Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

VKM Report 2015:09

Final health and environmental risk assessment of genetically modified soybean MON 89788

Scientific opinion on herbicide tolerant, genetically modified soybean MON 89788 from Monsanto Company for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (Application EFSA/GMO/NL/2006/36)

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

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Food/feed and environmental risk assessment of herbicide tolerant genetically modified soybean MON 89788 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (EFSA/GMO/NL/2006/36)

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Scientific opinion on herbicide tolerant, genetically modified soybean MON 89788 from Monsanto Company for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (Application EFSA/GMO/NL/2006/36)

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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 Dahl, Knut Tomas Dalen, Hilde-Gunn Hoen-Sorteberg, Olavi Junttila, Richard Meadow, Kåre M. Nielsen and Monica Sanden.

Acknowledgment

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

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

Competence of VKM experts

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

Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

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Abstract

Soybean MON 89788 expresses the *cp4 epsps* gene from the plant pathogenic bacterium *Agrobacterium tumefaciens* (*Rhizobium radiobacter*) sp. strain CP4. The encoded enzyme 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein confers tolerance to the active herbicidal substance glyphosate. Updated bioinformatics analyses of the inserted DNA and flanking sequences in soybean MON 89788 have not indicated a potential production of putative harmful proteins or polypeptides caused by the genetic modification. Genomic stability of the functional insert and consistent expression of the *cp4 epsps* gene, have been shown over several generations of soybean MON 89788. With the exception of the intended changes caused by the trans-genetically introduced trait, data from several field trials performed in USA and Argentina show that soybean MON 89788 is compositionally, morphologically and agronomically equivalent to its conventional counterpart and other commercial soybean varieties. A sub-chronic feeding study with rats, as well as a nutritional assessment trial with broilers has not revealed adverse effects of soybean MON 89788. These studies indicate that soybean MON 89788 is nutritionally equivalent to, and as safe as conventional soybean varieties. The CP4 EPSPS protein produced in soybean MON 89788 does not show sequence resemblance to known toxins or IgE-dependent allergens, nor has it been reported to cause IgE-mediated allergic reactions. Soybean is not cultivated in Norway, and there are no cross-compatible wild or weedy relatives of soybean in Europe.

Based on current knowledge, the VKM GMO Panel concludes that with the intended usage, there are no discernible safety concerns associated with soybean MON 89788 regarding human or animal health or to the environment 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 (former Norwegian Directorate for Nature Management) and the Norwegian Food Safety Authority (NFSA) to conduct final food, feed and environmental risk assessments of all genetically modified organisms (GMOs) and products containing or consisting of GMOs that are authorised in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC. The request covers scope(s) relevant to the Gene Technology Act. The request does not cover GMOs that VKM already has conducted its final risk assessments on. However, the Agency and NFSA requests VKM to consider whether updates or other changes to earlier submitted assessments are necessary.

The herbicide-tolerant genetically modified soybean MON 89788 (Unique Identifier MON-89788-1) from Monsanto Company is approved under Regulation (EC) No 1829/2003 for food and feed uses, import and processing since 4 December 2008 (Application EFSA/GMO/NL/2006/36, Commission Decision 2008/933/EC).

Soybean MON 89788 has previously been assessed as food and feed by the VKM GMO Panel commissioned by the Norwegian Food Safety Authority related to the EFSAs public hearing of the application EFSA/GMO/2006/36 in 2007 (VKM 2007). MON 89788 has also been evaluated by the VKM GMO Panel as a component of the stacked GM events MON 87701 x MON 89788 (EFSA/GMO/NL/2009/73) and MON 87705 x MON 89788 (EFSA/GMO/NL/ 2011/100) (VKM 2010, 2013).

The food, feed and environmental risk assessment of the soybean MON 89788 is based on information provided by the applicant in the application EFSA/GMO/NL/2006/36, and scientific comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment also considered other relevant peer-reviewed scientific literature.

The VKM GMO Panel has evaluated MON 89788 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. The Norwegian Scientific Committee for Food Safety 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 2011a), 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 2011c).

The scientific risk assessment of soybean MON 89788 includes molecular characterisation of the inserted DNA and expression of novel proteins, comparative assessment of agronomic and phenotypic characteristics, nutritional assessments, toxicology 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 89788 expresses the gene encoding the enzyme CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS), which is derived from the CP4 strain of *Agrobacterium tumefaciens* (updated scientific name: *Rhizobium radiobacter*), and renders soybean MON 89788 tolerant to the active herbicidal substance glyphosate.

Molecular characterisation

The applicant has provided sufficient analyses to characterise the DNA insert, number of inserts, integration site and flanking sequences in the soybean genome. The results show that one functional copy of the *cp4 epsps* gene only, is present in the soybean MON 89788 genome. No other functional vector genes were detected. Similarity searches in 2006, with databases of known toxins and allergens did not indicate potential production of harmful proteins or polypeptides caused by the genetic modification. Southern blot and segregation analyses show that the introduced gene elements are stably inherited and expressed over multiple generations, and consistent with the observed phenotypic characteristics of soybean MON 89788. The VKM GMO Panel concludes that the molecular characterisation of soybean MON 89788 does not indicate a safety concern.

Comparative assessments

The VKM GMO Panel has considered the available literature on compositional data and found that except for small intermittent variations, no biologically relevant differences exist between soybean MON 89788 and its corresponding control in the analyses of seed and forage. The field studies investigating composition of MON 89788 show no biologically relevant differences between GM crops treated and untreated with glyphosate. The data presented do not show unintended effects as a result of the genetic modification.

Based on current knowledge, the VKM GMO Panel concludes that with the exception of the introduced trait, soybean MON 89788 is compositionally, agronomically, morphologically and ecologically equivalent to its conventional counterpart, and other conventional soybean varieties.

Food and feed risk assessment

A 90-day sub-chronic toxicity study with rats, as well as a nutritional assessment trial with broilers fed diets containing soybean MON 89788 did not indicate any adverse effects. The CP4 EPSPS protein does not show sequence resemblance to known toxins or IgE-dependent allergens, nor has it been reported to cause IgE-mediated allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that soybean MON 89788 is nutritionally equivalent to and as safe as its conventional counterpart and other conventional soybean varieties.

Environmental assessment

Considering the intended uses of soybean MON 89788, excluding 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 89788.

Soybean MON 89788 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 89788. 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.

Overall conclusion

Based on current knowledge and considering the intended usage, the VKM GMO Panel concludes that soybean MON 89788 is as safe as its conventional counterpart and commercial soybean varieties. With the exception of the introduced trait, soybean MON 89788 is nutritionally, morphologically, agronomically and ecologically equivalent to conventional soybean varieties.

Likewise, the VKM GMO Panel concludes that soybean MON 89788 does not represent an environmental risk in Norway.

Key words: GMO, soybean (*Glycine max*), MON 89788, EFSA/GMO/NL/2006/36, herbicide tolerance, *cp4 epsps,* food and feed safety, environmental risk evaluation, Regulation (EC) No 1829/2003, VKM, risk assessment, Norwegian Scientific Committee for Food Safety, Norwegian Environment Agency

Sammendrag på norsk

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

Den genmodifiserte, herbicidtolerante soyalinjen MON 89788 (unik kode MON-89788-1) fra Monsanto Company ble godkjent til import, videreforedling og til bruk som mat og fôr under EU-forordning 1829/2003 4. desember 2008 (Kommisjonsbeslutning 2008/913/EU).

Soyalinjen MON 89788 ble første gang vurdert av VKMs faggruppe for GMO i 2007 (VKM 2007). Helserisikovurderingen ble utført på oppdrag av Mattilsynet i forbindelse med EFSAs offentlige høring av søknad EFSA/GMO/NL/2006/36 i 2007. VKMs faggruppe for GMO har også risikovurdert to soyahybrider der den genmodifiserte soyaen inngår som en av foreldrelinjene – MON 87701 x MON 89788 (EFSA/GMO/NL/2009/73) og MON 87705 x MON 89788 (EFSA/GMO/NL/2011/100) (VKM 2010, VKM 2013).

Risikovurderingen av den genmodifiserte soyalinjen er basert på uavhengige vitenskapelige publikasjoner og dokumentasjon som er gjort tilgjengelig på EFSAs nettside EFSA GMO Extranet. Vurderingen er gjort i henhold til tiltenkt bruk i EU/EØS-området, og i overensstemmelse med 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; 2010; 2011 a,b,c) 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, metabolitter, 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 89788 uttrykker *cp4 epsps*-genet fra jordbakterien *Agrobacterium tumefaciens* (syn. *Rhizobium radiobacter*). Genet koder for enzymet 5-enolpyruvylsikimat-3-fosfatsyntetase, som omdanner fosfoenolpyruvat og sikimat-3-fosfat til 5-enolpyruvylsikimat-3-fosfat, en viktig metabolitt i syntesen av aromatiske aminosyrer. I motsetning til plantens eget enzym er det bakterielle enzymet også aktivt ved nærvær av N-fosfonometylglycin (glyfosat). De transgene plantene vil derfor tolerere høyere doser av herbicider med virkestoffet glyfosat sammenlignet med konkurrerende ugras.

Molekylær karakterisering

Søkeren har oppgitt tilstrekkelige analysedata til å karakterisere de introduserte DNA-innskuddene, antallet integreringer, integreringssteder, og innskuddenes flankerende DNA-sekvenser i genomet til soya MON 89788. Resultatene viser at kun ett funksjonelt *cp4 epsps* gen er integrert i genomet til soyalinjen. Homologisøk fra 2006, med databaser over kjente toksiner og allergener, indikerer at genmodifiseringen ikke har ført til potensiell produksjon av skadelige proteiner eller polypeptider i soya MON 89788. Southern blot og segresjons - analyser viser at det introduserte genet er stabilt nedarvet og uttrykt over flere generasjoner, og i samsvar med de fenotypiske egenskapene til soya MON 89788. VKMs faggruppe for GMO konkluderer med at den molekylære karakteriseringen ikke indikerer noen helserisiko ved soya MON 89788.

Komparative analyser

VKMs faggruppe for GMO har vurdert tilgjengelig litteratur vedrørende soya MON 89788 og funnet at det, med unntak av små tilfeldige variasjoner i enkeltparametere målt i bønner og øvrig plantemateriale, ikke foreligger biologisk relevante forskjeller mellom den genmodifiserte soyaen og dens kontroll. Feltstudier viste ingen ernæringsmessig effekt av sprøyting med glyfosat på soya MON 89788. De rapporterte dataene viser ingen utilsiktede effekter som følge av genmodifiseringen.

Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO at soya MON 89788, med unntak av den introduserte egenskapen, er ernæringsmessig, agronomisk, morfologisk og økologisk vesentlig lik dens konvensjonelle motpart, samt andre konvensjonelle sorter.

Helserisiko

En 90-dagers sub-kronisk toksisitetsstudie med rotter og en ernæringsstudie utført med broilere gitt fôr inneholdende soya MON 89788, har ikke indikert helseskadelige effekter. CP4 EPSPS-proteinet viser 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 89788 er ernæringsmessig lik, og like trygg som, dens konvensjonelle motpart og andre konvensjonelle sorter.

Miljørisiko

Med bakgrunn i tiltenkt bruksområde for søknaden er miljørisikovurderingen av soyalinje MON 89788 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 89788 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.

Samlet vurdering

Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO at soya MON 89788, ved forskreven bruk, er like trygg som dens konvensjonelle motpart og andre konvensjonelle soyasorter. Soya MON 89788 er ernæringsmessig, morfologisk, agronomisk og økologisk ekvivalent med konvensjonell soya.

Likeledes finner faggruppen, ut i fra dagens kunnskap, at den omsøkte bruken av soya MON 89788 ikke vil medføre noen miljørisiko i Norge.

Abbreviations and/or glossary

ADF	Acid Detergent Fibre. The insoluble residue remaining after boiling a feed/food sample in acid detergent solution. It contains many insoluble (structural) fibre components – lignin, cellulose, silica – but also insoluble forms of nitrogen. It does not, however, contain hemicellulose. See also NDF.
ARMG	Antibiotic resistance marker gene
BC	Backcross. Backcross breeding is extensively used to move a single trait of interest (e.g. disease resistance gene) from a donor line into the genome of a preferred or "elite" line without losing any part of the preferred lines pre-existing genome. The plant with the gene of interest is the donor parent, while the elite line is the recurrent parent. BC_1, BC_2 etc. designates the backcross generation number.
BLAST	Basic Local Alignment Search Tool. Software that is used to compare nucleotide (BLASTn) or protein (BLASTp) sequences to sequence databases and calculate the statistical significance of matches, or to find potential translations of an unknown nucleotide sequence (BLASTx). BLAST can be used to understand functional and evolutionary relationships between sequences and help identify members of gene families.
bp	Basepair
Bt	*Bacillus thuringiensis*
CaMV	Cauliflower mosaic virus
Codex	Set by The Codex Alimentarius Commission (CAC), an intergovernmental body to implement the Joint FAO/WHO Food Standards Programme. Its principle objective is to protect the health of consumers and to facilitate the trade of food by setting international standards on foods (i.e. Codex Standards).
Cp4 epsps	The *5-enolpyruvylshikimate-3-phosphate synthase* gene from *Agrobacterium tumefaciens* strain CP4
CTP	Chloroplast transit peptide
DAP	Days after planting
DNA	Deoxyribonucleic acid
DT50	Time to 50% dissipation of a protein in soil
DT90	Time to 90% dissipation of a protein in soil
dw	Dry weight
dwt	Dry weight tissue

EC	European Commission
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EPSP	5-enolpyruvylshikimate-3-phosphate
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	Environmental risk assessment
E-score	Expectation score
EU	European Union
fa	Fatty acid
FAO	Food and Agriculture Organisation
FIFRA	US EPA Federal Insecticide, Fungicide and Rodenticide Act
Fitness	Describes an individual's ability to reproduce successfully relative to that of other members of its population.
fw	Fresh weight
fwt	Fresh weight tissue
GAT	Glyphosate N-acetyltransferase
GLP	Good Laboratory Practice
Glyphosate	Broad-spectrum systemic herbicide
GM	Genetically Modified
GMO	Genetically Modified Organism
GMP	Genetically Modified Plant
H	Hybrid
ha	Hectare
ILSI	International Life Sciences Institute
IPM	Integrated Pest Management
IRM	Insect Resistance Management
Locus	The position/area that a given gene occupies on a chromosome
LOD	Limit of detection

LOQ	Limit of quantification
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionisation-Time Of Flight. A mass spectrometry method used for detection and characterisation of biomolecules, such as proteins, peptides, oligosaccharides and oligonucleotides, with molecular masses between 400 and 350,000 Da.
mRNA	Messenger RNA
MS	Member States
MT/NFSA	Norwegian Food Safety Authority (Mattilsynet)
NDF	Neutral detergent fibre, The insoluble residue remaining after boiling a feed/food sample in neutral detergent solution. It contains most insoluble (structural) fibre components – cellulose, hemicellulose, lignin, silica, tannins and cutins. See also ADF.
Northern blot	A technique used to study gene expression by detection of RNA or mRNA separated in a gel according to size.
NTO	Non-target organism
Near-isogenic lines	Term used in genetics/plant breeding, and defined genetic lines that are identical except for differences at a few specific locations or genetic loci.
OECD	Organisation for Economic Co-operation and Development
ORF	Open Reading Frame; a molecular reading frame that can code for amino acids between two successive stop codons.
OSL	Over season leaf
OSR	Over season root
OSWP	Over season whole plant
PCR	Polymerase chain reaction, a technique to amplify DNA by copying
R0	First transformed generation, parent
RNA	Ribonucleic acid
RP	Recurrent parent
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis. Technique to separate proteins according to their approximate size
SAS	Statistical Analysis System
SD	Standard deviation
Southern blot	Method used for transfer of electrophoresis-separated DNA fragments to a filter membrane and possible subsequent fragment detection by probe hybridisation

Soybean Growth Stages	**Vegetative Stages** VE - Emergence VC - Cotyledon stage V1- First trifoliolate V2 – Second trifoliolate V3 – Third trifoliolate V(n) – nth trifoliolate	**Reproductive Stages** R1 – Beginning flowering R2 – Full flowering R3 – Beginning pod (pods 5 mm in top 4 nodes) R4 – Full pod (pods 2 cm in top 4 nodes) R5 – Beginning seed (seed 3 mm long in top 4 nodes) R6 – Full size seed (pod containing a green seed that fills the pod capacity in top 4 nodes on the main stem) R7 – Beginning maturity (one pod on the main stem has reached its mature pod colour) R8 – Full maturity (95 % of the pods on the plant have reach their full mature colour)
SPC	Soybean protein concentrate	
T-DNA	Transfer DNA, the transferred DNA of the tumour-inducing (Ti) plasmid of some species of bacteria such as *Agrobacterium tumefaciens* and *A. rhizogenes*, into plant's nuclear genome. The T-DNA is bordered by 25-base-pair repeats on each end. Transfer is initiated at the left border and terminated at the right border, and requires the *vir* genes of the Ti plasmid.	
TI	Trait integrated	
TMDI	Theoretical Maximum Daily Intake	
Transgene copy number	Defined as the number of exogenous DNA insert(s) in the genome. If the exogenous DNA fragment inserts only once at a single locus of the genome, it is a single copy transgenic event.	
U.S. EPA	United States Environmental Protection Agency	
VKM	Norwegian Scientific Committee for Food Safety	
Western blot	Technique used to transfer proteins separated by gel electrophoresis by 3-D structure or denaturated proteins by the length of the polypeptide to a membrane, where they might be identified by antibody labelling.	
WHO	World Health Organisation	

Background

On 7 November 2006, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands an application (Reference EFSA/GMO/NL/2006/36) for authorisation of the genetically modified herbicide tolerant soybean MON 89788 (Unique Identifier MON-89788-1), submitted by Monsanto Company 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/NL/2006/36 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 publicity 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 8 June 2007, EFSA declared the application as valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the 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 1929/2003, 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 September 2007 (VKM 2007). EFSA published its scientific opinion 2 July 2008 (EFSA 2008), and soybean MON 89788 was approved for food and feed uses, import and processing 4 December 2008 (Commission Decision 2008/933/EC).

