statistically significant difference between soybeans MON 87701 and A5547 fell, except for early stand count, within the range in levels defined by a set of reference soybean varieties describing the natural variation.

4.2. Conclusion

Based on the comparative analysis of soybean MON 87701, the conventional counterpart and several other commercial non-GM soybean varieties, the EFSA GMO Panel concludes that soybean MON 87701, as assessed in this application, is compositionally, phenotypically and agronomically not different from its conventional counterpart except for expressing the Cry1Ac protein and showing increased levels of vitamin E, and equivalent to commercial soybean varieties, except for the presence of the newly expressed protein (Cry1Ac).

5. Food/feed safety assessment

5.1. Evaluation of relevant scientific data

5.1.1. Product description and intended use[16]

The scope of application EFSA-GMO-BE-2010-79 is for food and feed use, import and processing of soybean MON 87701 within the European Union. Thus, soybean MON 87701 will be imported into the EU mixed with other soybean varieties and be used as food or feed, or for the production of a large number of derived products, as any commercial soybean variety. The main product for human use is soybean oil. Around 10 % of the heat-processed (toasted) defatted soybean meal goes to production of soybean products for human consumption, including flours, soybean protein concentrates and various textured products simulating meats, sea-foods and cheeses. The rest of the toasted defatted soybean meal goes to feed, in the European Union mainly to poultry, pig and cattle (OECD, 2001). Whole soybeans are used to produce soy sprouts, baked soybeans, and roasted soybeans. There is also a limited direct use of soybeans as animal feeds.

The genetic modification event present in soybean MON 87701 results in the expression of a new protein, the Cry1Ac protein, that confers protection against lepidopteran pests such as velvet bean caterpillar (*Anticarsia gemmatalis*), soybean looper (*Pseudoplusia includens*), soybean anxil borer (*Epinotia aporema*) and sunflower looper (*Rachiplusia nu*). Thus, the genetic modification is intended to improve agronomic performance only and is not intended to influence the nutritional characteristics, the processing characteristics and overall use of soybean as a crop.

5.1.2. Effects of processing[17]

Soybean MON 87701 will be used for production and manufacturing of food and feed products as any other commercial soybean variety. Taking into account the compositional analysis, providing no indication of relevant compositional changes, the EFSA GMO Panel has no reason to assume that the

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[15] Additional information July 2011
[16] Technical dossier/section D7.7
[17] Technical dossier/section D7.6

characteristics of soybean MON 87701 and derived processed products would be different from those of the respective products derived from conventional soybean varieties.

Soybean MON 87701 will be processed in the same manner as conventional soybeans. A heat treatment of soybean MON 87701 (190°C for 15.5 min) reduced the quantity of immunodetectable Cry1Ac protein to levels below the limit of detection, corresponding to a 94 % reduction compared to the level in non-treated MON 87701. It was suggested that the heat-induced losses likely are due to protein degradation and/or aggregation of the Cry1Ac proteins into insoluble complexes.

5.1.3.   Toxicology[18]

5.1.3.1.   Protein used for safety assessment

Due to the relatively low expression level of the Cry1Ac protein in soybean MON 87701 (see section 3.1.3.), and the very difficult task to isolate a sufficient quantity of purified protein from the GM soybean, the safety studies with the newly expressed protein were conducted with a Cry1Ac protein encoded by the *cry1Ac* gene from a specific strain of *B. thuringiensis* and expressed in *E. coli*. The structural similarity and physicochemical and functional equivalence of the Cry1Ac protein produced by *E. coli* to that produced in soybean MON 87701 was shown by N-terminal sequencing (Edman degradation), Western analysis with Cry1Ac specific antibodies, mobility in SDS-PAGE, proteolytic peptide mapping following MALDI-TOF mass spectrometry, glycosylation analysis and Cry1Ac biological activity. Together, these methods confirmed the equivalence of the bacterial and the plant Cry1Ac proteins. Based on the identified similarity in structure and equivalence in physico-chemistry and function between these proteins, the EFSA GMO Panel accepts the use of a Cry1Ac test material derived from *E. coli* for the degradation studies and safety testing of the Cry1Ac protein present in soybean MON 87701, as well as for use as a reference standard in the ELISA to estimate Cry1Ac expression levels in various tissues of soybean MON 87701.

5.1.3.2.   Toxicological assessment of the expressed novel protein in soybean MON 87701

The newly introduced gene in soybean MON 87701 is derived from the soil bacterium *Bacillus thuringiensis* subsp. *kurstaki*. The gene codes for a protein, Cry1Ac, which is insecticidal specifically against lepidopteran insects but is unknown to be toxic to humans and animals. Products of cotton MON 531, expressing a Cry1Ac protein that in amino acid sequence is 100 % identical to the protein expressed in soybean MON 87701, but contains four additional amino acids at the N-terminus of the MON 87701-produced protein, has been on the European market as existing food or feed, and food additive since the end of 2002, without any adverse effects to human health having been reported.

(a) <u>Acute toxicity testing</u>

The Cry1Ac protein produced in a recombinant *E. coli* strain did not induced adverse effects in an acute oral toxicity study in CD-1 mice administered a single dose of 1460 and 1290 mg/kg body weight to male and female animals, respectively.

