Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

VKM Report 2015: 08

Final health and environmental risk assessment of genetically modified soybean A5547-127

Scientific opinion on herbicide tolerant, genetically modified soybean A5547-127 from Bayer CropScience for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (Application EFSA/GMO/NL/2008/52)

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

Report from the Norwegian Scientific Committee for Food Safety (VKM) 2015: 08
Food/feed and environmental risk assessment of herbicide tolerant genetically modified soybean A5547-127 for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (EFSA/GMO/NL/2008/52)

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

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Assessed and approved

The opinion has been assessed and approved by the Panel on Genetically Modified Organisms. Members of the panel are: Åshild Andreassen (chair), Per Brandtzæg, Knut 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

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

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Abstract

Soybean A5547-127 expresses the *phosphinothricin-N-acetyltransferase* (*pat*) gene from the soil bacterium *Streptomyces viridochromogenes*. The encoded PAT protein confers tolerance to the active herbicidal substance glufosinate-ammonium. Bioinformatics analyses of the inserted DNA and flanking sequences in soybean A5547-127 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 *pat* gene have been shown over several generations of soybean A5547-127. With the exception of the intended changes caused by the trans-genetically introduced trait, data from field trials performed in the USA show that soybean A5547-127 is compositionally, morphologically and agronomically equivalent to its conventional counterpart and other commercial soybean varieties. A repeated dose toxicity study with rats and a nutritional assessment trial with broilers have not revealed adverse effects of soybean A5547-127. These studies indicate that soybean A5547-127 is nutritionally equivalent to and as safe as conventional soybean varieties. The PAT protein produced in soybean A5547-127 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 A5547-127 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 A5547-127 (Unique Identifier ACS-GMØØ6-4) from Bayer CropScience is approved under Regulation (EC) No 1829/2003 for food and feed uses, import and processing since 10 February 2012 (Application EFSA/GMO/NL/2008/52, Commission Decision 2012/81/EC).

Soybean A5547-127 has previously been assessed as food and feed by the VKM GMO Panel commissioned by the Norwegian Food Safety Authority and the Norwegian Environment Agency related to the EFSAs public hearing of the application EFSA/GMO/2008/52 in 2008 (VKM 2008).

The food, feed and environmental risk assessment of the soybean A5547-127 is based on information provided by the applicant in the application EFSA/GMO/NL/2008/52, 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 A5547-127 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 A5547-127 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 A5547-127 is derived from the conventional soybean variety A5547, which was transformed using particle bombardment. Soybean A5547-127 expresses the phosphinothricin-N-acetyltransferase (*pat*) gene, from the soil bacterium *Streptomyces viridochromogenes*. The encoded PAT protein confers tolerance to the active herbicidal substance glufosinate-ammonium.

Molecular characterisation

The applicant has provided sufficient analyses to characterise the DNA inserts, number of inserts, integration sites and flanking sequences in the soybean genome. The results show that one full length functional copy of the *pat* gene is present in the soybean A5547-127 genome. Similarity searches in 2007 and 2009, with databases of known toxins and allergens did not indicate any 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 several generations, and consistent with the observed phenotypic characteristics of soybean A5547-127. The VKM GMO Panel concludes that the molecular characterisation of soybean A5547-127 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 A5547-127 and its corresponding control A5547 in the analyses of seeds and various processed food and feed commodities. 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 A5547-127 is compositionally, agronomically and morphologically equivalent to its conventional counterpart and other conventional soybean varieties.

Food and feed risk assessment

A 14-day repeated dose toxicity study with rats fed PAT protein, as well as a nutritional assessment trial with broilers fed diets containing soybean A5547-127 did not indicate any

adverse effects. The PAT protein in A5547-127 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 A5547-127 is nutritionally equivalent to and as safe as its conventional counterpart and other conventional soybean varieties.

Environmental assessment

Considering the intended uses of soybean A5547-127, 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 A5547-127.

Soybean A5547-127 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 A5547-127. 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 A5547-127 is as safe as its conventional counterpart and other commercial soybean varieties. With the exception of the introduced trait, soybean A5547-127 is nutritionally, morphologically and agronomically equivalent to conventional soybean varieties.

Likewise, the VKM GMO Panel concludes that soybean A5547-127 does not represent a discernible environmental risk in Norway.

Key words: GMO, soybean (*Glycine max*), A5547-127, EFSA/GMO/NL/2008/52, herbicide tolerance, *pat,* food and feed safety, environmental risk, 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 A5547-127 (unik kode ACS-GMØØ6-4) fra Bayer CropScience ble godkjent til import, videreforedling og til bruk som mat og fôr under EU-forordning 1829/2003 10. februar 2012 (Kommisjonsbeslutning 2012/81/EU).

Soyalinjen A5547-127 ble første gang vurdert av VKMs faggruppe for GMO i 2008 (VKM 2008). Helse- og miljørisikovurderingen ble utført på oppdrag av Mattilsynet og Miljødirektoratet i forbindelse med EFSAs offentlige høring av søknad EFSA/GMO/NL/2008/52.