Soybean MON 89788 has also been evaluated by the VKM GMO Panel as a component of the stacked GM maize events MON 87701 x MON 89788 (EFSA/GMO/NL/2009/73) and MON 87705 x MON 89788 (EFSA/GMO/NL/2011/100)(VKM 2010, 2013).

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, 2011a), 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 varieties (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

Genetically modified soybean MON 89788 (Unique Identifier MON-89788-1) (Trade name Roundup Ready 2 Yield®) is an analogue to the well characterised soybean 40-3-2 also developed by Monsanto (VKM 2014). They mainly differ regarding transgene delivery; soybean 40-3-2 was transformed by particle acceleration, whereas soybean MON 89788 was developed by *Agrobacterium*-mediated transformation. Both soybean events express the same particular gene that provides a high tolerance to the broad spectrum systemic herbicide glyphosate, the active ingredient in Roundup. Roundup is widely used in a variety of weed control programs throughout most of the world.

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

In glyphosate-tolerant soybean MON 89788, the herbicide tolerance trait is generated in the plants through the addition of a bacterial *epsps* gene derived from the common plant pathogenic, soil bacterium *Agrobacterium tumefaciens* (updated scientific name: *Rhizobium radiobacter*) sp. strain CP4 (CP4 EPSPS). The enzyme produced from the *cp4 epsps* gene has a lower affinity to the herbicide compared with the innate soybean enzyme, and thus confers glyphosate-tolerance to the whole plant.

The genetic modification in soybean MON 89788 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 89788 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 2011a), 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 2011c).

The food, feed and environmental risk assessment of the genetically modified soybean MON 89788 is based on information provided by the applicant in the application EFSA/GMO/NL/2006/36, relevant peer-reviewed scientific literature, and scientific opinions and comments from EFSA and other member states made available on the EFSA website GMO Extranet.

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

2.1 Information related to the genetic modification

2.1.1 Description of the methods used for the genetic modification

MON 89788 was developed through *Agrobacterium*-mediated transformation of meristem tissue from the soybean cultivar A3244 with the binary vector PV-GMGOX20 (Figure 2.1.2-1). The PV-GMGOX20 vector contains the *cp4 epsps* coding sequence intended for transfer to the soybean genome.

Selected plants of the first generation (R_0) were self-pollinated to generate R_1 plants. The R_1 plants that were homozygous for the *cp4 epsps* insert and tolerant to glyphosate were advanced for further development, with the subsequent selection of progeny designated as soybean MON 89788. A flow chart over the development of soybean MON 89788 is shown in Figure AI-1 in Appendix I (AI).

2.1.2 Nature and source of vector used

The plasmid vector PV-GMGOX20 (Figure 2.1.2-1) is approximately 9.7 kb in size, containing the plasmid backbone region (~5.4 kb), and the transfer DNA (T-DNA) of ~ 4.3kb (Table 2.1.2-1). The T-DNA consists of the chimeric promoter FMV/Tsf1 which contains enhancer sequences (the Tsf1 leader and intron sequence) to regulate constitutive expression of the combined *CTP2/cp4 epsps* genes, and finally the *E9* 3' nontranslated sequence, which directs transcriptional termination and polyadenylation. CP4 EPSPS confers tolerance to glyphosate, and the CTP2 transit peptide directs transport of CP4 EPSPS to chloroplasts.

The plasmid backbone includes the bacterial gene *aadA* that confers resistance to the antibiotics spectinomycin and streptomycin. This gene was used during molecular cloning and for selection purposes prior to plant transformation. As this gene resides outside the T-DNA border regions, it is not expected to be transferred into the soybean genome.

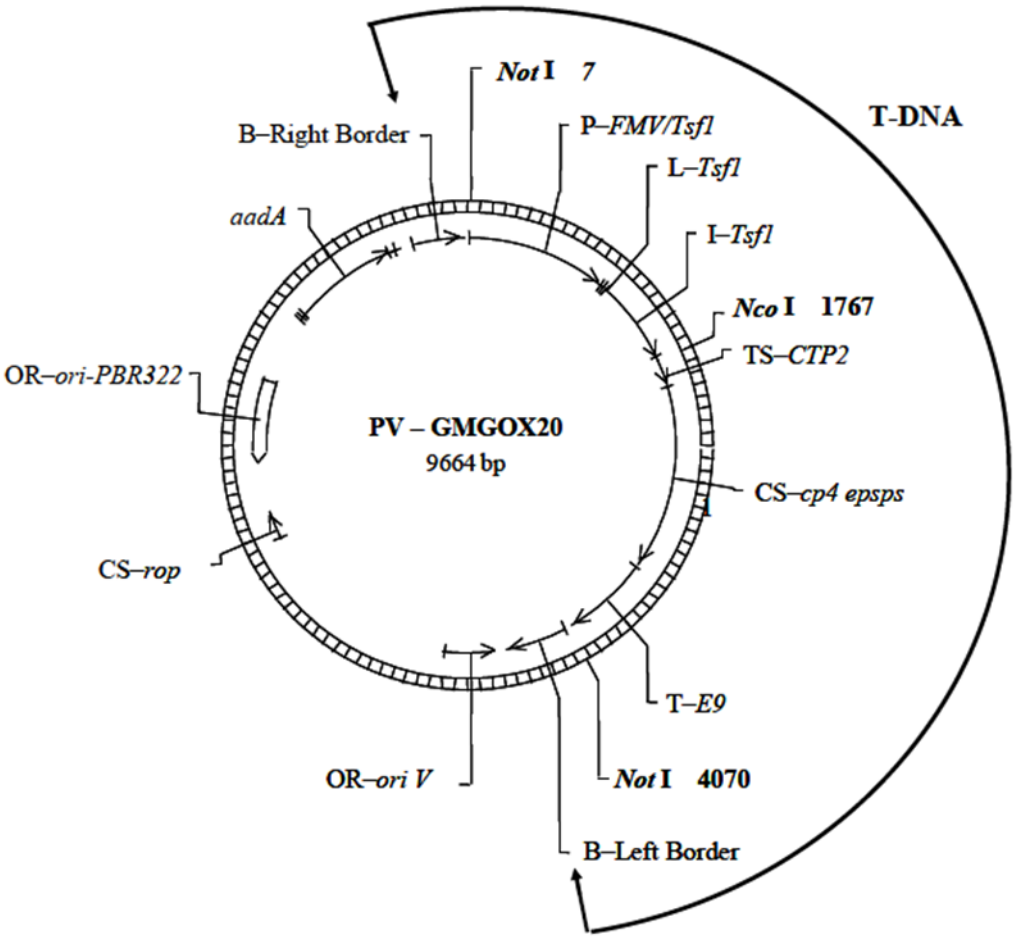

Figure 2.1.2-1. Circular map of plasmid PV-GMGOX20. The genetic elements and restriction sites for the enzymes used in the Southern analyses (with positions relative to the plasmid vector) are shown on the plasmid map. The region intended for insertion into the soybean genome (T-DNA) is highlighted on the exterior of the map. (Figure 1. in Technical dossier).

Table 2.1.2-1. Summary of genetic elements in the plasmid PV-GMGOX20 (Table 3. In Technical Dossier)

Genetic element[1, 2]	Position in plasmid	Function and/or reference
		T-DNA
Intervening sequence	1-51	Sequences used in DNA cloning
P–*FMV/Tsf1*	52-1091	Chimeric promoter consisting of enhancer sequences from the *35S* promoter of the Figwort Mosaic Virus (Richins *et al.*, 1987) and the promoter from the *Tsf1* gene of *Arabidopsis thaliana* encoding the elongation factor EF-1 alpha (Axelos *et al.*, 1989)
L–*Tsf1*	1092-1137	5' nontranslated leader sequence (exon 1) from the *Tsf1* gene of *Arabidopsis thaliana* encoding the elongation factor EF-1 alpha (Axelos *et al.*, 1989)
I–*Tsf1*	1138-1759	Intron from the *Tsf1* gene of *Arabidopsis thaliana* encoding the elongation factor EF-1 alpha (Axelos *et al.*, 1989)
Intervening sequence	1760-1768	Sequences used in DNA cloning
TS–*CTP2*	1769-1996	Sequences encoding the chloroplast transit peptide from the *ShkG* gene of *Arabidopsis thaliana* encoding EPSPS (Klee *et al.*, 1987)
CS–*cp4 epsps*	1997-3364	Codon optimized coding sequence of the *aroA* (*epsps*) gene from the *Agrobacterium* sp. strain CP4 encoding the CP4 EPSPS protein (Barry *et al.*, 1997; Padgette *et al.*, 1996)
Intervening sequence	3365-3406	Sequences used in DNA cloning
T–*E9*	3407-4049	3' nontranslated sequence from the ribulose-1,5-bisphosphate carboxylase small subunit (*RbcS2*) *E9* gene of pea (*Pisum sativum*) (Coruzzi *et al.*, 1984)
Intervening sequence	4050-4092	Sequences used in DNA cloning
B–Left border	4093-4534	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker *et al.*, 1983)
		Vector backbone
Intervening sequence	4535-4620	Sequences used in DNA cloning
OR–*ori V*	4621-5017	Origin of replication from the broad host range plasmid RK2 for plasmid maintenance in *Agrobacterium* (Stalker *et al.*, 1981)
Intervening sequence	5018-6525	Sequences used in DNA cloning

Table 2.1.2-1. Continued

Genetic element[1, 2]	Position in plasmid	Function and/or reference
CS–*rop*	6526-6717	Coding sequence for the repressor of primer (ROP) protein for maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989)
Intervening sequence	6718-7134	Sequences used in DNA cloning
OR–*ori-PBR322*	7135-7763	Origin of replication from pBR322 for plasmid maintenance in *E. coli* (Sutcliffe, 1978)
Intervening sequence	7764-8263	Sequences used in DNA cloning
aadA	8264-9152	Bacterial gene encoding an aminoglycoside-modifying enzyme, 3' (9)-O-nucleotidyl-transferase from the transposon Tn*7* (Fling *et al.*, 1985)
Intervening sequence	9153-9288	Sequences used in DNA cloning
		T-DNA
B–Right border	9289-9645	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA (Depicker *et al.*, 1982)
Intervening sequence	9646-9664	Sequences used in DNA cloning

1 Intervening sequences are not regarded as genetic elements.

2 P – Promoter; L – Leader; I – Intron; TS – Targeting sequence; CS – Coding sequence; T – 3' nontranslated transcriptional termination and polyadenylation signal sequences; B – Border region; OR – Origin of replication.

2.1.3 Source of donor DNA, size and intended function of each constituent fragment of the region intended for insertion

The genetic elements intended for insertion into the receiving soybean genome are comprised between the B-Right and B-Left T-DNA borders in plasmid PV-GMGOX20, as indicated in Figure 2.1.2-1 and Table 2.1.2-1. The left and right border elements are necessary for the efficient transfer of the T-DNA into the soybean host genome and were derived from *Agrobacterium tumefaciens* plasmids.

Starting from the B-Right border is the chimeric and transcriptionally constitutive promoter *FMV/Tsf1,* consisting of enhancer sequences from the Figwort Mosaic Virus *35S* promoter followed by the first exon and intron of *Tsf1* from *Arabidopsis thaliana.* These genetic elements enhance the constitutive expression of the *CTP2/cp4 epsps* coding sequence. The chloroplast transit peptide sequence, *CTP2,* which precedes the *cp4 epsps* coding sequence, is derived from the *Arabidopsis thaliana* endogenous *epsps* gene. This transit peptide directs the transport of the CP4 EPSPS protein to the chloroplast, which is where the plant EPSPS resides and where the site of aromatic amino acid biosynthesis is. EPSPS catalyses the conversion of shikimate-3-phosphate (S3P) and phosphoenolpyruvate (PEP) into 5-enolpyruvylshikimate-3-phosphate (EPSP), an intermediate required for the production of aromatic amino acids. The bacterial CP4 EPSPS in MON 89788 is a 47.6 kDa single polypeptide of 450 amino acids, and confers a high level of tolerance to glyphosate, unlike most native plant and other microbial EPSPS enzymes.

The final element, the *E9* sequence, contains the 3' nontranslated region of the ribulose-1,5-bisphosphate carboxylase small subunit (*RbcS2*) that directs transcriptional termination and polyadenylation of the *CTP2/cp4 epsps* mRNA.

2.2 Information relating to the GM plant

2.2.1 Description of the trait(s) and characteristics introduced or modified

Soybean MON 89788 contains a single functional gene encoding the CP4 EPSPS protein, which confers tolerance to glyphosate.

2.2.2 Information on the sequences actually inserted or deleted

2.2.2.1 The size and copy number of all detectable inserts, both complete and partial

The applicant analysed genomic DNA of MON 89788 by Southern blot to determine the insert number (number of integration sites of the transgene within the soybean genome) and copy number (number of repeats/copies of the transgene sequence within integration site(s)). The molecular analysis of MON 89788 is described in Dickinson et al. (2006).

Genomic DNA of soybean MON 89788 was digested with the restriction enzymes *Xmn* I, *Bpl* I, *Nco* I and/or *Not* I. Genomic DNA from the parent soybean cultivar A3244 and plasmid PV-GMGOX20, were used as negative and positive controls, respectively. Figure 2.2.2.1-1 shows a schematic representation of restriction sites, insert and genomic regions flanking the insertion in MON 89788.

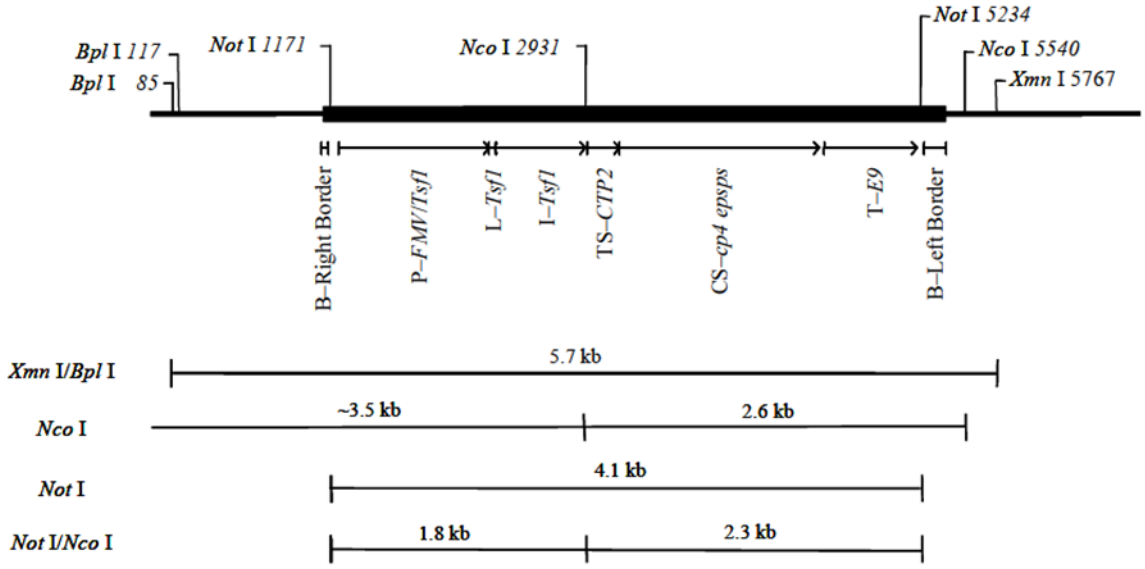

Figure 2.2.2.1-1. Schematic representation of the insert and genomic flanking sequences in MON 89788 (Figure 5. In Technical Dossier). A linear map of the insert and genomic DNA flanking the insert in MON 89788 is shown. The upper portion of the figure displays the genetic elements within the insert (thick rectangular bar), as well as the restriction sites used in Southern blot analyses (with positions relative to the soybean genome). Arrows underneath the designated insert indicate the direction of transcription. Shown on the lower portion of the map are the sizes of the DNA fragments after digestion with the respective restriction enzyme or with a combination of enzymes.