(b) <u>Degradation in simulated digestive fluids</u>

Digestion of the Cry1Ac protein (1182 amino acids) in a pepsin digestion assay (simulated gastric fluid) was studied *in vitro* by identifying peptide fragments using SDS-PAGE colloidal blue gel staining and Western analysis. The SDS-PAGE colloidal blue gel staining demonstrated that at least 99.7 % of the Cry1Ac protein produced in *E. coli* was fully degraded by pepsin-containing simulated

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[18] Technical dossier/section D7.8

gastric fluid of pH 1.2 within 30 seconds. In agreement with this finding, Western analysis showed that most (>95 %) of the Cry1Ac protein was digested in simulated gastric fluid within the same time frame. The digestion resulted in fragments (3.5-4 kDa) of the Cry1Ac protein visible for up to 60 min on the gel. These fragments of the Cry1Ac protein started at amino acid positions 415 and 882, respectively. Combining a two minute exposure of the Cry1Ac protein to simulated gastric fluid (producing complete degradation of the full length protein and appearance of the short fragment) with a subsequent exposure of the digest to simulated intestinal fluid (neutral pH, 1 min), resulted in complete disappearance of the shorter fragment. In simulated intestinal fluid, the full length Cry1Ac was digested below the limit of detection within five minutes, producing a trypsin-resistant core polypeptide (around 55 kDa) stable throughout the digestion.

(c) Bioinformatics studies

Searches for amino acid sequence homology of the Cry1Ac protein expressed in soybean MON 87701 with amino acid sequences of toxic proteins stored in an updated propriety data base using the FASTA sequence alignment tool, indicated significant homology only with other Cry proteins not toxic to humans and animals. Thus, no safety concerns for humans and animals were identified.

5.1.3.3.   Toxicological assessment of new constituents other than proteins

No new constituent other than the Cry1Ac protein is expressed in soybean MON 87701 and no relevant changes in the composition of soybean MON 87701 were detected by the compositional analysis.

5.1.3.4.   Toxicological assessment of the whole GM food/feed

No indication was found in the molecular analysis and in the comparative compositional, phenotypic and agronomic analysis that the genetic modification of soybean MON 87701 resulted in any unintended changes. According to the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006a), animal safety studies with the whole food/feed are not required in these cases .

However, the applicant supplied a 90-day feeding trial with Sprague-Dawley rats of the Crl:CD strain. This study was performed using a protocol adapted from OECD Guideline 408 (OECD, 1998), with 12 animals per sex and treatment. Diets were provided ad libitum and contained 30 % toasted and defatted soybean meal prepared from either soybean MON 87701, the conventional counterpart (A5547), or one of three conventional reference soybean varieties: Anand, UA4805 or Ozark. Diets were nutritionally balanced and analysed for its composition, in particular in relation to feed quality.

There were no differences in mean weekly body-weights and cumulative mean body weight gains between male animals of the group fed diets with processed MON 87701 soybean meal as compared to male rats administered diets with processed meal of soybean A5547. However, the mean weekly body-weights and cumulative body weight gains of female rats given the MON 87701-supplemented diet were consistently, and, in particular, from week 9 onwards, statistically significantly lower than in female rats administered diets with processed A5547 soybean meal. The study was confounded by the fact that the mean body-weight of the female rats receiving the MON 87701-based diet was already lower at the start of the study. Feed consumption was generally lower for female rats given the MON 87701-containing diet.

The applicant concluded that the observed differences in female body-weight and cumulative body-weight gain most likely were attributable to biological variation, and were not treatment-related. To confirm this interpretation of the result of the initial study (subsequently called study I), the applicant performed a second 90-day feeding study (study II) in rats. The second study used 20 rats/sex/group, and 15 % and 30 % incorporation of processed MON 87701 or A5547 soybean meal in the diet.

In study II, there were no relevant differences in body-weight, cumulative body-weight gain and feed intake in female rats receiving the MON 87701-containing diets as compared to females administered the A5547-containing diet (control diet). However, in this study the mean body-weight and cumulative body-weight gains in male rats receiving 15 % and 30 % MON 87701 soybean meal in the diet were statistically significantly higher than in rats receiving the control diets throughout a considerable part of the study. A statistically significantly higher feed consumption was observed during specific time periods in male animals receiving 15 % and 30 % MON 87701 soybean meal in the diets. On the basis of the results from study I and II, the EFSA GMO Panel considers it unlikely that the observed differences in animal body-weights in both studies are related to the exposure to processed meal of soybean MON 87701.

There were no treatment-related deaths in any of the two studies, and no relevant differences in clinical findings between test and control groups. Regarding haematology, coagulation parameters and serum chemistry, statistically significant differences between test and control groups were observed. Some of the differences only occurred in rats administered 15 % MON 87701 soybean meal in the diet of study II and were thus not considered treatment related. The differences found in animals receiving diets containing 30 % MON 87701 soybean meal (i.e. , lower mean haemoglobin level and eosinophil counts in female animals in study II; lower mean total protein and higher chloride levels in female animals in study I; lower mean phosphorus levels in males in study II) are regarded by the EFSA GMO Panel as incidental since the values were within the historical control ranges and there were no changes in related parameters which could indicate a specific organ toxicity. Furthermore, the differences were only observed in one of the two studies and not reproduced in the other one. In both studies, urinalysis did not show toxicologically relevant differences between groups.