2.2.2.2 Insert and copy number determination

The insert number was determined by digesting MON 89788 and A3244 DNA with the combination of restriction enzymes *Bpl* I and *Xmn* I, which cleave outside but not within the insert. These enzymes should release a restriction fragment containing the entire insert and adjacent plant genomic DNA (Figure 2.2.2.1-1). The number of restriction fragments detected should indicate the number of inserts present in MON 89788. The copy number (i.e. copies of the insert within a locus) was determined by digesting genomic MON 89788 DNA with the restriction enzyme *Nco* I. If MON 89788 contains one copy of the insert, Southern blots probed with the entire insert (after *Nco* I digestion) should therefore result in two bands, each representing a portion of the insert along with adjacent plant genomic DNA (Figure 2.2.2.1-1).

MON 89788 DNA digested with a combination of *Bpl* I and *Xmn* I produced a single band of ~5.7 kb, indicating that MON 89788 contains one insert located within a ~5.7 kb *Bpl* I / *Xmn* I restriction fragment (Figure 2.2.2.1-1). Digestion with *Nco* I produced two unique bands of 2.6 and ~3.5 kb. The positive control, plasmid PV-GMGOX20 DNA mixed with A3244 DNA, digested with *Not* I produced two bands of 4.1 kb and 5.6 kb, respectively, as expected from the two restriction sites for *Not* I within the plasmid (~ 9.7kb) (Figure 2.1.2-1).

The banding patterns from the Southern analyses indicate that only a single copy of the insert is present in soybean MON 89788.

2.2.2.3 Assessment of the cp4 epsps cassette integrity

The applicant has assessed the integrity of the inserted *cp4 epsps* coding sequence and associated genetic elements by digesting MON 89788 DNA with *Not* I or with a combination of *Not* I and *Nco* I and probing the Southern blots with the individual genetic elements of the the *cp4 epsps* gene cassette. Digestion with *Not* I was expected to generate a single 4.1 kb restriction fragment containing the *cp4 epsps* gene cassette, while digestion with the combination of *Not* I and *Nco* I was expected to generate two restriction fragments of 1.8 kb and 2.3 kb (2.2.2.1-1). The 1.8 kb fragment contains the *FMV/Tsf1* promoter, the *Tsf1* leader and the *Tsf1* intron, whereas the 2.3 kb fragment contains the *CTP2* targeting sequence, the *cp4 epsps* coding sequence and the *E9 3'* nontranslated region. Plasmid PV-GMGOX20 DNA digested with *Not* I or with a combination of *Not* I and *Nco* I and mixed with A3244 DNA was used as a positive hybridisation control and size estimator. Each individual Southern blot was examined with the probes: *FMV/Tsf1* promoter + *Tsf1* leader, *Tsf1* intron, *CTP2* targeting sequence + *cp4 epsps* coding sequence or the *E9 3'* nontranslated sequence, respectively.

According to the analysis data, all hybridisations showed the expected banding patterns without any major deviations, indicating that the integrity of the entire T-DNA insert is preserved in soybean event MON 89788.

2.2.2.4 Assessment of potential elements from the plasmid PV-GMGOX20 backbone in MON 89788

The applicant has performed a Southern blot analysis in which DNA from MON 89788 and A3244 were digested with either the combination of the restriction enzymes *Bpl*I and *Xmn*I, or with *Nco*I. A mix of Plasmid PV-GMGOX20 DNA and A3244 DNA was digested with *Not*I and used as a positive control. The blot was hybridised simultaneously with three overlapping probes that spanned the backbone sequence of PV-GMGOX20. DNA from the negative control A3244 digested with a combination of *Bpl*I and *Xmn*I or with *Nco*I showed no detectable hybridisation bands. Plasmid PV-GMGOX20 DNA mixed with A3244 DNA and digested with *Not*I produced the expected band at 5.6 kb. MON 89788 DNA digested with either a combination of *Bpl*I and *Xmn*I or with *Nco*I showed no detectable hybridisation signal. The results indicate that MON 89788 does not contain any detectable backbone sequence from the plasmid PV- GMGOX20.

2.2.2.5 The organisation of the inserted genetic material including its sequence data and that of flanking 5' and 3' regions

The organisation of the elements within the insert in MON 89788 was assessed by the applicant by PCR amplification followed by DNA sequencing analyses (Dickinson et al., 2006). Several PCR primers were designed with the intent to amplify three overlapping DNA fragments spanning the entire length of the insert and the associated flanking genomic DNA (Figure 2.2.2.5-1). Next these PCR products (amplicons) were subjected to DNA sequencing to determine the organisation of the genetic elements within the insert of MON 89788. Genomic DNA from soybean A3244 and plasmid PV-GMGOX20 were used as negative and positive controls in the PCR reactions, respectively.

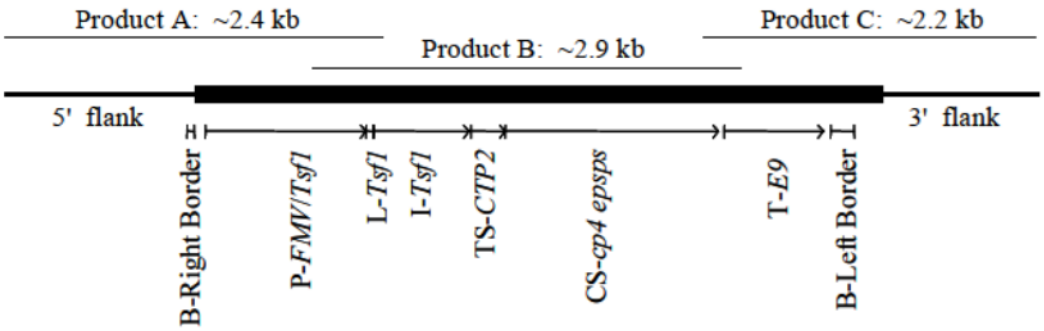

Figure 2.2.2.5-1. Sizes and relative position of the three overlapping DNA fragments (designated products A, B and C) used to span the entire length of the insert and associated flanking genomic DNA in MON 89788. (Adopted from Figure 12 in Technical Dossier)

PCR reactions with the genomic DNA from A3244, did not generate any PCR product, whereas plasmid PV-GMGOX20 produced an expected PCR product of ~2.9 kb. PCR reactions with soybean genomic DNA from MON 89788 produced products of ~2.4 kb for product A, ~2.9 kb for product B and ~2.2 kb for product C. According to the applicant, the

amplification of the predicted size PCR products from MON 89788 indicate that the arrangement and linkage of the elements in the insert are consistent with the PV-GMGOX20 plasmid map and with the map of the insert depicted in Figure 2.2.2.1-1.

According to the applicant, the consensus sequence of the insert and flanking genomic DNA was generated by compiling data from numerous sequencing reactions conducted on the PCR products A, B and C. This analysis resulted in 4303 base pairs (bp) beginning at base 9604 in the right border region of PV-GMGOX20, and ending at base 4242 in the left border region of PV-GMGOX20. A 1103 bp of soybean genomic DNA flanking the 5' end of the insert and 1060 bp of soybean genomic DNA flanking the 3' end of the insert were also determined. According to the applicant these analyses confirmed the presence and organisation of the integrated genetic elements in MON 89788 as described in Table 2.2.2.5-1.

Table 2.2.2.5-1. Summary of genetic elements in the MON 89788 insert (Table 4. In Technical Dossier)

Genetic element[1,2]	Position in sequence[3]	Size (kb)	Function and/or reference
Sequence flanking 5′ end of the insert	1-1103	1.10	Soybean nuclear genomic DNA
B–Right border	1104-1145	0.04	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA (Depicker *et al.*, 1982)
Intervening sequence	1146-1215	0.07	Sequences used in DNA cloning
P–*FMV/Tsf1*	1216-2255	1.04	Chimeric promoter consisting of enhancer sequences from the *35S* promoter of the Figwort Mosaic Virus (Richins *et al.*, 1987) and the promoter from the *Tsf1* gene of *Arabidopsis thaliana* encoding the elongation factor EF-1 alpha (Axelos *et al.*, 1989)
L–*Tsf1*	2256-2301	0.05	5' nontranslated leader (exon 1) from the *Tsf1* gene of *Arabidopsis thaliana* encoding the elongation factor EF-1 alpha (Axelos *et al.*, 1989)
I–*Tsf1*	2302-2923	0.62	Intron from the *Tsf1* gene of *Arabidopsis thaliana* encoding the elongation factor EF-1 alpha (Axelos *et al.*, 1989)
Intervening sequence	2924-2932	0.01	Sequences used in DNA cloning
TS–*CTP2*	2933-3160	0.23	Sequences encoding the chloroplast transit peptide from the *ShkG* gene of *Arabidopsis thaliana* encoding EPSPS (Klee *et al.*, 1987)
CS–*cp4 epsps*	3161-4528	1.37	Codon optimized coding sequence of the *aroA* (*epsps*) gene from the *Agrobacterium* sp. strain CP4 encoding the CP4 EPSPS protein (Barry *et al.*, 1997; Padgette *et al.*, 1996)
Intervening sequence	4529-4570	0.04	Sequences used in DNA cloning
T–*E9*	4571-5213	0.64	3' nontranslated sequence from the ribulose-1,5-bisphosphate carboxylase small subunit (*RbcS2*) *E9* gene of pea (*Pisum sativum*) (Coruzzi *et al.*, 1984)
Intervening sequence	5214-5256	0.04	Sequences used in DNA cloning
B–Left border	5257-5406	0.15	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker *et al.*, 1983)
Sequence flanking 3′ end of the insert	5407-6466	1.06	Soybean nuclear genomic DNA

[1] Intervening sequences and genomic flanking sequences are not regarded as genetic elements.
[2] B – Border; P – Promoter; L – Leader; I – Intron; TS – Targeting sequence; CS – Coding sequence; T – 3' nontranslated transcriptional termination and polyadenylation signal sequences.
[3] Numbers correspond to the sequence in Figure 5 that represents the insert in MON 89788 and adjacent genomic DNA.

2.2.2.6 In the case of deletion(s), size and function of the deleted region(s)

Not applicable

2.2.3 Information on the expression of the inserted sequence

CP4 EPSPS protein levels in tissues derived from MON 89788 were determined by ELISA. The levels of the CP4 EPSPS protein in over-season leaf (OSL), seed, root and forage were measured in tissues collected from MON 89788 produced in replicated field trials across five Argentinean and five US field locations during the 2004-2005 and 2005 growing seasons, respectively (Mozaffar and Silvanovich 2006, Pineda and Silvanovich 2006). CP4 EPSPS protein levels for all tissue types were calculated on a µg/g protein per gram fresh weight (fw) basis. Moisture content was determined in each tissue type and protein levels were converted to a dry weight (dw) basis by calculation. For MON 89788, the mean CP4 EPSPS protein levels across Argentinean sites for OSL1, OSL2, OSL3, OSL4, seed, root and forage were 280, 340, 310, 460, 170, 100 and 290 µg/g dw, respectively (Table AI-1). The mean CP4 EPSPS protein levels across US sites for OSL1, OSL2, OSL3, OSL4, seed, root and forage were 300, 340, 330, 290, 150, 74 and 220 µg/g dw, respectively (Table AI-2). The data show that the CP4 EPSPS protein levels measured in each tissue are comparable across the two growing seasons and locations.

2.2.3.1 Part of the plant where the insert is expressed

Production of the CP4 EPSPS protein is expected to occur in all tissues since the *FMV/Tsf1* promoter should drive expression in all plant parts.

2.2.3.2 Expression of potential fusion proteins and analyses of open reading frames

The applicant has conducted bioinformatic analyses in 2006 to assess the potential for allergenicity, toxicity or bioactivity of potential putative polypeptides encoded by the DNA spanning the junctions of the soybean genomic DNA and the 5' and 3' ends of the inserted DNA in MON 89788 (McClain and Silvanovich 2006). Sequences spanning both junctions were translated from stop codon to stop codon in all six reading frames. Hypothetical polypeptides from each reading frame were compared to allergen (AD6), toxin (TOXIN5) and public domain (ALLPEPTIDES) database sequences using bioinformatic tools. The FASTA sequence alignment tool was used to assess structural relatedness between the query sequences and any protein sequence in the AD6, TOXIN5 and ALLPEPTIDES databases. In addition to structural similarity, each putative polypeptide was screened for short (eight amino acid) polypeptide matches with sequences from the databases using a pair-wise comparison algorithm. In these analyses, eight linearly contiguous and identical amino acids were defined as immunologically relevant, where eight constitutes the typical minimum sequence length likely to represent an immunological epitope. According to the applicant, no biologically relevant structural similarities to allergens, toxins or bioactive proteins were observed for any of the hypothetical polypeptides from the alignment searches. A result from the short polypeptide matching search on the other hand (ALLERGENSEARCH program) did produce one sequence match within the AD6 database. According to the applicant, this protein sequence was unnamed and uncharacterised for any allergenic potential and was considered not to have allergenic potential as determined by an independent expert allergy review panel[3]. Therefore, excluding this putative eight amino acid match, the applicant concluded that there were no immunologically significant epitopes present in any of the reading frames at either DNA-insert junction.

[3] The allergen, gliadin and glutenin sequence database (AD6) was assembled from sequences found on the FARRP (Food Allergy Research and Resource Program Database) allergen database dated January 2006, located at http://www.allergenonline.com (University of Nebraska).

2.2.4 Genetic stability of the insert and phenotypic stability of the GM plant

2.2.4.1 Genetic stability of the insert in MON 89788

To assess the stability of the insert in MON 89788, the applicant has performed Southern blot analyses of DNA obtained from four generations of MON 89788. The DNA samples were subjected to digestion with the restriction enzyme *Nco*I. Genomic DNA from soybean A3244,

and the plasmid PV-GMGOX20 mixed with A3244 DNA, were used as negative and positive controls, respectively. The blot was hybridised simultaneously with three overlapping probes that cover the entire T-DNA region of plasmid PV-GMGOX20. The negative control showed no detectable hybridisations, whereas the positive control produced the expected bands of 4.1 and 5.6 kb. The hybridisation of MON 89788 DNA produced the two bands of 2.6 kb and ~3.5 kb as shown previously (Figure 2.2.2.1-1). The results show the stability of the insert over four generations of MON 89788.

2.2.4.2 Phenotypic stability of the glyphosate tolerant trait in MON 89788

During the development of MON 89788, phenotypic segregation data were produced and analysed across several generations (Table AI-3). The presence of the glyphosate tolerance trait in individual plants was evaluated by CP4 EPSPS ELISA and/or treatment with glyphosate. The presence and copy number of the *cp4 epsps* gene in the R1 generation of MON 89788 was determined by quantitative PCR analysis (Bubner and Baldwin, 2004; Schmidt and Parrott, 2001).

First, R0 plants were self-pollinated after which the resulting R1 seeds were germinated and tested for glyphosate-tolerance and presence of the CP4 EPSPS protein. Selected R1 plants that survived the glyphosate treatment (29 out of 43, Table AI-3) were subjected to quantitative PCR analysis and the plants that were homozygous for the *cp4 epsps* gene were selected. These plants were then self-pollinated to give rise to a population of homozygous R2 plants. The segregation ratio for R2 and the subsequent generations were expected to be 100% positive (1:0 for positive:negative plants) for the glyphosate-tolerance trait.

The phenotypic segregation ratio was assessed by means of a Chi-square (X^2) analysis, which was conducted on the R1 generation to determine heritability of the *cp4 epsps* expression cassette in MON 89788. This analysis tests the observed to expected trait segregation ratio according to Mendel's principles of inheritance.

The analysis indicated no significant differences between the observed and expected phenotypic ratio for MON 89788. Following the selection of the plants homozygous for the insert containing the *cp4 epsps* gene, the subsequent generations were no longer segregating and the expected and observed segregation ratios were identical. The results of the analyses are consistent with the finding of a single chromosomal insertion of the *cp4 epsps* gene that segregates according to Mendel's laws of genetics, and also consistent with the molecular characterisation data indicating a single insertion site of the *cp4 epsps* gene in the soybean genome. The phenotypic stability is further supported by the CP4 EPSPS protein levels measured in the Argentinian and US field trials described in Mozaffar and Silvanovich 2006 (generation R6 of MON 89788), and Pineda and Silvanovich 2006 (generation R7 of MON 89788).