Macroscopic examinations at necropsy did not reveal relevant changes in both studies. Regarding organ weights in study I, male rats given the MON 87701-diet showed reduced absolute spleen weights, and altered spleen weight/final body weight and spleen weight/brain weight ratios as compared to male rats in the control group. However, the values for these parameters in the MON 87701 soybean meal-exposed animals were comparable to the values for these parameters in male rats receiving the reference diets and in historical controls. Furthermore, there were no histopathological findings in the microscopic examination of this organ. The differences in spleen weight parameters observed in males are therefore not considered to be related to the exposure to processed MON 87701 soybean meal. The statistically significant differences in absolute organ weights observed in females (i.e. lower brain, kidney, liver and spleen weights) are considered to be a consequence of the lower body weight and lower body weight gain during the study (see above) since the respective organ weights relative to body weights were not affected. Furthermore, there were no histological alterations in these organs which would indicate adverse effects.

Study II showed several statistically significant differences between animals that had received 15 % MON 87701 soybean meal in the diet and animals of the control group (15 % processed soybean meal of A5547). Such differences (kidney, liver and thyroid/parathyroid weight in males, adrenal gland weight relative to brain weight in females) were not observed in the high-dose group (30 % soybean MON 87701 meal), and are thus considered as incidental. The applicant considered these observations to be a consequence of the slightly higher body weights in males given 15 % MON 87701 in the diet. In the high dose group, statistically significantly lower epididymide and teste weights (both relative to final body weight) were noted in male rats, and higher mean thyroid/parathyroid weights (absolute and relative to final body weight and brain weight) in female rats. The differences were small, and the group means of these parameters were within the range in group means of the historical controls receiving 30 % processed soybean meal. Since no differences for these parameters were found in study I and no histopathological alterations were observed in that study, and there were also no clinical or gross pathological findings in the repeated study, the observed differences in epididymide, teste and thyroid/parathyroid weights in study II are not considered related to the exposure to MON 87701 soybeans. Microscopic examinations of other organs and tissues carried out in study I revealed no relevant differences between the test and control group (no microscopic examinations were carried out in study II).

The EFSA GMO Panel concludes that administration of diets containing 30 % processed meal of soybean MON 87701 to rats did not cause adverse effects.

5.1.4. Allergenicity[19]

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 (Codex Alimentarius, 2009; EFSA, 2006a; EFSA Panel on Genetically Modified Organisms (GMO), 2010).

5.1.4.1.   Assessment of allergenicity of the newly expressed proteins

The *cry1Ac* gene originates from *B. thuringiensis* subsp. *kurstaki,* a soil-borne and plant-interacting micro-organism that is not known to be allergenic. The Cry1Ac protein is expressed in various tissues of soybean MON 87701, except roots, and has been quantified at a number of growth stages during the growing season (see section 3.1.3.). The most relevant tissue for the assessment of food allergenicity is the seed, which contains around 5 μg Cry1Ac/g dry weight (i.e. around 0.0013 % of total soybean protein).

A bioinformatics-supported comparison of the amino acid sequence of the Cry1Ac protein with the sequences of known allergens, gliadins, and glutenins, collected in an updated proprietary database based on the FARRP database, was performed. This analysis included both overall sequence alignments using the FASTA algorithm and searches for short identical stretches of at least eight contiguous amino acids. In the overall sequence alignment, no identity higher than 35 % in polypeptides of 80 or more amino acids was found between the Cry1Ac protein and known allergens. Similarly, no identical sequence of eight contiguous amino acids was detected. As described above, Cry1Ac is degraded under simulated gastric and intestinal conditions. Based on these results, the GMO Panel considers that the newly expressed Cry1Ac protein is unlikely to be allergenic in the intended conditions of exposure.

5.1.4.2.   Assessment of allergenicity of the whole GM plant or crop

Allergenicity of the whole crop could be increased as an unintended effect of the random insertion of the transgene in the genome of the recipient, for example through qualitative or quantitative modifications of the pattern of expression of endogenous allergens. However, given that the comparative compositional and agronomical analysis revealed soybean MON 87701 not to be different from the conventional counterpart except for soybean MON 87701 expressing the Cry1Ac protein and showing increased levels of vitamin E, no increased allergenicity is anticipated for soybean MON 87701. Because soybean is a recognised allergenic food, the applicant performed extensive *in vitro* allergenicity studies with extracts of soybeans MON 87701, A5547 (the conventional counterpart of soybean MON 87701), and 17 different commercial soybean varieties. The IgE-binding of soybean proteins to sera from 13 individuals clinically documented allergic to soybean, and 5 non-allergic individuals were quantified with an ELISA method in order to demonstrate that the allergenicity potential of soybean MON 87701 is not altered in comparison to conventional soybean varieties. Whereas proteins from none of the soybean varieties showed binding to sera from non-allergic individuals, most serum samples from allergic individuals had similar reactivity to proteins in extracts from soybean MON 87701 and soybean A5547. The applicant

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[19] Technical dossier/section D7.9

supplied two-dimensional (2D) electrophoresis of water extracts of soybean MON 87701 and A5547 followed by Western blotting with human IgE antibodies from allergic individuals to further address the potential for changes in endogenous allergenicity of soybean 87701. These studies demonstrated no meaningful qualitative and quantitative difference in the IgE-binding patterns to proteins of extracts derived from soybean MON 87701 and soybean A5547 for all the sera studied except for the serum of one very high IgE-responder allergic individual. The differences observed in the *in vitro* studies with this particular allergic individual may be due to the heterogeneity of the human IgE response of the sera samples and differences in the endogenous protein expression in the crop and do not raise concern under the intended conditions of use. Therefore, the EFSA GMO Panel is of the opinion that these studies do not indicate a consistent and biologically significant modification of the overall allergenicity of soybean MON 87701 as compared to that of its conventional counterpart.

5.1.5. Nutritional assessment of GM food/feed[20]

As the molecular characterisation of soybean MON 87701 identified no unintended effects of the genetic modification, the newly expressed Cry1Ac protein was found safe for higher animals, and the comparative compositional and agronomical analysis revealed soybean MON 87701 to be equivalent to commercial soybean varieties except for soybean MON 87701 expressing the Cry1Ac protein, a nutritional equivalence to conventional soybeans can be assumed. According to the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006a), long-term livestock feeding studies with the whole food/feed are not required in these cases.

The applicant provided a 42-day broiler chicken feeding study (Cobb × Cobb 500) with defatted soybean meal performed according to generally accepted guidelines (ILSI, 2003). The study consisted of nine treatment groups: one group received soybean MON 87701, another group received soybean MON 87701 × MON 89788 (not included in the analysis for soybean MON 87701), another group received soybean A5547 (conventional counterpart of MON 87701), and the other six groups different conventional non-GM soybean varieties.

Each treatment consisted of 60 male and 60 female broilers allocated in pens of 12 chickens per pen, that were reduced to 10 birds/pen at day 7. A randomised complete block design with 5 blocks of 18 pens was used. Animals were fed adjusted diets containing approximately 33 % (w/w) of soybean meal in the starter diet (day 0-21) and 30 % soybean meal in the grower/finisher diet (day 21-42). The diets were formulated based on nutrient requirements recommended by the National Research Council (NRC, 1994), and were quality controlled, including confirmation that levels of pesticides and mycotoxins were below threshold levels of concern for feeding studies.

The different treatment groups showed a chicken mortality between 0.8 % and 5.0 % during the first 7 days of the study, mainly due to bacterial infection and dehydration, and between 0.0 % and 5.0 % during day 7-42 of the study, being most deaths due to ascites or sudden death syndrome. A total mortality rate of 10 % for the soybean MON 87701-fed chickens may be incidental but is not considered good practice by the EFSA GMO Panel for scientific studies devoted to nutritional wholesomeness/safety testing.[21] However, feeding of broiler chickens with products of soybean MON 87701 had no effects on feed intake, final body weight (around 2.5 kg per animal), and weight gain of broilers. Only adjusted feed conversion rates differed slightly between the treatment groups, ranging from 1.52 to 1.56 kg feed per kg weight gain for the various groups. The lowest value was obtained from broiler chickens fed soybean MON 87701 (but only in females). No difference was observed in the various parameters of carcass yield, neither in fat, protein and moisture content of breast and thigh meat. However, there was a diet-sex interaction for three of the parameters tested: percent fat pad

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[20] Technical dossier/section D7.10
[21] Additional information July 2010

weight and thigh weight were slightly reduced in males, and percent chilled weight was slightly reduced in females.[22]

In conclusion, the broiler feeding study identified no relevant difference in broiler performance, carcass yield or meat composition between chickens fed diets containing extracted soybean meal produced from soybean MON 87701 and the conventional counterpart or other conventional soybean varieties. The data confirm the results of the comparative compositional analysis that indicated that soybean MON 87701 is compositionally and, therefore, implicitly as nutritious as commercial soybean varieties, including the conventional counterpart.

5.1.6. Post-market monitoring of GM food/feed

The risk assessment concluded that no data have emerged to indicate that soybean MON 87701 is less safe than its conventional counterpart. In addition, soybean MON 87701 is as nutritious as conventional soybean. Therefore, and in line with the Guidance Document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed (EFSA, 2006a), the EFSA GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

5.2. Conclusion

The Cry1Ac is degraded in simulated digestive and intestinal fluids, and bioinformatics-supported studies demonstrated that the Cry1Ac protein show no homology to known toxic and allergenic proteins. No toxicity of the Cry1Ac protein was observed in an acute toxicity study in mice where the protein was administered orally at a high dose. The result of 90-day feeding studies with toasted defatted soybean meal from MON 87701 in rats did not raise concern. Whole-product testing of soybean extracts to sera from soy-allergic patients showed that the overall allergenicity of the whole plant had not been changed. A 42-day feeding study on broiler chickens showed that soybean MON 87701 is as nutritious as conventional counterpart and other soybean varieties included in the study. In conclusion, the EFSA GMO Panel is of the opinion that soybean MON 87701 is as safe and as nutritious as its conventional counterpart and commercial soybean varieties in the context of its intended use.

6. Environmental risk assessment and monitoring plan

6.1. Environmental risk assessment

The scope of this application EFSA-GMO-BE-2010-79 is for food and feed uses, import and processing and does not include cultivation. Considering the intended uses of soybean, the environmental risk assessment is concerned with the indirect exposure mainly from manure and faeces from animals fed with soybean MON 87701 and with the accidental release into the environment of viable grains of soybean MON 87701 during transportation and processing.

Soybean MON 87701 has been developed for protection against certain lepidopteran pests (i.e. *A. gemmatalis, P. includens, E. aporem* and *R. nu w*hich are not present in European fauna). Insect resistance is achieved by the expression of the *B. thuringiensis* derived Cry1Ac protein.

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[22] Additional information December 2010

6.1.1.   Unintended effects on plant fitness due to the genetic modification[23]

Cultivated soybean (*Glycine max* (L.) Merr.) is a species in the subgenus *Soja* of the genus *Glycine*. The species originated from eastern Asia and is a highly domesticated crop (Lu, 2005). The major worldwide soybean producers are the United States (US), Brazil, Argentina, China, North Korea and South Korea. In the European Union , soybean is mainly cultivated in Austria, Italy, France, Hungary and Romania (Dorokhov et al., 2004).[24]

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

Field trials with soybean MON 87701 were carried out by the applicant across 16 locations in the US in 2007 and 8 locations in Argentina in 2007/2008 as described in section 4.1.3. As mentioned above, no statistically significant difference was observed in the combined site analysis of the field trials data of the 2007 season. The combined site analysis of the 2007/2008 field data identified three statistically significant differences in early stand count, seed moisture and test weight of harvested seeds.