2.3 Conclusion

The applicant has provided sufficient analyses to characterise the DNA insert, number of inserts, integration site and flanking sequences in the soybean genome. The results show that one functional copy of the *cp4 epsps* gene only, is present in the soybean MON 89788 genome. No other functional vector genes were detected. Similarity searches in 2006, with databases of known toxins and allergens did not indicate potential production of harmful proteins or polypeptides caused by the genetic modification. Southern blot and segregation analyses show that the introduced gene elements are stably inherited and expressed over multiple generations, and consistent with the observed phenotypic characteristics of soybean MON 89788. The VKM GMO Panel concludes that the molecular characterisation of soybean MON 89788 does not indicate a safety concern.

3 Comparative assessments

3.1 Choice of comparator and production of material for the comparative assessments

In the compositional, agronomic and morphological assessment studies (designated study A and study B; see below), the GM soybean MON 89788 was compared to the non-transgenic Asgrow variety A3244 (conventional counterpart; control), which is a soybean with background-genetics similar to MON 89788. In addition to the control, 2-3 conventional soybean varieties (references) were included in the field trial at each site to develop a 99% tolerance interval for each analyte. A total of 12 different reference varieties were investigated across the various sites. All sites were planted following a randomised complete block design with three replications at each site. Soybean MON 89788 was treated with glyphosate herbicides at the recommended doses for commercial use, while the conventional control A3244 and the 12 conventional soybean varieties (Stine/ST3600, Stine/ST3870, Asgrow/A3525, Asgrow/A3559, Asgrow/A2553 Asgrow/A3204, Stine/ST2788, Asgrow/A2804, Stine/ST3300, Asgrow/A2704, Stine/ST2800, Asgrow/A2833) were treated with other commercial herbicides. The field trials were carried out in Argentina in the season 2004-2005 and in the US in 2005, each season and year at five different geographical sites.

Compositional data from glyphosate treated MON 89788, the conventional control, and conventional references were presented in the original application. Because the studies were conducted prior to the most recent EFSA guidance (2011), the design of the field studies did not include MON 89788 not treated with glyphosate. Because of this, Monsanto provided additional data in response to the Panel's request for compositional analysis of MON 89788 not treated with glyphosate herbicide. Samples from two additional field studies (designated study C and study D; see below) were analysed to assess compositional equivalence. These two studies represent 10 field sites and two growing seasons. In these studies, untreated MON 89788 was compared to MON 89788 treated with glyphosate. Further statistical analyses, combining the data from these two compositional studies (C and D) with compositional data from the two studies (A and B) submitted in the original application, were conducted to compare the untreated and treated MON 89788 to the conventional control A3244. Also this design is not according to the EFSA guidelines, especially because the conventional control was not included in these additional studies.

Extensive composition data on soybean available from the ILSI crop composition database was in these case considered useful by the applicant to assess the biological relevance of statistically significant differences ($p<0.05$) observed between untreated and treated MON 89788 in the context of the natural variability in soybean composition.

An overview over the different field studies are presented in Table AII-1. Study A was conducted in Argentina, where the soybeans were planted at five locations in 2004 and

harvested in 2005. Study B, C and D was performed in the USA, in regions that are representative of commercial soybean production. Thus the comprehensive (combined site) statistical comparison represents four growing seasons, 20 field sites and 94 samples.

Statistical analysis

Statistical analyses of the compositional data were conducted using a mixed model analysis of variance with data from each of five sites (within-site) and a combination of all five field sites (combined-site). Each individual analyte for MON 89788 was compared to the A3244 control, for each of the five sites (within-site) and across-sites. The statistical significance was defined at the level of $p < 0.05$. There were a total of 42 components statistically evaluated (the initial 56 analytes minus 14 for which >50% of the observations were below the limit of quantification (LOQ). A total of 126 comparisons were made: 42 components each statistically analysed in three ways (single sites individually plus all sites combined).

3.1.1 Comments of the VKM GMO Panel regarding study design and statistical analyses

The applicant has presented data from four different field studies as basis for their comparative assessment of soybean MON 89788 to its control and to reference varietals. None of the studies were performed according to EFSA's most recent guidelines (EFSA, 2011), studies A and B having been carried out prior to the publication of these guidelines. In an effort to conform to the recent EFSA (2011) guidelines, studies C and D were carried out. These studies cannot be considered independently of studies A and B, however, since no conventional control or reference varieties were included, and study D did not include replicates, only random samples from different field sites.

The VKM GMO Panel recognises serious flaws in the study design. However, for lack of any other data concerning compositional assessment, the data were included for the overall evaluation of the field studies A-D by the applicant, and is therefore mentioned in this assessment. For details see Tables AII-5 and AII-6

3.2 Compositional analysis

3.2.1 Field trials performed in Argentina during the 2004-2005 season – study A

Compositional analyses were conducted on seed and forage tissues collected from five field sites in Argentina during the 2004-2005 growing seasons (Lundry et al., 2006a). A total of 60 different analytes were analysed in seed and forage tissues. The compositional analysis of forage samples included proximates (protein, fat, ash and moisture), acid detergent fiber (ADF) and neutral detergent fiber (NDF). The concentration of other carbohydrates was estimated by calculation.

The compositional analysis of seed samples included proximates (protein, fat, ash and moisture), ADF, NDF, as well as amino acids, fatty acids (C8-C22), phytic acid, trypsin inhibitor, isoflavones and lectins. The concentration of other carbohydrates was estimated by calculation. The content of vitamins in seeds was not analysed in study A. The OECD (2012) recommends that vitamins are analysed in novel foods and feeds.

Of the 60 analytes analysed, 14 minor fatty acids had more than 50% of the analytical values below the assay quantification limit. These fatty acids are known to occur at low or non-detectable levels in soybean oil (Codex Standard, 2005) and were not included in the statistical analyses (Technical dossier). For the remaining 46 analytes, statistical analysis was conducted comparing MON 89788 with the A3244 control. Within-site analyses showed no statistical significant differences for 94% of the comparisons (216 out of 230). The 14 significant differences were distributed over 12 analytes (Table AII-2). Two (seed linolenic acid and forage moisture) were observed at two sites and the remaining 10 (seed serine, ash, methionine, palmitic acid, linoleic acid, moisture, isoleucine, valine, glycitein and forage ash) were observed only at one site. For seed linolenic acid, the magnitude of the differences in the within-site analyses (MON 89788 minus A3244) was less than 4% and the differences were observed at two individual sites. For forage moisture, the magnitude of differences was less than 3% and the mean of MON 89788 was higher than the mean of A3244 at one site, while lower at another site.

There was no consistent trend for the differences observed in either forage moisture or seed linolenic acid and these differences were not corroborated by the across-site analysis. Moreover, the analyte levels were within the 99% tolerance interval for traditional soybean varieties.

According to the applicant, the observed statistical differences for seed linolenic acid and forage moisture are likely to reflect the natural compositional variability in soybeans. Furthermore, the moisture content of any crop depends on local, pre-harvest climate conditions and any post-harvest treatment like drying or storing. Due to this, any statistically significant differences in moisture content have little biological relevance. To take variable moisture content into account and to enable the comparison of the other results, the fresh weight values should be based on dry weight (e.g. % dw). This appears to have been carried out for the data from this and the other field trials B-D (see below). For the remaining 10 analytes, where significant differences were observed in one site, the differences between MON 89788 and A3244 were not reproducible across-sites and no consistent trends were observed (Table AII-2). All mean levels of MON 89788 analytes were well within the 99% tolerance interval for traditional soybeans that were grown concurrently in all sites (Technical dossier).

3.2.2 Field trials performed in USA during the 2005 season – study B

According to the dossier submitted by the applicant, seed and forage tissues of MON 89788 and control (A3244) were harvested from soybeans grown in three replicated plots at each of five field sites across the US during the 2005 growing season.

A total of 63 different analytes were analysed in seed and forage tissues. The compositional analysis of forage samples included proximates (protein, fat, ash and moisture), ADF, NDF and other carbohydrates by calculation.

The compositional analysis of seed samples included proximates (protein, fat, ash and moisture), ADF, NDF, as well as amino acids, fatty acids (C8-C22), phytic acid, trypsin inhibitor, isoflavones, lectins, raffinose, stachyose, vitamin E, and carbohydrates by calculation (Technical dossier, Lundry et al., 2006b). Vitamin K1 was not analysed, although according to OECD soybean oil is considered to be a source for humans of vitamin K (OECD, 2012). Of the 63 analytes analysed, 14 minor fatty acids had more than 50% of the analytical values below the assay quantitation limit (not shown). These fatty acids are known to occur at low or non-detectable levels in soybean oil (Codex Standard, 2005) and were not included in the statistical analyses.

For the remaining 49 analytes, statistical analysis was conducted comparing MON 89788 with the A3244 control. Within-site analyses indicated that there were no statistically significant differences in 91% of the comparisons (223 out of 245) between MON 89788 and A3244.

The 22 significant differences found in the seed samples were distributed over 20 different analytes. For the following seventeen analytes one observation of phenylalanine, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, eicosenoic acid, behenic acid, ADF, carbohydrates, fat, stachyose, genistein, seed moisture, and NDF were statistically significantly different, while 2 observations for raffinose showed statistically significant differences.

In the across-site (all sites combined) analyses, few differences were detected. For the three other analytes, that is forage moisture (1 observation), seed daidzein (1 observation) and seed glycitein (2 observations), the statistically significant differences between MON 89788 and the control were corroborated by the across-site analysis of these analytes (Table AII-3). In addition, a statistically significant difference was observed for vitamin E in the across-site analysis, although no differences were seen in the individual sites for this analyte. The differences for all four analytes in the across-site analysis, however, were small (1.6% – 11%) and the mean levels of these analytes in MON 89788 were well within the 99% tolerance intervals for traditional soybeans (Table AII-3). Furthermore, the mean levels of MON 89788 seed daidzein, glycitein and vitamin E are also within the ranges for traditional soybeans reported in the International Life Science Institute Crop Composition Database, as well as in published literature.

In forage no differences were found in ADF, NDF or other proximates. The only difference was found in moisture content. The mean levels of forage moisture for both MON 89788 and A3244 were below that of the ILSI and literature ranges; however, the difference in moisture between MON 89788 and A3244 was only 1.6%. As stated above, the moisture content of any crop depends on local, pre-harvest climate conditions and post-harvest treatment like drying or storing. Due to this, any statistically significant differences in moisture content have little biological relevance.

3.2.3 Field trials performed in the USA during the 2007 season – study C

Untreated and glyphosate treated MON 89788 soybeans were harvested from replicated plots at two field sites in the US during the 2007 growing season and subjected to compositional analysis (seeds only) (Taylor et al., 2008a). The Stonington site provided four replicates of treated MON 89788 and six replicates of untreated MON 89788; the Oxford site provided five replicates of treated MON 89788 and four replicates of untreated MON 89788. No conventional control or reference varieties were included in the field trial design. The results were therefore compared with composition data on soybean from the ILSI crop composition database. The VKM GMO Panel recognises the shortcomings of study C. However, the data were included by the applicant for the combined-site evaluation of the field studies A-D, and is therefore mentioned in this assessment.

According to the applicant the analyses of the combined-site data set showed no statistically significant differences ($p>0.05$) for 93% of the analytes (39 of the 42). Any differences observed were considered within the range of values found in the ILSI database and literature, and therefore not considered biologically relevant (Table AII-4).

3.2.4 Field trials performed in the USA during the 2006 season – study D

MON 89788 soybeans untreated and treated with glyphosate were harvested from unreplicated plots at eight diverse field sites across the USA during the 2006 growing season and subjected to compositional analysis (Taylor et al., 2008b). No conventional control or reference varieties were included in the field trial design. The VKM GMO Panel recognises serious flaws in the study design. However, the data were included by the applicant for the combined-site evaluation of the field studies A-D, and is therefore mentioned in this assessment. For details concerning study D results see Tables AII-5 and AII-6.

3.2.5 Combined site statistical analysis of untreated and treated MON 89788 and conventional control (A3244) samples from studies A, B, C and D

A subset of key soybean analytes was selected based on the importance of the analytes from either a nutritional and/or a food and feed safety perspective, taking into account the guidance provided by the OECD consensus document on compositional considerations for

new varieties of soybean (OECD, 2001). Only the key nutrients and anti-nutrients were used for this combined sites analysis of all field studies, since the purpose was to assess from a statistical perspective whether untreated and treated MON 89788 and the conventional control, A3244, can all be considered to belong to a common population (Technical dossier, Responses to EFSA questions). The results are presented in Table AII-7.

The statistical analysis consisted of a calculation of means and standard errors along with 95% confidence intervals for a subset of key analytes for untreated and treated MON 89788 and the conventional control. The subset of analytes included: the nutritionally important components in soy (total fat, protein, carbohydrates by calculation, moisture, ash, ADF, NDF), the 10 essential amino acids, the most abundant fatty acids (palmitic, stearic, oleic, linoleic and linolenic acids), the isoflavones (diadzein, genistein and glycitein) and vitamin E; and the important components from a food/feed safety perspective (trypsin inhibitor, lectins and phytic acid). The essential amino acids were measured and expressed as % dw (dry weight) while the fatty acids given as % total fatty acids as the unit of measurement. The sample size for vitamin E was lower since vitamin E was not analysed in study A. The confidence interval, which combines the analyte mean along with the standard error of the analyte mean, estimated the population mean. For each analyte, the confidence intervals associated with the untreated and treated MON 89788 and the conventional control A3244 were compared numerically to assess whether the individual means and standard errors were similar or different. Although this assessment of the overlap of the confidence intervals does not replace a regular ANOVA test, it can be used to show whether the means are similar. The means, standard errors and 95% confidence interval results presented in Table AII-7 demonstrate that, except for threonine, tryptophan and NDF, the means were similar and the confidence intervals overlapped. The confidence intervals for untreated MON 89788 were either just below (threonine) or just above (tryptophan) the intervals for treated MON 89788 and the conventional control A3244. The confidence intervals for NDF in untreated MON 89788 were just below the intervals for the conventional control A3244.

3.3 Agronomic traits and GM phenotype

The application EFSA/GMO/NL/2006/36, covering authorisation of soybean MON 89788 for all food and feed uses, include data on agronomic and morphological characteristics from the field trials performed in the USA. The field trials were conducted at 17 locations during the 2005 growing season to evaluate morphological, agronomic and ecological interaction characteristics. According to the applicant, these 17 locations provided a diverse range of environmental and agronomic conditions representative of the majority of commercial soybean production regions in the USA, including regions where MON 89788 soybean would be anticipated to be produced. A randomized complete block design with three replications was employed for the comparisons and analyses. Three to four commercial reference varieties were included for each trial site (in total 23 varieties for all trial sites). Glyphosate herbicide was not applied to the experimental plots.

Plant growth, development and yield characteristics were assessed under field and laboratory condition to identify unintended phenotypic effects or ecological interactions in MON 89788 soybean relative to the conventional control A3244 and commercially available soybean. The purpose of these evaluations was to assess whether the introduction of the glyphosate-tolerant trait altered the morphological and agronomic characteristics or the plant-insect, plant-disease, or plant-abiotic stressor interactions of MON 89788 soybean compared to the control. Certain growth, reproduction, and preharvest seed loss characteristics (such as lodging and pod shattering) can be used for an assessment of enhanced weed potential of MON 89788 soybean.

A total of 11 different **phenotypic characteristics** were evaluated. For the across-site analyses, no significant differences were detected between MON 89788 and A3244 soybean for early stand count, seedling vigor, days to 50% flowering, flower colour, lodging, pod shattering, final stand count, seed moisture, seed test weight, or yield. The only significant difference detected in the across-site analyses was plant height, which was reduced in MON 89788 as compared to the control A3244 (77.9 vs. 82.0 cm). The reduction in plant height was noted at four of the seven sites, but the mean value observed for MON 89788 falls within the range of values observed for the commercial soybean varieties (48.8 to 108.2 cm). Furthermore, the magnitude of the difference in plant height is small (approximately 5%), and decreased plant height is unlikely to contribute to increased weed potential.