The EFSA GMO Panel considers that the differences observed in early stand count, seed moisture, and weight of harvested grains are unlikely to affect the overall fitness and weed potential of the GM soybean, except under infestation conditions of specific target organisms.

Seed germination and dormancy characteristics, pollen morphology and viability were evaluated as described in section 4.1.3.

These field trial and laboratory data do not show altered agronomic performance that would indicate any change in fitness and invasiveness or weediness of soybean MON 87701 compared to conventional soybean varieties, except under infestation conditions of specific target organisms.

In addition to the data presented by the applicant, the EFSA GMO Panel is not aware of any scientific report of increased spread and establishment of GM soybean and any change in survival capacity, including overwintering (Dorokhov et al., 2004, Owen, 2005, Bagavathiannan and Van Acker, 2008, Lee et al., 2009).

Survival of soybean plants outside cultivation areas is mainly limited by a combination of low competitiveness, absence of a dormancy phase, and susceptibility to plant pathogens and cold climate conditions. Since these general characteristics are unchanged in soybean MON 87701, it can be considered that soybean MON 87701 has no altered survival, multiplication or dissemination characteristics, except under infestation conditions by specific target pests. Therefore, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects of the soybean MON 87701 in Europe will not be different to that of conventional soybean varieties.

6.1.2.   Potential for gene transfer[25]

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

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[23] Technical dossier/sections D4, D9.1 and D9.2
[24] http://epp.eurostat.ec.europa.eu/portal/page/portal/agriculture/data/database
[25] Technical dossier/section D6

(a) Plant to bacteria gene transfer

Genomic DNA is a component of many food and feed products derived from soybean. It is well documented that DNA present in food and feed becomes substantially degraded during digestion in the human or animal gastrointestinal tract. However, a low level of exposure of fragments of ingested DNA, including the recombinant fraction of such DNA, to microorganisms present in the digestive tract of humans, domesticated animals, and other animals feeding on soybean MON 87701 is expected.

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

A successful horizontal transfer would require stable insertion of the transgene sequences into a bacterial genome and a selective advantage conferred to the transformed host. The only known mechanism that facilitates horizontal transfer of non-mobile, chromosomal DNA fragments into bacterial genomes is homologous recombination (HR). HR requires the presence of stretches of similar DNA sequences between the recombining DNA molecules and, in addition to substitutive gene replacement, facilitates the insertion of non-homologous DNA sequences if their flanking regions share sequence similarity with bacterial sequences in the recipient.

Soybean MON 87701 contains genetic elements with identity or high similarity to those of bacteria. The coding sequence of Cry1Ac is a synthetic gene which is highly similar to corresponding genes from Cry1Ac producing *B. thuringiensis* and the flanking regions of the recombinant gene insert contain approximately 50 and 260 bp long sequences of the right and left border of the Ti-plasmid of *A. tumefaciens*, respectively. Both species, *A. tumefaciens* and *B. thuringiensis,* are not considered to be prevalent in the main receiving environment, i.e. the gastrointestinal tract of humans or animals. Both occur in soil, and in addition, *B. thuringiensis* has been frequently isolated from guts of insects (Jensen et al., 2003).

On a theoretical basis (i.e. without any study providing experimental evidence for horizontal gene transfer (HGT) in the case of GM food and feed derived from soybean MON 87701 or any other GM plant) it can be assumed that, as an extremely rare event, homologous recombination may occur between the recombinant *cry1Ac* gene and the *cry1Ac* gene of *B. thuringiensis* present in the environment. Such substitutive recombination events are unlikely to provide a selective advantage for the recipient organisms (EFSA, 2009). Double homologous recombination of the flanking regions with those on natural Ti-plasmids of *A. tumefaciens* would result in gene replacement, by which the recipient would loose its capability of crown gall formation (loss of auxin, cytokinin and opine synthesizing genes).

In addition to homology-based recombination processes, illegitimate recombination that does not require the presence of DNA similarity between the recombining DNA molecules is theoretically possible. However, the transformation rates for illegitimate recombination were considered to be 10^{10}-fold lower than for homologous recombination (Hülter and Wackernagel, 2008; EFSA, 2009). Illegitimate recombination events have not been detected in studies that have exposed bacteria to high concentrations of GM-plant DNA (EFSA, 2009). Thus this process in comparison to HR is not considered to significantly contribute to horizontal gene transfer events. In comparison to the above described homology-facilitated recombination processes, the contribution of illegitimate recombination is extremely low.

The *cry1Ac* gene of soybean MON 87701 is regulated by a eukaryotic plant promoter (derived from the *A. thaliana RbcS4*). The expression level of eukaryotic promoters in bacteria is variable, but often

inefficient (Warren et al., 2008). The expression of the *prRBCS4-cry1ac* construct in bacteria is unknown.

In a worst case scenario, considering the possibility of expression, an *A. tumefaciens* recipient would become capable of producing an insecticidal Cry1Ac protein. The exposure of bacterial communities to the recombinant gene in soybean MON 87701 must, however, be seen in the context of the natural occurrence and level of exposure to alternative sources of similar genes to which bacterial communities are continually exposed. Due to its specific life-style as a soil bacterium and plant pathogen, in contrast to the life-style of *B. thuringiensis*, which colonizes insect guts and infects specific target insects, the EFSA GMO Panel considers unlikely that *A. tumefaciens* would gain selective advantage from such a HGT by double homologous recombination.

The EFSA GMO Panel concludes that the *cry1Ac* gene from soybean MON 87701 may, on a theoretical basis, be transferred by double homologous recombination to *A. tumefaciens* or to *B. thuringiensis*. However, since both *A. tumefaciens* and *B. thuringiensis* are not considered to be members of the gut microbiota, exposure to recombinant DNA of MON 87701 is considered to be very low. Due to the natural occurrence of *cry1Ac* in the environment, a low level gene transfer to *A. tumefaciens* or gene replacement in *B. thuringiensis* is not regarded to confer a novel selective advantage. Considering its intended use as food and feed and the above assessment, the EFSA GMO Panel has therefore not identified a concern associated with a HGT from MON 87701 to bacteria.

(b) Plant to plant gene transfer

Considering the intended uses of soybean MON 87701 and the physical characteristics of soybean seeds, a possible pathway of gene dispersal is from seed spillage and pollen of occasional feral GM soybean plants originating from accidental seed spillage mainly during transportation and/or processing.

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

Soybean is an annual, almost completely self-pollinating crop in the field which has a percentage of cross-pollination usually lower than 1 % (Weber and Hanson, 1961; Caviness, 1966; Ray et al., 2003; Lu, 2005; Yoshimura et al., 2006; Abud et al., 2007). Soybean pollen dispersal is limited because the anthers mature in the bud and directly pollinate the stigma of the same flower (OECD, 2000). However, cross-pollination rates as high as 6.3 % have been reported for closely spaced plants (Ray et al., 2003), suggesting the potential of some within-crop gene flow. These results indicate that natural cross-pollination rates can fluctuate significantly among different soybean varieties under particular environmental conditions such as favourable climate for pollination and abundance of pollinators (Gumisiriza and Rubaihayo, 1978; Kikuchi et al., 1993; Ahrent and Caviness, 1994; Ray et al., 2003; Lu, 2005).

Plant to plant gene transfer could therefore occur under the following scenario: imports of soybean MON 87701 seeds (while most soybean MON 87701 seeds will be processed in countries of production), processing outside of importing ports, transportation in regions of soybean production in Europe, spillage of GM seeds mainly during transportation, germination and development of spilled seeds within soybean fields or in very close vicinity of cultivated soybean fields, overlap of flowering periods and environmental conditions favouring cross-pollination. The likelihood of all these conditions occurring and thereby resulting in cross-pollination between GM soybean plants and cultivated soybean is therefore extremely low. Apart from seed production areas, GM plants and plants derived from out-crossing with this GM soybean will not persist overtime. Dispersal of soybean seeds by animals is not expected due to the characteristics of the seed, but accidental release into the environment of seeds may occur (e.g. during transportation and processing for food, feed and industrial uses). However, cultivated soybean seeds rarely display any dormancy characteristics and grow only under certain environmental conditions as volunteers in the year following cultivation (OECD, 2000). Even in soybean fields, seeds usually do not survive during the winter due to predation, rotting, germination resulting in death, or due to management practices prior to planting the subsequent crop (Owen, 2005).

The EFSA GMO Panel takes into account that this application does not include cultivation of the soybean within the EU so that the likelihood of cross-pollination between cultivated soybean and occasional soybean plants resulting from seed spillage is considered extremely low. However, in countries cultivating this GM soybean and producing seed for export, there is a potential for admixture in seed production and thus the introduction of GM seeds through this route.

In conclusion, since soybean MON 87701 has no altered survival, multiplication or dissemination characteristics, the EFSA GMO Panel is of the opinion that the likelihood of unintended environmental effects as a consequence of spread of genes from soybean MON 87701 in Europe will not differ from that of conventional soybean varieties.

6.1.3. Interactions of the GM plant with target organisms[26]

Due to the intended uses of soybean MON 87701, which exclude cultivation, and due to the low level of exposure to the environment, potential interactions of the GM plant with target organisms were not considered an issue by the EFSA GMO Panel.

6.1.4. Interactions of the GM plant with non-target organisms[27]

Due to the intended uses of soybean MON 87701, which exclude cultivation, and due to the low level of exposure to the environment, potential interactions of the GM plant with non-target organisms were not considered an issue by the EFSA GMO Panel.

However, the EFSA GMO Panel evaluated whether the Cry1Ac protein might potentially affect non-target organisms by entering the environment through manure and faeces from animals fed this GM soybean. Cry proteins are degraded by enzymatic activity in the gastrointestinal tract, meaning that only a very low amount of these proteins would remain intact to pass out in faeces. This was demonstrated for Cry1Ab (Einspanier et al., 2004; Lutz et al., 2005; Lutz et al., 2006; Wiedemann et al., 2006; Guertler et al., 2008; Paul et al., 2010). There would, subsequently, be further degradation of the protein in the manure and faeces due to microbiological proteolytic activity.

In addition, there will be further degradation of Cry proteins in soil reducing the possibility for exposure of potentially sensitive non-target organisms. While Cry proteins may bind to clay minerals

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[26] Technical dossier/sections D8 and D9.4
[27] Technical dossier/section D9.5

and humic substances in soil, thereby reducing their availability to micro-organisms for degradation, there are no indications of persistence and accumulation of Cry proteins from GM crops in soil (reviewed by Icoz and Stotzky, 2008). The EFSA GMO Panel is not aware of evidence of released Bt toxins protein causing significant negative effects on soil micro-organisms.