The **ecological interactions** of MON 89788 in relation to its conventional control were also determined across sites. Of the 12 insect categories, 18 disease categories and 10 abiotic stressors evaluated in the studies, only one difference between soybean MON 89788 and its comparator was noted. This was related to a reduced severity of symptoms caused by leafhopper in MON 89788 plots as compared to control plots at a single trial site and a single point in time. The leafhopper susceptibility of the plant fell within the range observed among commercial soybean varieties. In addition, quantitative data were collected on the abundance of specific pests and beneficial insects, and the prevalence of plant damage. Three out of the 66 comparisons performed were statistically significantly different for insect abundance (pests and beneficial insects), which was more or less the number of statistical differences expected due to random variation in the samples taken. No difference was noted between soybean MON 89788 and the control with respect to plant damage.

In addition, dormancy and germination characteristics of MON 89788 were shown to be unaltered compared to the conventional control (Phillips & Kendrick, 2006, unpublished). No differences in pollen characteristics, more specifically pollen morphology and viability, were detected when comparing MON 89788 to the traditional soybean control (Rosenbaum, 2006, unpublished).

3.4 Conclusion

The VKM GMO Panel has considered the available literature on compositional data and found that except for small intermittent variations, no biologically relevant differences exist between soybean MON 89788 and its corresponding control in the analyses of seed and forage. The few observed statistical differences between MON 89788 and A3244 are likely to reflect the natural variability of the analytes since their mean levels for MON 89788 were well within the 99% tolerance intervals for conventional reference varieties and within the ranges in the ILSI-Crop Composition Database. The field studies investigating composition of MON 89788 show no biologically relevant differences between GM crops treated and untreated with glyphosate. The data presented do not show unintended effects as a result of the genetic modification.

Based on current knowledge, the VKM GMO Panel concludes that with the exception of the introduced trait, soybean MON 89788 is compositionally, agronomically, morphologically and ecologically equivalent to its conventional counterpart, and other conventional soybean varieties.

4 Food and feed safety assessment

4.1 Previous evaluations by the VKM GMO panel

In an earlier risk assessment of soybean MON 89788, the VKM GMO Panel concluded that based on data from feeding studies with rats and broilers fed processed soybean meal, soybean MON 89788 is nutritionally equivalent to and as safe as conventional soybean varieties (VKM 2006).

The CP4 EPSPS protein has previously been assessed by VKM in several genetically modified glyphosate tolerant crop varieties including soybean (MON 89788, MON 87705, MON 87701 x MON 89788, MON 87769 x MON 89788 and 305423 x 40-3-2), cotton, oilseed rape and several maize events (e.g. NK603, NK603 x MON810; 1507 x NK603; MON 863 x NK603; MON 1445 x MON 531, GA21; and more).

4.2 Product description and intended uses

Soybean MON 89788 was first cultivated in the USA and Canada in 1996, and subsequently cultivated in Argentina in 2011, Brazil in 2010, Canada in 1999, Japan in 2006 and Uruguay in 2012. Soybean MON 89788 was commercialised as food and/or feed in Argentina (2011), Australia (2004, food), Brazil (2010), Canada (2000), China (2010), Colombia (2012, feed), EU (2008), India (2014), Japan (2001 food, 2003 feed), Malaysia (2012), Mexico (2003, food), New Zealand (2004, food), Philippines (2009), Russian Federation (food 2008, feed

2007), Singapore (2014, food), South Africa (2001), South Korea (2009), Taiwan (food, 2007), Thailand (food 2013), Turkey (feed 2011), Uruguay (cultivation, 2012).

The genetic modification in soybean 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. Conventional soybean protein concentrate is a common ingredient in salmon feed formulation in Norway (www.mattilsynet.no). Since 2008, NFSA has given four fish feed producers in Norway extended exemption from seeking approval of GM products. The exemption applies to processed, non-viable feed products from 19 different GM varieties. In October 2014, this exemption was not extended.

Soybean MON 89788 has been used to produce food and feed since 1998. According to the applicant the commercial experience since 1998 has confirmed that the production and processing of MON 89788 does not differ from the production and processing of the equivalent foods and feeds, originating from traditional soybean.

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 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). Whole soybeans are utilised to produce food products such as soy sprouts, baked soybeans, roasted 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.

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 meal.

The soybean MON 89788 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.

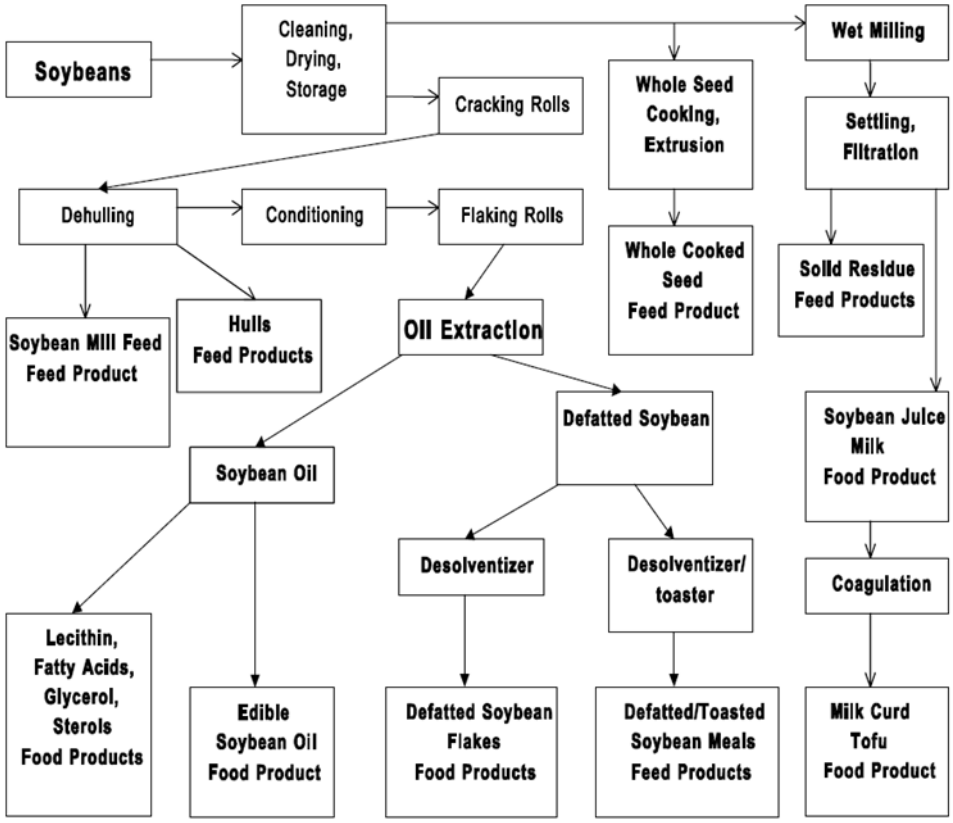

Figure 4.2-1. Processing of soybean, adapted from Waggle and Kolar, 1997, Technical dossier

4.3 Effects of processing

The processing steps that are 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 degrees, 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. Intermediate temperatures (55°C) will reduce the activity of the CP4 EPSPS enzyme, while it will be completely inactivated at higher temperatures (65° and 75°C). pH values < 4, will also reduce enzymatic activity (Effective range of enzyme is pH 4-11). The CP4 EPSPS enzyme is degraded in foods like tofu and soybean paste (Kim et al. 2006; Wu et al. 2012 a, b; Tian et al 2014). The heat treatments used by Wu et al. (2012) were boiling, autoclaving or heating by microwaves. Autoclaving, when used to manufacture soy drink, textured vegetable protein, soybean meal, etc. generated more degradation of CP4 EPSPS-protein then boiling and microwave treatment. The processing methods used by Tian et al. (2014) were dry heat treatment, wet heat treatments and extrusion. They used different temperatures (e.g 75°C to 135°C) and different times (3 to 30 minutes). Degradation of the *cp4 epsps* gene and CP4 EPSPS protein depended on time and temperature. As temperature rose from 90°C to 150°C the CP4 EPSPS protein content was reduced from 4,19 % to 0.54 %, and was not detectable at 165°C. The 483-bp *cp4 epsps* gene was not detected after dry heating, wet heating, or extrusion at 120°C with a 39% moisture content (Tian et al. 2014).

Similar results were found by Fernandes et al. (2013) when baking the maize bread broa (a Portuguese oven baked bread made with polenta) containing 11% flour from maize event TC1507 and 20% flour from maize event MON 810. Under these conditions the majority of DNA and proteins in maize was denatured, which also applies to the CP4 EPSPS protein and *cp4 epsps* gene in processed maize products (Dien et al., 2002, Hammond & Jez 2011, Fernandes et al., 2013).

Another study quantified the levels of CP4 EPSPS proteins in full fat soybean meal (FFSBM). Only trace levels of CP4 EPSPS was found in the FFSBM product, and in extruded salmon feeds containing the FFSBM, the protein was reported to be non-detectable (<0.1 %) (Sanden et al., 2005).

4.4 Toxicological assessment of soybean MON 89788

The toxicological assessment is based on results available from studies with mice, rats and broilers. These studies have utilised various formulations of soybean MON 89788 (e.g. as soybean meal in diets or protein concentrates) or purified CP4 EPSPS protein produced in *Escherichia coli.* Protein concentrate is about 70% soy protein and is basically defatted soy flour without the water-soluble carbohydrates and ethanol-soluble antinutritional factors. The formulation used is indicated under each study presentation.

In addition to the safety testing conducted by the applicant, a safety testing program has been conducted on soybean MON 89788 within the Russian Federation, summarised in Tutelyan (2013). The available English transcript describes the program as compliant with the Russian national requirements: MY 2.3.2.2306-07 “Medico-biological safety assessment of genetically-engineered and modified organisms of plant origin”. The content of these requirements and the exact design of the respective studies have however been difficult to assess for the VKM GMO panel, since this information is only available in Russian. Still, the testing conducted in the Russian Federation is deemed valuable for the risk assessment of soybean MON 89788. This is due to the programme being rather extensive with several studies conducted and many parameters monitored. Also, the studies are of particular interest since these are the only studies conducted with a soy protein concentrate, a main ingredient in Norwegian fish feed formulations. A brief summary is presented in Appendix IV.

4.4.1 Acute toxicity testing

Humans and animals have a history of safe consumption of the endogenous plant protein EPSPS, and the CP4 EPSPS protein is structurally and functionally similar to the EPSPS. CP4 EPSPS producing crops have been consumed as food and feed since 1996 without apparent adverse effects being linked to the consumption.

Due to the low levels of CP4 EPSPS protein in soybean MON 89788 and the difficult task of isolating a sufficient quantity of purified CP4 EPSPS from the soybean, the acute toxicity testing studies were conducted with a CP4 EPSPS protein encoded by the *cp4 epsps* gene from *Agrobacterium* sp. strain CP4, expressed in *Escherichia coli*. The structural similarity and physicochemical and functional equivalence between the CP4 EPSPS protein produced by *E. coli* and the protein produced by soybean MON 89788 was shown by N-terminal sequencing (Edman degradation), Western blot analysis, mobility in SDS-PAGE, MALDI-TOF mass spectrometry, glycosylation analysis and CP4 EPSPS enzymatic activity (Technical dossier). All these methods confirmed the equivalence between the bacterial and the plant CP4 EPSPS proteins. Based on the identified similarity in structure and equivalence in physicochemistry and function between these proteins, the VKM GMO Panel accepts the use of CP4 EPSPS test material derived from *E. coli* for the degradation studies and safety testing of the CP4 EPSPS protein present in soybean MON 89788 and as a reference standard in the ELISA used to estimate CP4 EPSPS levels in various tissues of soybean MON 89788.

Submitted data demonstrated low levels of CP4 EPSPS protein in soybean MON 89788 (approx. 0.167 µg CP4 EPSPS/mg fresh weight whole seed (range 0.086-0.270) and approx. 0.502 µg/mg in leaf (range 0.321-0.618)). The protein was not detectable in soybean oil and showed no meaningful amino acid sequence homology to known toxic proteins (UK-ACNFP, 1995). *In vitro* digestion studies with simulated gastric fluid, demonstrated that CP4 EPSPS is rapidly degraded under conditions mimicking the stomach (Harrison et al., 1996). Digestion of the CP4 EPSPS protein in simulated gastric fluid was studied *in vitro* by following the CP4 EPSPS enzymatic activity, and by identifying peptide fragments using SDS-PAGE colloidal blue gel staining and Western blot analysis methods. The SDS-PAGE colloidal blue

gel staining demonstrated that at least 98% of the CP4 EPSPS protein produced in *E. coli* was fully degraded by pepsin-containing simulated gastric fluid at pH 2 within 15 seconds. In agreement with this finding, Western blotting showed that most of the CP4 EPSPS protein was digested in simulated gastric fluid within the same time frame. Similarly, studies on the function of CP4 EPSPS exposed to simulated gastric fluid revealed that the enzymatic activity was reduced by more than 90% within 15 seconds.

4.4.1.1 Single dose toxicity study of CP4 EPSPS

The applicant provided data from a single dose toxicity study with 10 male and 10 female DC-1 mice per treatment group conducted in compliance with GLP regulations (MSL 13077, 1993;. Harrison et al., 1996). Doses of 49, 154 or 572 mg CP4 EPSPS protein produced in *E. coli* were administered orally by gavage and a control group with the same number of male and female mice received the vehicle only. Animals were terminated on days 8-9 and macroscopic examination of internal organs was carried out at necropsy. All animals survived and there were no indications of adverse effects up to the highest dose tested.

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 and is therefore not taken into account in this risk assessment (EFSA 2011).

4.4.1.2 Toxicological assessment of new constituents other than proteins

No new constituent other than the CP4 EPSPS protein is expressed in soybean MON 89788 and no relevant changes in the composition of soybean MON 89788 were detected by the compositional analysis.

4.4.2 A 90 day sub-chronic toxicity study

The applicant provided data from a GLP-compliant sub-chronic 90 day feeding study of 20 rats of each sex of the Sprague-Dawley strain Crl:CD® (SD) (Kirkpatrick., 2007).Diets were formulated to be comparable to the Certified Rodent Lab diet, containing 15% soybean meal, with increasing substitution of the conventional soybean meal with 5-15% MON 89788 (see below). Each diet was administered ad libitum to the rats from approximately 6-8 weeks of age. The composition of the diet and its quality, including herbicide residues, were reported. Whereas soybean MON 89788 had been sprayed with glyphosate, the soybean control (A3244) had been sprayed with other conventional herbicides.

All three groups were given a diet containing 15% processed soybean meal (w/w), as this is the concentration of soybean meal in the Certified Rodent Lab diet. The high dose group was given a diet containing 15% soybean MON 89788, the low dose group was given a diet containing 10% A3244 and 5% MON 89788, and the control group received 15% a diet containing soybean meal from the control A3244.

In parallel, the applicant also performed a similar feeding study with six different diets containing 15% soybean meal from commercial reference varieties. Also, data from historical controls (fed diets with 15% processed soybean meal) were used when assessing the relevance of any statistically significant differences observed. The feed and water was presented ad libitum.

During the study, animals were observed twice daily for mortality and moribidity. Detailed physical examinations, including behavioural observations outside the home cage, individual body weights and food consumption were recorded weekly. Clinical pathology evaluations (haematology, serum chemistry and urinalysis) were conducted on samples collected from 10 animals/sex/group at the scheduled necropsy date (study week 13). Complete necropsies were extended on all animals and appropriate organs were weighed at the scheduled necropsy date. Selected tissues were examined microscopically from all animals that were fed control and high dose diets.

All animals survived the treatments. Inclusion of soybean MON 89788 in the diet had no influence on feed intake, body weights, and behaviour of the animals. There were also no haematological changes noted, and in relation to serum chemistry the only statistically significant alteration in males, was increased mean triglyceride levels (88 vs 63 mg/dL) in rats receiving the diet containing 10% A3244 + 5% MON 89788, compared to the control diet (15% A3244). The triglyceride level was unchanged both in the 15% MON 89788 dose group in males and in both dose groups in females. The increased triglyceride level in four male rats was higher than normal. Similar increased levels were randomly observed in rats fed different reference soybean varieties. All were within the range reported for the historical controls (11-170 mg/dL). In female rats, the only influence on serum chemistry was a slightly reduced calcium level (10.9 vs 10.6 mg/dL) in rats receiving 15% soybean MON 89788. As the reduction was small (<3%), and the calcium level fell within the range of calcium values for the rats given the six reference soybean varieties and were within the historical control range (7.7-14.3 mg/dL), this observation is considered to be of no toxicological concern. Urinalysis parameters were comparable in treated and control rats.

At necropsy, there were no differences in macroscopic findings related to the treatment. The only diet-related difference was a significantly reduced relative brain weight (adjusted for final body weight) in males receiving 5% soybean MON 89788. There was no influence on this parameter in the males receiving the high-dose group or in any females receiving any diet. The significance was probably due to dividing a slight, non-significantly reduced (2.8%) mean absolute brain weight with a slight, non-significantly increased (4.0%) mean body weight. Furthermore, the values fell within the values obtained in rats given the reference soybean diets and the historical controls. Microscopic studies revealed no alterations related to the tested soybean.