Considering the scope of the application, it can be concluded that the exposure of potentially sensitive non-target organisms to the Cry1Ac protein is likely to be very low and of no biological relevance.

6.1.5.   Interactions with the abiotic environment and biogeochemical cycles[28]

Due to the intended uses of soybean MON 87701, which exclude cultivation, and due to the low level of exposure to the environment, potential interaction of the GM plant with the abiotic environment and biogeochemical cycles were not considered an issue by the EFSA GMO Panel.

6.2.   Post-market environmental monitoring[29]

The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC 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 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.

Monitoring is related to risk management, and thus a final adoption of the monitoring plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gives its opinion on the scientific content of the monitoring plan provided by the applicant (EFSA, 2006b). The potential exposure to the environment of soybean MON 87701 would be through manure and faeces from animals fed soybean MON 87701 or through accidental release into the environment of GM soybean seeds (e.g. during transportation and processing). The EFSA GMO Panel is aware that, due to physical characteristics of soybean seed and methods of transportation, accidental spillage cannot be excluded. Therefore, the EFSA GMO Panel recommends that appropriate management systems are introduced to actively monitor the occurrence of feral soybean plants in areas where soybean spillage and plant establishment are likely to occur as proposed in the EFSA Guidance Document (EFSA, 2006a) and the scientific opinion of the EFSA GMO Panel on post-market environmental monitoring (EFSA, 2006b).

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

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

The EFSA GMO Panel is of the opinion that the scope of the monitoring plan proposed by the applicant is in line with the intended uses of soybean MON 87701 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The

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[28] Technical dossier/sections D9.8 and D10
[29] Technical dossier/section D11

EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

6.3. Conclusion

The scope of the application ifs for food and feed uses, import and processing of soybean MON 87701 and excludes cultivation. Considering the intended uses, the environmental risk assessment is concerned with indirect exposure mainly through manure and faeces from animals fed seeds produced by soybean MON 87701 and with the accidental release into the environment of viable seeds of soybean MON 87701 (e.g. during transportation and processing).

In case of accidental release into the environment of viable seeds of soybean MON 87701 (e.g. during transportation and processing), there are no indications of an increased likelihood of establishment and spread of feral soybean MON 87701 plants, except under infestation conditions of specific target organisms. In addition, the low levels of environmental exposure of these GM soybean plants and the newly expressed protein through other routes indicate that the risk to non-target organisms is extremely low. The unlikely but theoretically possible transfer of the recombinant gene from soybean MON 87701 to environmental bacteria does not raise concern due to the lack of a selective advantage in the context of its intended uses. The scope of the post-market environmental monitoring plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean MON 87701.

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

The EFSA GMO Panel also recommends that appropriate management systems should be in place to restrict seeds of soybean MON 87701 entering cultivation as this would require specific approval under Directive 2001/18/EC or Regulation (EC) No 1829/2003.

CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was requested to carry out an evaluation of a scientific risk assessment of the soybean MON 87701 for food and feed uses, import and processing in accordance with Regulation (EC) No 1829/2003.

The EFSA GMO Panel is of the opinion that the molecular characterisation data provided for soybean MON 87701 are sufficient to conclude on this part of the risk assessment evaluation. The results of the bioinformatic analyses of the inserted DNA and the flanking regions do not raise safety concerns. The levels of Cry1Ac protein in soybean MON 87701 have been sufficiently analysed in various tissues and the stability of the genetic modification has been demonstrated. The EFSA GMO Panel considers that the molecular characterisation does not indicate a safety concern.

Based on the comparative analysis of soybean MON 87701, the conventional counterpart (A5547) and several other commercial soybean varieties, the EFSA GMO Panel concludes that soybean MON 87701, as assessed in this application, is compositionally, phenotypically and agronomically not different from its conventional counterpart except for having an increased vitamin E content (still within the normal range of soybeans) and expressing the Cry1Ac protein. Except for expressing the Cry1Ac protein, soybean MON 87701 is also compositionally and agronomically equivalent to commercial soybean varieties.

The Cry1Ac protein expressed in MON 87701 is degraded in simulated digestive and intestinal fluids, and bioinformatics-supported studies demonstrated that the Cry1Ac protein show no homology to known toxic and allergenic proteins. No toxicity of the Cry1Ac protein was observed in an acute toxicity study in mice where the protein was administered orally at a high dose.

The result of 90-days feeding studies in rats with processed soybean MON 87701 meal did not raise concern. Whole-product testing of soybean extracts to sera from soy-allergic patients demonstrated unchanged overall allergenicity of the whole plant. A 42-day feeding study on broiler chickens showed that defatted soybean meal from MON 87701 is as nutritious as meal from the conventional counterpart and other soybean varieties included in the study.

Considering the intended uses of soybean MON 87701, which exclude cultivation, there is no requirement for scientific assessment on possible environmental effects associated with the cultivation of this GM soybean. In case of accidental release into the environment of viable seeds of soybean MON 87701 (e.g.; during transportation and processing), there are no indications of an increased likelihood of establishment and spread of feral soybean plants, except under infestation conditions of specific target organisms. In addition, the low levels of environmental exposure of these GM soybean plants and the newly expressed protein through other routes indicate that the risk to non-target organisms is extremely low. The unlikely but theoretically possible transfer of the recombinant gene from soybean MON 87701 to environmental bacteria does not raise concern due to the lack of a selective advantage in the context of its intended uses. The scope of the post-market environmental monitoring plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean MON 87701. The EFSA GMO Panel is aware that, due to physical characteristics of soybean seed and methods of transportation, accidental spillage cannot be excluded. Therefore, the EFSA GMO Panel recommends that, within general surveillance, appropriate management systems are introduced to actively monitor the occurrence of feral soybean plants in areas where soybean spillage and plant establishment are likely to occur.