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 IgE-dependent allergic reactions in already sensitised persons and whether the transformation may have altered the allergenic properties of the modified food. A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (CAC, 2003; EFSA, 2006a; EFSA 2011).

4.4.3.1 Assessment of allergenicity of the newly expressed proteins

The *cp4 epsps* gene originates from *Agrobacterium sp.* strain CP4*,* a common soil-bacteria and plant-pathogen that is not known to be allergenic. A bioinformatics-supported comparison of the amino acid sequence of the CP4 EPSPS protein with the sequences of known IgE-dependent allergens, gliadins, and glutenins has been performed. This analysis included both overall sequence alignments with the FASTA algorithm and searches for short identical stretches of at least eight contiguous amino acids. No homology higher than 35% was found between CP4 EPSPS and allergens. As described above, CP4 EPSPS is rapidly degraded under simulated gastric conditions. Based on these results, the VKM GMO Panel considers that the newly expressed CP4 EPSPS protein is unlikely to be allergenic.

Bioinformatic analyses have been conducted according to Codex Alimentarius guidelines of 2003. Searches for amino acid sequence homology of the CP4 EPSPS protein expressed in soybean MON 89788 with amino acid sequences of toxic and general proteins stored in databases indicated significant homology only with other EPSPS- and related- proteins. No sequence homology with known IgE-dependent allergens or toxic proteins was found.

4.4.3.2 Assessment of allergenicity of the whole GM plant

Because the soybean is a recognised allergenic food, the applicant performed extensive *in vitro* allergenicity studies with extracts of soybean MON 89788, A3244 (a conventional soybean variety with background genetics similar to soybean MON 89788), and 24 different commercial varieties (both non-GM and GM).

The IgE-binding of soybean proteins to sera from 16 patients allergic to soybean, and 5 non-allergic individuals was quantified with a validated ELISA method to examine if the allergenic potential of soybean MON 89788 is altered compared with conventional soybean varieties. Whereas none of the soybean varieties showed IgE-binding to sera from non-allergic patients, all but one serum from allergic patients had similar reactivity to extracts from soybeans MON 89788 and A3244. Furthermore, the reactivity was within the tolerance interval defined by the reactivity to the 14 commercial non-GM soybean varieties. The deviating serum showed IgE-binding to A3244 that did not fulfil the acceptance criteria of

the ELISA method (variability within each triplicate sample ≤25 %), and was therefore removed from the analysis.

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 89788.

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 2010c), adjuvants are substances that, when co-administered with an antigen increases the immune response to that antigen and therefore might increase the risk of allergic reactions. Adjuvanticity has not been routinely considered in the assessment of allergenicity of GMOs.

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 sensitisation (Brandtzaeg & Tolo, 1977; Lim & Rowley, 1982).

The CP4 EPSPS-protein has not been reported to have adjuvant properties.

4.5 Nutritional assessment of GM food and feed

Compositional analyses of soybean MON 89788 indicate nutritional equivalence to the non-GM control with a comparable genetic background, and to the published range of values in the literature (see 3.2). The nutritional equivalence of soybean MON 89788 and control was further shown by the 42-day broiler feeding study described in 4.5.2.

According to the updated version of the EFSA guidance for risk assessment of food and feed from genetically modified plants (EFSA, 2011a), the experimental design should always include the following test materials: the GM plant exposed to the intended herbicide, the non-GM comparator treated with conventional herbicide management regimes, and the GM plant treated with the conventional herbicide management regimes. The broiler chicken study provided by the applicant is not in accordance with the suggested experimental design in the last EFSA guidance document on risk assessment (EFSA, 2011a). The Norwegian GMO Panel agrees on the importance of including GM plants treated both with and without the intended herbicide in comparative analyses (composition, agronomic traits, food and feed safety assessments), but recognises that the applicant submitted the application prior to the last guidance document from EFSA.

4.5.1 Intake information/exposure assessment

The human soybean oil consumption in Europe was calculated at 6.3-7.0 g/person/day, based on FAO Statistics from 1997 to 2001. Assuming that 54% of the soybean oil was derived from soybean MON 89788, the estimated average exposure of the European consumer to products of soybean MON 89788 would be approximately 3.4-3.7 g/person/ day (Technical dossier).

Soy beans 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.

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 89788 meal in the EU would be 21% for broilers, 18% for pigs and 12% for dairy cattle (Technical dossier).

In Norway, almost 1.5 mill tons of fish feed was produced in 2012 and soybean protein concentrate (SPC) is one important protein source in salmon feeds (Directorate of Fisheries, Biomass statistics 2013). The average inclusion level of SPC in feed for Atlantic salmon is 25%, total SPC used for fish feed production in 2013 was calculated to be approximately 375 000 tons (Annual Sustainability report, Skretting, 2013).

Assuming that 100% of the SPC was derived from soybean MON 89788, the estimated average exposure of Atlantic salmon (post smolt, 200 g) to products of soybean MON 89788 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 GMOs (*e.g.* below 0.9%) (Spilsberg et al 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: the 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 has provided a 42-day broiler feeding study (Ross × Ross 308) performed according to generally accepted guidelines at the time (ILSI, 2003), and consisting of eight treatments groups (MSL-20422, Technical report). One group received soybean MON 89788, another group received the control non-GM soybean A3244 and six other groups received other commercial non-GM soybean varieties (A2824, A2804, A4324, A3469, A3559, and

ST3870). Each treatment group consisted of 50 male and 50 female broilers (in pens of 10 birds/pen and pens in a randomised complete block design) fed diets containing approximately 33% (w/w) of soybean meal in the starter diet and 30% soybean meal in the grower/finisher diet. The diets contained meal from soybean MON 89788 sprayed with glyphosate, and meal from soybean A3244 and conventional varieties sprayed with conventional herbicides, diets were quality controlled and formulated based on nutrient and pesticide analyses performed before diet formulation.

Mortality was comparable in all treatments of the study, being around 4% in the group receiving soybean MON 89788, which is close to rates commonly reported for broilers in feeding studies. There were no effects observed on body weight, feed conversion and carcass yield in this study.

4.6 Conclusion

A 90-day sub-chronic toxicity study with rats, as well as a nutritional assessment trial with broilers fed diets containing soybean MON 89788 did not indicate any adverse effects. The CP4 EPSPS protein does not show sequence resemblance to known toxins or IgE-dependent allergens, nor has it been reported to cause IgE-mediated allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that soybean MON 89788 is nutritionally equivalent to and as safe as its conventional counterpart and other conventional soybean varieties.

5 Environmental risk assessment

Considering the scope of the application EFSA/GMO/NL/2006/36, the environmental risk assessment is concerned with the accidental release into the environment of viable soybean MON 89788 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 (OECD, 2000; Bramlage et al., 1978). 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 89788 will increase the potential to establish feral populations. A series of field trials with soybean MON 89788 was conducted by the applicant at several locations in the USA and Puerto Rica (1991-1994), Argentina (1993-1994), Canada (1993-1994), France (1994),

and Italy (1994, 1996, and 1997) to compare the agronomic performance and field characteristics of soybean MON 89788 with its comparators (see section 3.3). With the exception of targeted responses to the presence of glyphosate herbicides, the agronomic, morphological and ecological field trial data did not show major changes in plant characteristics indicating altered fitness, persistence and invasiveness of soybean MON 89788 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 89788, or changes to its survivability (including over-wintering), persistence or invasive capacity. Because the general characteristics of soybean MON 89788 are unchanged, the herbicide tolerance is not likely to provide a selective advantage in Norway. The VKM GMO Panel is of the opinion that the likelihood of unintended environmental effects based on establishment and survival of soybean MON 89788 will not differ from that of conventional soybean varieties.

5.2 Potential for gene transfer

A prerequisite for gene transfer is the availability of pathways for the transfer of genetic material, either through horisontal 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 89788. 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 (Nielsen et al. 2000; De Vries & Wackernagel, 2002, reviewed in EFSA, 2004, 2009a; Bensasson et al., 2004; 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 89788 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 & 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. (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 gene from soybean MON 89788 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 *cp4 epsps* gene from MON 89788 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 for the soil bacteria, 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, 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 89788 will not present a risk of spread of transgenes to organic or conventionally grown varieties, wild populations or closely related species in Norway.

5.3 Interactions between the GM plant and target organisms

Considering the intended uses of soybean MON 89788, excluding cultivation and the absence of target organisms, potential interactions of the GM plant with target organisms were not considered an issue by the VKM GMO Panel.

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

Considering the intended uses of soybean MON 89788, excluding cultivation, potential interactions of the GM soybean with non-target organisms were 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 89788, 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 89788, excluding 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 89788.

Soybean MON 89788 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 of seeds from soybean MON 87988 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 are 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.

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 2011c). 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 89788 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 89788.

7 Conclusions

Molecular characterisation

The applicant has provided sufficient analyses to characterise the DNA insert, number of inserts, integration site and flanking sequences in the soybean genome. The results show that one functional copy of the *cp4 epsps* gene only, is present in the soybean MON 89788 genome. No other functional vector genes were detected. Similarity searches in 2006, with databases of known toxins and allergens did not indicate potential production of harmful proteins or polypeptides caused by the genetic modification. Southern blot and segregation analyses show that the introduced gene elements are stably inherited and expressed over multiple generations, and consistent with the observed phenotypic characteristics of soybean MON 89788. The VKM GMO Panel concludes that the molecular characterisation of soybean MON 89788 does not indicate a safety concern.

Comparative assessments

The VKM GMO Panel has considered the available literature on compositional data and found that except for small intermittent variations, no biologically relevant differences exist between soybean MON 89788 and its corresponding control in the analyses of seed and forage. The few observed statistical differences between MON 89788 and A3244 are likely to reflect the natural variability of the analytes since their mean levels for MON 89788 were well within the 99% tolerance intervals for conventional reference varieties and within the ranges in the ILSI-Crop Composition Database. The field studies investigating composition of MON 89788 show no biologically relevant differences between GM crops treated and untreated with glyphosate. The data presented do not show unintended effects as a result of the genetic modification.

Based on current knowledge, the VKM GMO Panel concludes that with the exception of the introduced trait, soybean MON 89788 is compositionally, agronomically, morphologically and ecologically equivalent to its conventional counterpart, and other conventional soybean varieties.

Food and feed risk assessment

A 90-day sub-chronic toxicity study with rats, as well as a nutritional assessment trial with broilers fed diets containing soybean MON 89788 did not indicate any adverse effects. The CP4 EPSPS protein does not show sequence resemblance to known toxins or IgE-dependent allergens, nor has it been reported to cause IgE-mediated allergic reactions.

Based on current knowledge, the VKM GMO Panel concludes that soybean MON 89788 is nutritionally equivalent to and as safe as its conventional counterpart and other conventional soybean varieties.

Environmental assessment

Considering the intended uses of soybean MON 89788, excluding 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 89788.

Soybean MON 89788 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 of seeds from soybean MON 87988 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.

Overall conclusion

Based on current knowledge and considering the intended usage, the VKM GMO Panel concludes that soybean MON 89788 is as safe as its conventional counterpart and commercial soybean varieties. With the exception of the introduced trait, soybean MON 89788 is nutritionally, morphologically, agronomically, and ecologically equivalent to conventional soybean varieties.

Likewise, the VKM GMO Panel concludes that soybean MON 89788 does not represent an environmental risk in Norway.

8 Data gaps

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

As the broad-spectrum herbicides are sprayed on the plant canopy and spraying often takes place later in the growing season than is the case with selective herbicides associated with conventional crops, the residue and metabolite levels of herbicides in plants with tolerance to glyphosate could be higher compared to plants produced by conventional farming practices. There are however limited amounts of data available on pesticide residues in HT crops.

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

9 References

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Appendix I

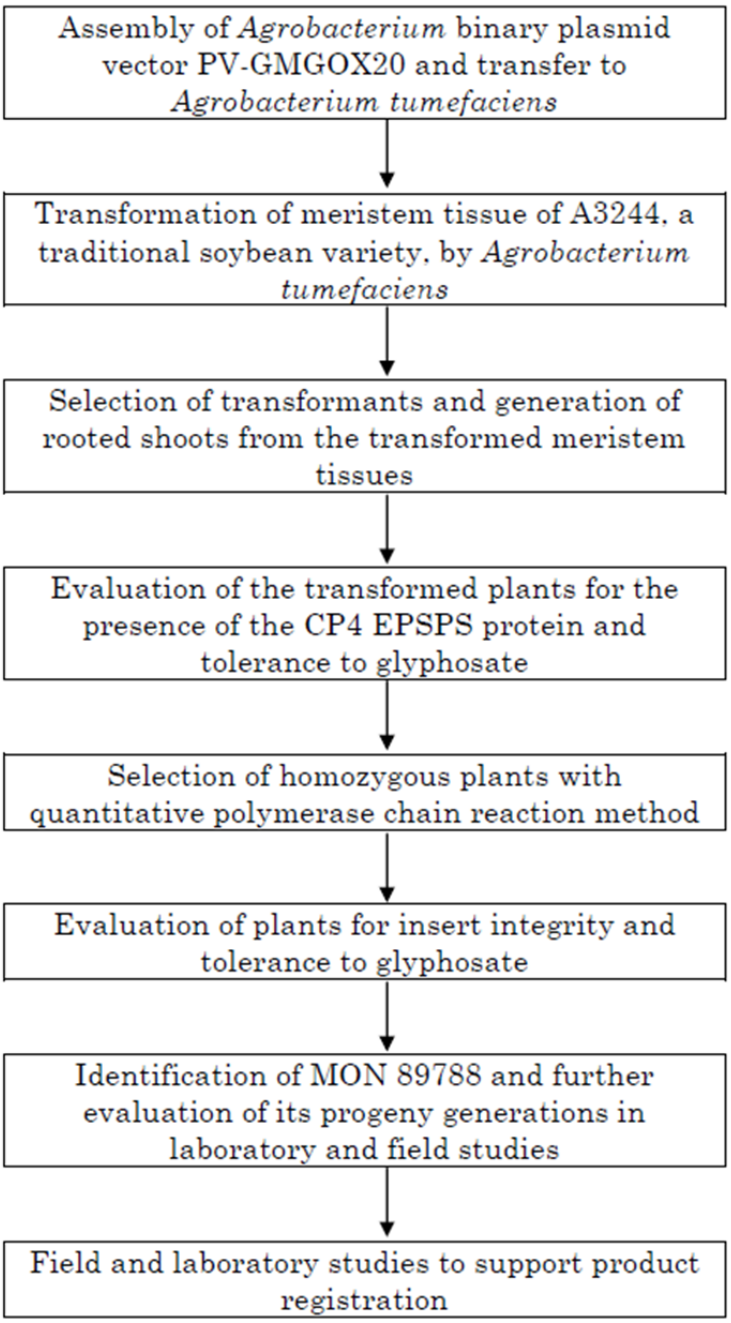

Figure AI-1. Flow chart of the major steps in the development of soybean MON 89788 (Figure 2. in Technical Dossier)

Table AI-1. Summary of CP4 EPSPS protein levels in tissue collected from MON 89788 produced in the Argentinean 2004-2005 growing season. (Table 6 in Technical Dossier)

Tissue type[1]	CP4 EPSPS µg/g fw (SD)[2]	Range[3] (µg/g fw)	CP4 EPSPS µg/g dw (SD)[4]	Range (µg/g dw)	LOQ / LOD (µg/g fw)
OSL1	55 (13)	27 – 70	280 (71)	140 – 370	0.57 / 0.26
OSL2	68 (12)	48 – 86	340 (61)	250 – 440	0.57 / 0.26
OSL3	61 (16)	40 – 86	310 (75)	200 – 420	0.57 / 0.26
OSL4	90 (29)	48 – 160	460 (130)	250 – 780	0.57 / 0.26
Seed	150 (28)	120 – 230	170 (33)	130 – 260	0.34 / 0.26
Root	32 (5.7)	23 – 43	100 (26)	63 – 160	0.57 / 0.11
Forage	73 (9.3)	56 – 93	290 (25)	240 – 330	0.57 / 0.10

[1] For all tissues, n = 15. OSL1 to OSL4 represent over-season leaves collected at the following developmental stages: OSL1 – at the V3 to V4 growth stage; OSL2 – at the V6 to V8 growth stage; OSL3 – at the V9 to V10 growth stage; OSL4 – at the V11 to V13 growth stage. Forage and root tissue samples were collected at the R6 (full seed) growth stage. Seed samples were collected at the R8 growth stage.

[2] Protein quantities are expressed as mean micrograms (µg) of CP4 EPSPS protein per gram (g) of tissue on a fresh weight (fw) basis. The arithmetic mean and standard deviation (SD) were calculated for each tissue type across all sites.