In conclusion, the EFSA GMO Panel considers that the information available for soybean MON 87701 addresses the scientific comments raised by the Member States and that the soybean MON 87701, as described in this application, is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment in the context of its intended uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of Belgium, received 17 May 2010, concerning a request for placing on the market of Soybean MON 87701 submitted by Monsanto under Regulation (EC) No 1829/2003.

2. Acknowledgement letter, dated 4 June 2010, from EFSA to the Competent Authority of Belgium (Ref. CGL/RM/PB/KL/lg (2010) 4896301).

3. Letter from EFSA to applicant, dated 11 June 2010, delivering the 'Statement of Validity' for application EFSA-GMO-BE-2010-79, Soybean MON 87701 submitted by Monsanto under Regulation (EC) No 1829/2003 (Ref. PB/KL/CE/mt (2010) 4923433).

4. Letter from EFSA to applicant, dated 21 June 2010, requesting additional information and stopping the clock (Ref. PB/KL/JA/shv (2010) 4937036).

5. Letter from applicant to EFSA, received 1 July 2010, providing additional information.

6. Letter from EFSA to applicant, dated 12 November 2010, requesting additional information and maintaining the clock stopped (Ref. PB/KL/JA/mt (2010) 5312865).

7. Letter from applicant to EFSA, received 3 January 2011, providing additional information.

8. Letter from EFSA to applicant, dated 4 March 2011, requesting additional information and maintaining the clock stopped (Ref. PB/KL/JA/lg (2011) 5610867).

9. Letter from applicant to EFSA, received 15 March 2011, providing additional information.

10. Letter from EFSA to applicant, dated 31 May 2011, restarting the clock (Ref. PB/KL/JA/mt (2011) 5803594).

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Appendix III

Soy products

By Dagrunn Engeset and Inger Therese Lillegaard

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

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

Reason for the choice of menus

The milk allergy menu

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

The vegan menu

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

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

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

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

7 days vegan menu, high preference for soy products

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

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

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

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

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

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

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

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

Sunday:
Breakfast: soy sausages, chapatti, onion, pickles, tomato juice, tea
Lunch: tofu wrap (like lunch Tuesday)
Snack: fruit salad
Dinner: Vegan meatballs (chickpeas, tofu, water, rolled oats, wheat flour) in tomato sauce, spaghetti, mixed salad, soda, soy chocolate dessert
Supper: vegan cheesecake with raspberries (cheese cream topping: soy cream cheese, tofu, sugar, lemon), coffee

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

Monday:
Breakfast: Oat porridge (like vegan)
Lunch: Bread with salami and soy cheese, tomato/cucumber/bell pepper, orange juice, coffee
Snack: Banana, walnuts
Dinner: Sausages without milk, mashed potatoes with soy milk, mixed salad, water
Supper: Coarse bread, boiled egg, pickled herring, milk free margarine, mayonnaise, soy milk

Tuesday:
Breakfast: Bread breakfast (like Monday lunch)
Lunch: Bread lunch (like Monday supper)
Snack: Smoothie (like vegan)
Dinner: Vegetable soup (like vegan Thursday)
Supper: omelette with bread, soy milk, tea

Wednesday:
Breakfast: Weetabix with soy milk
Lunch: Bread lunch (like Monday supper)
Snack: Banana and nuts
Dinner: Meat balls, mushy peas, potatoes, carrots, sauce, lingonberry jam, water
Supper: Oat porridge (like vegan)

Thursday:
Breakfast: Smoothie (soy milk, strawberries, banana, apple juice)
Lunch: Bread lunch (like Monday supper)
Snack: Soy yoghurt with nuts, grapes
Dinner: Fish gratin made with soy milk, carrots, bacon, water, soy chocolate dessert
Supper: oat porridge (like vegan)

Friday:
Breakfast: Corn flakes with soy milk, coffee, orange juice
Lunch: Tomato soup with macaroni (without milk), white bread, water
Snack: Milk chocolate without milk, cashew nuts, raspberries
Dinner: Lasagne (cheese sauce of soy milk and soy cheese), mixed salad, pita bread, wine, water, soy ice cream

Supper: Pizza with soy cheese, beer, potato chips

<u>Saturday</u>:
Breakfast: Egg and bacon, bread, orange juice, coffee
Lunch: Mixed salad with chicken and tofu, pita bread, water
Snack: Smoothie (like Thursday breakfast)
Dinner: Rice porridge made with soy milk, mutton ham, lemonade
Supper: Taco with soy sour cream and soy cheese, beer

<u>Sunday</u>:
Breakfast: Omelette with soy cheese, bread, cucumber/bell pepper, orange juice, tea
Lunch: waffle with soy milk (ordinary waffle with egg where soy milk replaces milk), jam, soy sour cream, coffee with soy cream and sugar
Snack: Milk free milk chocolate, nuts, fruit
Dinner: Salmon with potato, soy sour cream, cucumber, carrots, water, fruit salad
Supper: Vegan cheesecake with raspberries, coffee