[3] Minimum and maximum values were determined for each tissue type across all sites.

[4] Protein quantities are expressed as mean micrograms (µg) of CP4 EPSPS protein per gram (g) of tissue on a dry weight (dw) basis. The dry weight values were calculated by dividing the fresh weight values by the dry weight conversion factors obtained from moisture analysis data.

Table AI-2. Summary of CP4 EPSPS protein levels in tissue collected from MON 89788 produced in the US 2005 growing season (Table 7 in Technical Dossier)

Tissue type[1]	CP4 EPSPS µg/g fw (SD)[2]	Range[3] (µg/g fw)	CP4 EPSPS µg/g dw (SD)[4]	Range (µg/g dw)	LOQ / LOD (µg/g fw)
OSL1	54 (7.8)	40 – 66	300 (51)	220 – 380	0.57 / 0.26
OSL2	60 (10)	42 – 80	340 (55)	250 – 440	0.57 / 0.26
OSL3	58 (11)	40 – 79	330 (94)	200 – 520	0.57 / 0.26
OSL4	75 (17)	60 – 110	290 (48)	210 – 390	0.57 / 0.26
Seed	140 (20)	98 – 170	150 (22)	110 – 180	0.34 / 0.26
Root	22 (6.0)	13 – 38	74 (27)	41 – 150	0.57 / 0.11
Forage	59 (14)	41 – 94	220 (51)	140 – 330	0.57 / 0.10

[1] For forage, n = 14; for all other tissues, n = 15. OSL1 to OSL4 represent over-season leaves collected at the following developmental stages: OSL1 – at the V3-V4 growth stage; OSL2 – at the V6-V8 growth stage; OSL3 – at the V10-V12 growth stage; OSL4 – at the V14-V16 growth stage. Forage and root tissue samples were collected at the R6 (full seed) growth stage. Seed samples were collected at the R8 growth stage.

[2] Protein quantities are expressed as mean micrograms (µg) of CP4 EPSPS protein per gram (g) of tissue on a fresh weight (fw) basis. The arithmetic mean and standard deviation (SD) were calculated across all sites.

[3] Minimum and maximum values were determined for each tissue type across all sites.

[4] Protein quantities are expressed as mean micrograms (µg) of CP4 EPSPS protein per gram (g) of tissue on a dry weight (dw) basis. The dry weight values were calculated by dividing the fresh weight values by the dry weight conversion factors obtained from moisture analysis data.

Table AI-3. Glyphosate-tolerant trait segregation patterns of MON 89788 (Table 10 in Technical Dossier)

Generation	Number of plants (% Germ.)[1]	Expected[2]		Observed[3]		Chi-square
		Positive	Negative	Positive	Negative	
R_1	43	32.25	10.75	29	14	1.31[4]
R_2	58	58	0	58	0	Fixed
R_3	240 (80%)	192	0	192[5]	0	Fixed
R_3	240 (85%)	204	0	204[5]	0	Fixed
R_3	240 (85%)	204	0	204[5]	0	Fixed

[1] Percent germination based on visual estimation (plant stand, in 5% increments).

[2] Expected number of glyphosate-tolerant plants.

[3] Observed number of glyphosate-tolerant plants by ELISA and/or glyphosate application.

[4] Not significant at $p \leq 0.05$ (Chi-square = 3.84 at 1 df).

[5] Number of plants (observed positives) was calculated based on the number of seed planted × percent germination.

Appendix II

Table AII-1. List of MON 89788MON 89788 Composition Studies (From Technical dossier, Add_info_composition)

Study Letter Designation	Study Number	Soybean Crop Season [a]	Number of Field Locations	Total Number of Samples Analyzed [b]	References
A	05-01-30-29	2004/2005	5	30	Lundry *et al.*, 2006a
B	05-01-30-32	2005	5	29	Lundry *et al.*, 2006b
C	REG-07-277	2007	2	19	Taylor *et al.*, 2008a
D	RAR-08-007	2006	8	16	Taylor *et al.*, 2008b
Totals		4 seasons	20	94	

[a] Soybean crop season in which field trial was planted and harvested. Studies B, C and D were conducted in the U.S.A. Study A was conducted in Argentina, where it was planted in 2004 and harvested in 2005.

[b] Total number of samples analyzed includes untreated and treated MON 89788 and conventional control, A3244.

Table AII-2.Summary of differences between levels of analytes in MON 89788, A3244 and traditional varieties for the Argentina field trails during the 2004-2005 growing seasons (Technical dossier)

Analytical component (Units)	MON 89788 Mean	A3244 Mean	Difference (MON 89788 minus A3244) % of A3244	Difference (MON 89788 minus A3244) p-value	MON 89788 [Range]	Traditional tol. int.[1] [Range]
Statistical differences observed in more than one site						
Seed linolenic acid: Site 1 (% dw)	1.56	1.53	2.09	0.044	[1.53 – 1.60]	[1.06 – 2.08]
Seed linolenic acid: Site 2 (% dw)	1.64	1.58	3.82	0.016	[1.62 – 1.66]	[1.06 – 2.08]
Forage moisture: Site 1 (% fw)	72.17	70.63	2.17	0.006	[72.00 – 72.40]	[64.50 – 81.35]
Forage moisture: Site 2 (% fw)	72.37	74.17	-2.43	0.013	[71.90 – 72.80]	[64.50 – 81.35
Statistical differences observed in one site						
Seed serine (% dw)	2.18	2.24	-2.42	0.032	[2.16 – 2.19]	[1.72 – 2.51]
Seed ash (% dw)	5.93	5.73	3.34	0.027	[5.88 – 5.99]	[3.87 – 7.17]
Seed methionine (% dw)	0.58	0.57	2.14	0.03	[0.58 – 0.59]	[0.46 – 0.70]
Seed 16:0 palmitic acid (% dw)	18.82	20.41	-7.79	0.002	[18.42 – 19.17]	[15.38 – 21.95]
Seed 18:2 linoleic acid (% dw)	9.25	8.79	5.24	0.019	[9.2 – 9.28]	[5.16 – 13.37]
Seed moisture (% fw)	11.8	10.97	7.6	0.029	[11.60 – 12.00]	[6.73 – 12.59]
Forage ash (% fw)	7.77	8.54	-9.01	0.019	[7.42 – 8.25]	[5.74 – 9.95]
Seed isoleucine (% dw)	1.94	1.9	1.85	0.017	[1.92 – 1.94]	[1.40 – 2.14]
Seed valine (% dw)	2.05	2.01	2.13	0.048	[2.03 – 2.07]	[1.48 – 2.26]
Seed glycitein (µg/g dw)	81.11	69.83	16.16	0.045	[80.00 – 83.16]	[0 – 238.94]

[1] With 95% confidence, the tolerance interval contains 99% of the values expressed in the population of commercial varieties. Negative limits were set to zero.

Table AII-3. Summary of statistical differences between levels of analytes in MON 89788, A3244 and traditional varieties for the U.S.A. field trials during the 2005 growing season (Technical dossier)

Analytical component (Units)	MON 89788 Mean	A3244 Mean	Difference (MON 89788 minus A3244) % of A3244	Difference (MON 89788 minus A3244) p-value	MON 89788 [Range]	Traditional tol. int. [1] [Range]
Statistical differences observed in across-site analyses						
Forage moisture (% fw)	72.07	73.21	-1.55	0.006	[67.90 – 77.60]	[60.84 – 83.36]
Seed daidzein (µg/g dw)	993.67	1073.57	-7.44	0.021	[631.32 – 1571.41]	[0 – 1925.63]
Seed glycitein (µg/g dw)	91.77	102.61	-10.56	0.037	[53.78 – 162.52]	[0 – 287.45]
Seed vitamin E [1](mg/100 g dw)	2.71	2.52	7.41	0.015	[1.88 – 3.72]	[0 – 7.00]
Statistical differences observed in more than one site and not in the across-site analysis						
Seed raffinose: site 1 (% dw)	0.65	0.81	-20.02	0.024	[0.58 – 0.71]	[0 – 1.01]
Seed raffinose: site 2 (% dw)	0.42	0.33	25.45	0.035	[0.40 – 0.43]	[0 – 1.01]
Statistical differences observed in one site and not in the across-site analysis						
Seed phenylalanine (% dw)	2	2.01	-0.41	0.014	[2.00 – 2.01]	[1.70 – 2.45]
Seed palmitic acid (% dw)	2.21	2.4	-7.73	0.004	[2.17 – 2.25]	[1.32 – 2.64]
Seed stearic acid (% dw)	0.76	0.81	-5.43	0.024	[0.75 – 0.77]	[0.37 – 1.28]
Seed oleic acid (% dw)	3.3	3.68	-10.31	0.001	[3.24 – 3.36]	[2.06 – 6.43]
Seed linoleic acid (% dw)	10.27	11.02	-6.86	0.005	[10.06 – 10.42]	[7.75 – 11.22]
Seed linolenic acid (% dw)	1.45	1.55	-6.16	0.029	[1.41 – 1.48]	[0.84 – 1.69]

Table AII-4 Summary of statistically significant differences between levels of analytes in untreated and treated MON 89788 for the USA field trials during the 2007 growing season, combined sites analysis and from one single site (From Technical dossier, Add_info_composition).

Analytical Component (Units) [1]	Untreated MON 89788 Mean	Treated MON 89788 Mean	Mean Difference (Untreated minus Treated) % of Treated	Mean Difference (Untreated minus Treated) p-Value	Untreated MON 89788 (Range)	ILSI Database (Range)[2]
Statistically Significant Differences Observed in Combined-Site Analyses						
Seed Proximate						
Carbohydrates (% dw)	36.65	37.43	-2.09	0.031	(35.76 - 38.75)	(29.60 - 50.20)
Seed Antinutrient						
Stachyose (% dw)	2.59	2.43	6.35	0.012	(2.26 - 3.01)	(1.21 - 3.50)
Trypsin Inhibitor (TIU/mg dw)	26.81	29.76	-9.92	<0.001	(23.41 - 32.12)	(19.59 - 118.68)
Statistically Significant Differences Observed in the Stonington, IL Site						
Seed Fatty Acid						
16:0 Palmitic (% Total FA)	12.27	12.24	0.20	0.047	(12.23 - 12.31)	(9.55 - 15.77)
18:1 Oleic (% Total FA)	21.50	22.00	-2.24	0.023	(21.16 - 21.84)	(14.30 - 32.20)
18:2 Linoleic (% Total FA)	53.91	53.54	0.69	0.030	(53.71 - 54.15)	(42.30 - 58.80)
18:3 Linolenic (% Total FA)	6.91	6.77	2.14	0.031	(6.86 - 7.00)	(3.00 - 12.52)
20:0 Arachidic (% Total FA)	0.36	0.37	-2.19	0.023	(0.36 - 0.37)	(0.16 - 0.48)
20:1 Eicosenoic (% Total FA)	0.22	0.23	-3.92	0.004	(0.22 - 0.23)	(0.14 - 0.35)
22:0 Behenic (% Total FA)	0.38	0.39	-2.29	0.004	(0.37 - 0.39)	(0.28 - 0.60)
Seed Vitamin						
Vitamin E (mg/100 g dw)	3.91	4.15	-5.93	0.044	(3.78 - 4.12)	(0.19 – 6.17)
Seed Antinutrient						
Phytic Acid (% dw)	0.86	0.74	16.74	0.044	(0.78 - 0.93)	(0.63 - 1.96)
Trypsin Inhibitor (TIU/mg dw)	29.11	31.84	-8.56	0.015	(7.95 - 32.12)	(19.59 - 118.68)

[1] dw = dry weight; FA = fatty acid, [2] ILSI Crop Composition database, 2006

Table AII-5 Summary of statistically significant differences between levels of analytes in untreated and treated MON 89788 for the USA. field trials during the 2006 growing season (Combined Sites) (From Technical dossier, Add_info_composition).

			Mean Difference (Untreated minus Treated)			
Analytical Component (Units)[1]	Untreated MON 89788 Mean	Treated MON 89788 Mean	% of Treated	p-Value	Untreated MON 89788 (Range)	ILSI Database (Range)[2]
Statistically Significant Differences Observed						
Seed Amino Acid						
Tryptophan (% dw)	0.48	0.49	-2.53	0.026	(0.46 – 0.49)	(0.36 – 0.50)
Seed Fatty Acid						
17:0 Heptadecanoic (% Total FA)	0.10	0.11	-4.34	0.036	(0.092 – 0.11)	(0.085-0.15)
Seed Antinutrient						
Stachyose (% dw)	2.87	3.04	-5.43	0.040	(2.49-3.28)	(1.21-3.50)

[1] dw = dry weight; FA = fatty acid, [2] ILSI Crop Composition database, 2006

Table AII-6 Literature and ILSI ranges for components in soybean seed (From Technical dossier, Add_info_composition).

Tissue Component[1]	Literature Range[2]	ILSI Range[3]
Proximates (% dw)		
Ash	4.61-5.94[b]; 4.29-5.88[a]	3.885-6.994
Carbohydrates	29.3-41.3[a]	29.6-50.2
Fat, total	198-277[c] g/kg dw; 160-231[d] g/kg dw	8.104-23.562
Moisture (% fw)	5.3-8.73[a], 5.18-14.3[b]	4.7-34.4
Protein	329-436[c] g/kg dw; 360-484[d] g/kg dw	33.19-45.48
Fiber (% dw)		
Acid detergent fiber (ADF)	not available	7.81-18.61
Neutral detergent fiber (NDF)	not available	8.53-21.25
Crude fiber	5.74-7.89[a]	4.12-13.87
Amino Acids	**(g/100g dw)[a]**	**(g/100g dw)[h]**
Alanine	1.60– 1.86	1.513-2.104
Arginine	2.56 – 3.46	2.285-3.400
Aspartic acid	4.18 – 4.99	3.808-5.122
Cystine/Cysteine	0.54 – 0.66	0.370-0.808
Glutamic acid	6.64 – 8.16	5.843-8.201
Glycine	1.60 - 1.87	1.458-1.997
Histidine	0.98 – 1.16	0.878-1.175
Isoleucine	1.65 – 1.95	1.539-2.077
Leucine	2.81 – 3.37	2.590-3.622
Lysine	2.47 – 2.84	2.285-2.839
Methionine	0.51 – 0.59	0.431-0.681
Phenylalanine	1.78 – 2.19	1.632-2.346
Proline	1.86 – 2.23	1.687-2.284
Serine	1.96 – 2.28	1.106-2.484
Threonine	1.51 – 1.73	1.139-1.862
Tryptophan	0.56 – 0.63	0.3563-0.5016
Tyrosine	1.35 – 1.59	1.016-1.613
Valine	1.71 – 2.02	1.597-2.204
Methionine	0.51 – 0.59	0.431-0.681
Phenylalanine	1.78 – 2.19	1.632-2.346
Proline	1.86 – 2.23	1.687-2.284
Serine	1.96 – 2.28	1.106-2.484
Fatty Acids	**(% dw)**	**(% total)**
12:0 Lauric	not available	0.082-0.132
14:0 Myristic	not available	0.071-0.238
16:0 Palmitic	1.44-2.31[f]	9.55-15.77
16:1 Palmitoleic	not available	0.086-0.194

Table AII-6 cont. Literature and ILSI ranges for components in soybean seed (From Technical dossier, Add info composition).

Tissue Component[1]	Literature Range[2]	ILSI Range[3]
Fatty Acids (continued)	**(% dw)**	**(% total)**
17:0 Heptadecanoic	not available	0.085-0.146
17:1 Heptadecenoic	not available	0.073-0.087
18:0 Stearic	0.54-0.91[f]	2.7-5.88
18:1 Oleic	3.15-8.82[f]	14.3-32.2
18:2 Linoleic	6.48-11.6[f]	42.3-58.8
18:3 Linolenic	0.72-2.16[f]	3.00-12.52
20:0 Arachidic	0.04-0.7[f]	0.163-0.482
20:1 Eicosenoic	not available	0.14-0.35
20:2 Eicosadienoic	not available	0.077-0.245
22:0 Behenic	not available	0.277-0.595
Vitamins (mg/100g)	**fw[i]**	**mg/100g dw**
Vitamin E	0.85[g]	0.19-6.17
Anti-Nutrients		
Lectin (H.U./mg fw)	0.8-2.4[a]	0.090-8.460
Trypsin Inhibitor (TIU/mg dw)	33.2-54.5[a]	19.59-118.68
Phytic Acid (%dw)	not available	0.634-1.960
Isoflavones	**mg/100g fw**	**mg/kg dw**
Daidzein	9.88-124.2[e]	60.0-2453.5
Genistein	13-150.1[e]	144.3-2837.2
Glycitein	4.22-20.4[e]	15.3-310.4
Bio-Actives (%dw)		
Raffinose	not available	0.212-0.661
Stachyose	not available	1.21-3.50

[1] fw=fresh weight; dw=dry weight

[2] Literature range references:
[a] (Padgette *et al.*, 1996);
[b] (Taylor *et al.*, 1999);
[c] (Maestri *et al.*, 1998);
[d] (Hartwig and Kilen, 1991);
[e] U.S. Department of Agriculture, Agricultural Research Service. 2007a. USDA Iowa State University Database on the isoflavone content of foods, Release 1.4, Nutrient Data Laboratory Web site, http://www.ars.usda.gov/ba/bhnrc/ndl.
[f] OECD 2001. Consensus document on compositional considerations for new varieties of soybean: key food and feed nutrients and anti-nutrients. Organization for Economic Co-operation and Development, Environmental Health and Safety Publications. Paris, France. ENV/JM/MONO (2001)15.
[g] U.S. Department of Agriculture, Agricultural Research Service. 2007b. USDA National Nutrient database for standard reference, Release 20, Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/ba/bhnrc/ndl [accessed January 29, 2008].
[h] Data converted from mg/g dw to g/100g dw; g/100g dw is exactly equal to % dw
[i] Moisture value = 8.54g/100g.

[3] ILSI. 2006. International Life Sciences Institute Crop Composition Database. Version 3.0 http://www.cropcomposition.org. Search criteria soybean seed, all locations, all years, all proximates, amino acids, fatty acids, vitamins, bio-actives, fiber, dry weight other than moisture [Accessed January, 2008].

Conversions: % dw x 104 = µg/g dw; mg/g dw x 103 = mg/kg dw; mg/100g dw x 10 = mg/kg dw; g/100g dw x 10 = mg/g dw

Table AII-7. Statistical Analysis of Composition Data[a] for MON 89788 Untreated and Treated and Conventional Control for Studies A, B, C, and D (From Technical dossier, Add_info_composition).

Analytes	Type	Sample Size	Mean	Standard Error	Lower 95% CB	Upper 95% CB
Proximates (% dw)						
Ash	Control/A3244	10	5.33	0.14	5.01	5.64
Ash	MON89788/Treated	20	5.26	0.08	5.08	5.43
Ash	MON89788/Untreated	10	5.24	0.05	5.13	5.36
Carbohydrates	Control/A3244	10	37.52	0.45	36.51	38.53
Carbohydrates	MON89788/Treated	20	37.76	0.32	37.08	38.43
Carbohydrates	MON89788/Untreated	10	38.08	0.71	36.48	39.68
Fat	Control/A3244	10	17.21	0.46	16.18	18.25
Fat	MON89788/Treated	20	17.29	0.24	16.78	17.80
Fat	MON89788/Untreated	10	17.55	0.59	16.21	18.88
Moisture	Control/A3244	10	9.22	0.56	7.95	10.50
Moisture	MON89788/Treated	20	10.09	0.73	8.55	11.62
Moisture	MON89788/Untreated	10	10.95	1.41	7.77	14.13
Protein	Control/A3244	10	39.95	0.50	38.81	41.08
Protein	MON89788/Treated	20	39.70	0.33	39.01	40.39
Protein	MON89788/Untreated	10	39.13	0.35	38.34	39.91
Amino Acids (g/100g dw)						
Arginine	Control/A3244	10	3.009	0.059	2.875	3.144
Arginine	MON89788/Treated	20	3.055	0.036	2.979	3.131
Arginine	MON89788/Untreated	10	3.101	0.047	2.995	3.207
Histidine	Control/A3244	10	1.061	0.012	1.034	1.087
Histidine	MON89788/Treated	20	1.055	0.007	1.040	1.071
Histidine	MON89788/Untreated	10	1.041	0.008	1.024	1.059
Isoleucine	Control/A3244	10	1.814	0.020	1.767	1.860
Isoleucine	MON89788/Treated	20	1.820	0.012	1.795	1.845
Isoleucine	MON89788/Untreated	10	1.813	0.015	1.778	1.848
Leucine	Control/A3244	10	3.131	0.034	3.054	3.208
Leucine	MON89788/Treated	20	3.109	0.023	3.061	3.157
Leucine	MON89788/Untreated	10	3.041	0.026	2.982	3.100
Lysine	Control/A3244	10	2.597	0.024	2.543	2.650
Lysine	MON89788/Treated	20	2.579	0.015	2.547	2.611
Lysine	MON89788/Untreated	10	2.535	0.017	2.497	2.573
Methionine	Control/A3244	10	0.553	0.010	0.531	0.574
Methionine	MON89788/Treated	20	0.551	0.007	0.537	0.565
Methionine	MON89788/Untreated	10	0.558	0.004	0.549	0.568

Table AII-7 cont. Statistical Analysis of Composition Data[a] for MON 89788 Untreated and Treated and Conventional Control for Studies A, B, C, and D (From Technical dossier, Add_info_composition).

Analytes	Type	Sample Size	Mean	Standard Error	Lower 95% CB	Upper 95% CB
Amino Acids (g/100g dw) - continued						
Phenylalanine	Control/A3244	10	2.066	0.023	2.013	2.120
Phenylalanine	MON89788/Treated	20	2.075	0.016	2.042	2.107
Phenylalanine	MON89788/Untreated	10	2.062	0.019	2.019	2.104
Threonine	Control/A3244	10	1.593	0.016	1.557	1.628
Threonine	MON89788/Treated	20	1.563	0.011	1.539	1.586
Threonine	MON89788/Untreated	10	1.515	0.008	1.496	1.533
Tryptophan	Control/A3244	10	0.419	0.013	0.390	0.447
Tryptophan	MON89788/Treated	20	0.446	0.011	0.423	0.470
Tryptophan	MON89788/Untreated	10	0.483	0.003	0.477	0.490
Valine	Control/A3244	10	1.914	0.023	1.863	1.966
Valine	MON89788/Treated	20	1.909	0.013	1.881	1.936
Valine	MON89788/Untreated	10	1.886	0.015	1.853	1.920
Fatty Acids (% total)						
16:0 Palmitic	Control/A3244	10	11.96	0.07	11.80	12.12
16:0 Palmitic	MON89788/Treated	20	11.89	0.10	11.68	12.10
16:0 Palmitic	MON89788/Untreated	10	11.76	0.21	11.27	12.24
18:0 Stearic	Control/A3244	10	4.611	0.080	4.430	4.792
18:0 Stearic	MON89788/Treated	20	4.523	0.074	4.368	4.679
18:0 Stearic	MON89788/Untreated	10	4.304	0.081	4.120	4.488
18:1 Oleic	Control/A3244	10	20.30	0.36	19.49	21.10
18:1 Oleic	MON89788/Treated	20	20.57	0.34	19.86	21.28
18:1 Oleic	MON89788/Untreated	10	20.70	0.42	19.76	21.64
18:2 Linoleic	Control/A3244	10	53.67	0.38	52.81	54.54
18:2 Linoleic	MON89788/Treated	20	53.88	0.30	53.24	54.52
18:2 Linoleic	MON89788/Untreated	10	54.58	0.35	53.79	55.36
18:3 Linolenic	Control/A3244	10	8.544	0.302	7.861	9.228
18:3 Linolenic	MON89788/Treated	20	8.176	0.221	7.712	8.639
18:3 Linolenic	MON89788/Untreated	10	7.666	0.242	7.118	8.213
Vitamins (mg/100g dw)						
Vitamin E	Control/A3244	5	2.444	0.219	1.835	3.053
Vitamin E	MON89788/Treated	15	2.624	0.219	2.155	3.094
Vitamin E	MON89788/Untreated	10	2.682	0.334	1.925	3.438

Table AII-7 cont. Statistical Analysis of Composition Data[a] for MON 89788 Untreated and Treated and Conventional Control for Studies A, B, C, and D (From Technical dossier, Add_info_composition).

Analytes	Type	Sample Size	Mean	Standard Error	Lower 95% CB[a]	Upper 95% CB
Anti-Nutrients						
Lectin (H.U./mg fw)	Control/A3244	10	3.00	0.70	1.41	4.59
Lectin (H.U./mg fw)	MON89788/Treated	20	3.12	0.43	2.22	4.01
Lectin (H.U./mg fw)	MON89788/Untreated	10	2.90	0.52	1.73	4.07
Trypsin Inhibitor (TIU/mg dw)	Control/A3244	10	33.47	1.93	29.10	37.84
Trypsin Inhibitor (TIU/mg dw)	MON89788/Treated	20	35.27	1.46	32.22	38.32
Trypsin Inhibitor (TIU/mg dw)	MON89788/Untreated	10	31.33	2.26	26.22	36.45
Phytic Acid (% dw)	Control/A3244	10	0.91	0.08	0.73	1.08
Phytic Acid (% dw)	MON89788/Treated	20	0.89	0.05	0.79	0.99
Phytic Acid (% dw)	MON89788/Untreated	10	0.91	0.04	0.81	1.00
Isoflavones (mg/kg dw)						
Daidzein	Control/A3244	10	1122	93	912.8	1332
Daidzein	MON89788/Treated	20	1129	78	965.1	1292
Daidzein	MON89788/Untreated	10	1228	135	922.7	1533
Genistein	Control/A3244	10	979.7	79.1	800.8	1159
Genistein	MON89788/Treated	20	983.9	69.9	837.5	1130
Genistein	MON89788/Untreated	10	1009.3	125.7	725.0	1294
Glycitein	Control/A3244	10	93.55	5.90	80.20	106.9
Glycitein	MON89788/Treated	20	96.18	4.74	86.25	106.1
Glycitein	MON89788/Untreated	10	114.82	7.35	98.19	131.4
Fiber (% dw)						
Acid Detergent Fiber	Control/A3244	10	15.40	0.77	13.67	17.13
Acid Detergent Fiber	MON89788/Treated	20	14.75	0.65	13.38	16.11
Acid Detergent Fiber	MON89788/Untreated	10	12.63	0.67	11.12	14.14
Neutral Detergent Fiber	Control/A3244	10	17.37	0.71	15.78	18.97
Neutral Detergent Fiber	MON89788/Treated	20	15.27	0.63	13.95	16.59
Neutral Detergent Fiber	MON89788/Untreated	10	13.14	0.46	12.10	14.18

[a] 95% Confidence Intervals – Variability Used is Site-to-Site Variability
CB= Confidence Bound

Appendix III

Soy products

By Dagrunn Engeset and Inger Therese Lillegaard

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

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

Reason for the choice of menus

The milk allergy menu

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

The vegan menu

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

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

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

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

7 days vegan menu, high preference for soy products

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

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

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

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

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

Supper: bread with peanut butter, soy cheese, bell pepper, coffee with soy cream, orange juice

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

Saturday:
Breakfast: Soy smoothie (as previous)
Lunch: Soy waffle (like Friday snack)
Snack: Milk chocolate without milk, cashew nuts, raspberries
Dinner: Vegetarian bean casserole, pita bread, wine, water, soy chocolate dessert
Supper: Vegan pizza (marguerita with soy cheese), beer, potato chips

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

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

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

Tuesday:
Breakfast: Bread breakfast (like Monday lunch)
Lunch: Bread lunch (like Monday supper)
Snack: Smoothie (like vegan)

Dinner: Vegetable soup (like vegan Thursday)
Supper: omelette with bread, soy milk, tea

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

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

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

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

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

Appendix IV

A brief summary of studies performed by Tutelyan VA (2013):

Assessment of Potential Toxicity of GM Soybean Line MON 89788MON 89788 in Chronic Experiment in Rats

A 182-day study was conducted on two groups of male Wistar rats (50 animals in each group). The test group received soybean MON 89788MON 89788 with their feed while controls received a conventional soybean variety. Soybean in the form of defatted flour was included in the feed at the rate of ~8 g/rat/24 h (approximately 40% of the feed, assuming a feed intake of 20g/24h). Analyses of the organs were carried out after 30 and 182 days. During the experiment, palatability of the feed, body mass, and overall conditions of the animals were monitored.

During the experiment, no mortality was observed in test or control group, and overall condition of the animals was satisfactory. Weekly weight gain of both groups of rats corresponded to the gain level characteristic of animals of this breed and age. Results of morphological, hematological, and biochemical analyses indicate absence of any toxic effect of GM soybean line MON 89788MON 89788. Macro- and microscopic examination of internal organs of the test group rats and control group rats revealed no pathological alterations at 30 and 182 days from the onset of the experiment. Similarly, morphological examination detected no significant differences in the internal organs of the rats in both groups. Biochemical analyses of serum and urine detected a few significant differences of some factors, varying within the limits of physiological fluctuations typical for rats. Taking into account the absence of a tendency to maintain differences between groups, changes within normal range can be ascribed to individual fluctuations. Values of all studied parameters fell within the limits of physiological fluctuations characteristic of rats.

Despite the extensive nature of this study, measuring both conventional and also less conventional parameters, the VKM GMO panel thinks the study has certain limitations. For example, only male rats are included in the study. But still, the study is considered supporting to the conclusion.

Genotoxicity Studies of the GM Soybean Line MON 89788MON 89788 in Experiment in Mice

A 30-day *in vivo* genotoxicity study was conducted in male C57Bl/6 mice, sensitive to genotoxic influence. Soybean (defatted flour) was included in the feed at the rate of 3–3.2 g/mouse/24 h (approximately 65% of the feed, assuming a feed intake of 5g/24h). Evaluation of potential genotoxicity included identification of DNA damage by the method of gel-electrophoresis of isolated cells (DNA-comet assay) of bone marrow, kidneys, liver,

rectum and by detection of mutagenic activity by counting of chromosomal aberrations in metaphase cells of bone marrow.

After 30 days, results of cytogenetic analysis of mice bone marrow average parameters of chromosomal aberrations in control and test groups showed no significant difference and did not exceed spontaneous mutagenesis level characteristic of mice of this strain. DNA structure damage in cells of bone marrow, kidneys, liver, and rectum of the test group did not differ from similar parameters in the control group. The investigators concluded that the DNA integrity and level of chromosomal aberrations in the mouse demonstrate absence of any genotoxic effect of GM soybean line MON 89788MON 89788 compared with its conventional soybean.

Assessment of Potential Impact on Immune System of GM Soybean Line MON 89788MON 89788 in Experiment in Mice

A 45 days experiment was conducted on mice of strains CBA and C57Bl/6. Soybean (defatted flour) was included in the feed at the rate of 3–3.2 g/mouse/24 h (approximately 65% of the feed, assuming a feed intake of 5g/24h). Evaluation of immunomodulating and sensitising properties was carried out in four different tests: (1) effect on the humoral component of the immune system detected with hemagglutinin levels in response to sheep erythrocytes; (2) effect on the cellular component of the immune system, through delayed hypersensitivity reaction of the response to sheep erythrocytes; (3) sensibilisation effect, through a histamine sensitivity test; (4) response to infection by Salmonella typhimurium.

The assessment of the humoral component of the immune system demonstrated that development of antibodies in response to injection of sheep erythrocytes in mice of the control group was similar to that in the test group (both in CBA and C57Bl/6 strains), which demonstrates absence of any immunomodulating effect of GM soybean line MON 89788MON 89788 compared with its traditional counterpart. In the assessment of the condition of the cellular component of the immune system in terms of delayed hypersensitivity test, no immunomodulating effect of GM soybean line MON 89788MON 89788 was detected. In the assessment of sensibilisation effect and response effect to Salmonella typhimurium, no negative influence of GM soybean was detected. The investigators concluded that the assessment of potential impact of GM soybean line MON 89788MON 89788 on the immune system of mice demonstrate absence of any immunomodulating and sensibilization effect of the GM soybean line MON 89788MON 89788 compared with its traditional counterpart.

Assessment of Potential Allergenicity of GM Soybean Line MON 89788MON 89788 in Rats

A 29 days allergenicity experiment was conducted in male Wistar rats. Soybean (defatted flour) was added to the feed at the rate of 3.3 g/rat/24 h (approximately 17% of the feed, assuming a feed intake of 20g/24h), excluding equivalent in caloric value and nutrient materials content quantity of oatmeal and grain mixture. Allergenicity was assessed in a model of generalised anaphylaxis.

Severity of anaphylactic shock reaction in rats of the test group was not statistically significant different from the severity of reaction in rats of the control group and the results were within the range of typical values (30–60% lethality), which are usually observed at administration of anaphylaxis-inducing dose of ovalbumin to sensitised rats. Factors characterising intensity of humoral immune responses were not significantly different between groups. The investigators concluded that the allergenicity studies on GM soybean line MON 89788MON 89788 in rats demonstrate absence of an allergenic effect of the given GM soybean line compared with its traditional counterpart.