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 A5547-127 har fått innsatt et *pat*-gen fra jordbakterien *Streptomyces viridochromogenes*. Genet koder for enzymet fosfinotricin acetyltransferase (PAT), som acetylerer og inaktiverer glufosinat-ammonium, virkestoffet i fosfinotricin-herbicider av typen Finale® og Liberty®. Fosfinotricin er et ikke-selektivt kontaktherbicid som hemmer glutaminsyntetase. Enzymet deltar i assimilasjonen av nitrogen og katalyserer omdanning av glutamat og ammonium til aminosyren glutamin. Hemming av glutaminsyntetase fører til akkumulasjon av ammoniakk, og til celledød i planten. De genmodifiserte soyaplantene vil derfor tolerere høyere doser av plantevernmidler med virkestoffet glufosinat-ammonium 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 A5547-127. Resultatene viser at kun ett funksjonelt *pat* gen er integrert i genomet til soyalinjen. Homologisøk fra 2007 og 2009, med databaser over kjente toksiner og allergener, indikerer at genmodifiseringen ikke har ført til potensiell produksjon av skadelige proteiner eller polypeptider i soya A5547-127. 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 A5547-127. VKMs faggruppe for GMO konkluderer med at den molekylære karakteriseringen ikke indikerer noen helserisiko ved soya A5547-127.

Komparative analyser

VKMs faggruppe for GMO har vurdert tilgjengelig litteratur vedrørende soya A5547-127 og funnet at det, med unntak av små tilfeldige variasjoner i enkeltparametere målt i bønner og noen prosesserte komponenter til bruk i mat og fôr, ikke foreligger biologisk relevante forskjeller mellom den genmodifiserte soyaen og dens kontroll. De rapporterte dataene viser ingen utilsiktede effekter som følge av genmodifiseringen.

Ut i fra dagens kunnskap konkluderer VKMs faggruppe for GMO at soya A5547-127, med unntak av den introduserte egenskapen, er ernæringsmessig, agronomisk, og morfologisk vesentlig lik dens konvensjonelle motpart, samt andre konvensjonelle soyasorter.

Helserisiko

En 14 dagers toksisitetsstudie med rotter gitt PAT-protein i fôret, og en ernæringsstudie utført med broilere gitt fôr inneholdende soya A5547-127, har ikke indikert helseskadelige effekter. PAT-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 A5547-127 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 soyalinjen A5547-127 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 A5547-127 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 A5547-127, ved forskreven bruk, er like trygg som dens konvensjonelle motpart og andre konvensjonelle soyasorter. Soya A5547-127 er ernæringsmessig, morfologisk, og agronomisk ekvivalent med konvensjonell soya.

Likeledes finner faggruppen, ut i fra dagens kunnskap, at den omsøkte bruken av soya A5547-127 ikke vil medføre noen miljørisiko i Norge.

Abbreviations and explanations

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.
Aspirated grain fractions	Plant parts obtained during normal aspiration of cereal and oil seed crops in the handling of the product consisting primarily of plant parts, including glumes and contain not more than 15 percent ash (dirt), The American Feed Control Officials definition
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 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).
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
NFSA / MT	Norwegian Food Safety Authority (Mattilsynet)
NDF	Neutral detergent fibre, measure of fibre used for animal feed analysis. NDF measures most of the structural components in plant cells (i.e. lignin, hemicellulose and cellulose), but not pectin.
Northern blot	Northern blot is 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, in molecular genetics defined as a reading frame that can code for amino acids between two stop codons (without stop codons).

OSL	Over season leaf
OSR	Over season root
OSWP	Over season whole plant
pat	*Phosphinothricin-Acetyl-Transferase* gene
PAT	Phosphinothricin-Acetyl-Transferase *protein*
PCR	Polymerase chain reaction, a technique to amplify DNA by copying it
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**	**Reproductive Stages**
	VE - Emergence	R1 – Beginning flowering
	VC - Cotyledon stage	R2 – Full flowering
	V1- First trifoliolate	R3 – Beginning pod (pods 5 mm in top 4 nodes)
	V2 – Second trifoliolate	R4 – Full pod (pods 2 cm in top 4 nodes)
	V3 – Third trifoliolate	R5 – Beginning seed (seed 3 mm long in top 4 nodes)
	V(n) – nth trifoliolate	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)

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	Transgene copy number is 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
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 3 April 2008, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands an application (Reference EFSA/GMO/NL/2008/52) for authorisation of the genetically modified herbicide tolerant soybean A5547-127 (Unique Identifier ACS-GMØØ6-4), submitted by Bayer CropScience 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/2008/52 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 18 July 2008, 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 October 2008 (VKM 2008). EFSA published its scientific opinion 10 May 2011 (EFSA 2011d), and soybean A5547-127 was approved for food and feed uses, import and processing 10 February 2012 (Commission Decision 2012/81/EC).

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 A5547-127 (Unique Identifier ACS-GMØØ6-4) was developed to provide tolerance to the herbicidal active substance glufosinate-ammonium by the introduction of a gene coding for the phosphinothricin N-acetyltransferase enzyme (PAT) from the soil bacterium *Streptomyces viridochromogenes*.

Glufosinate-ammonium inhibits glutamine synthetase, leading to glutamine deficiency, ammonia accumulation and eventually to plant death. The PAT protein catalyses the conversion of glufosinate-ammonium to N-acetyl glufosinate. N-acetyl glufosinate is an inactive form that does not bind to glutamine synthetase allowing plants to grow in the presence of glufosinate-ammonium.

The genetic modification in soybean A5547-127 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 A5547-127 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 A5547-127 is based on information provided by the applicant in the application EFSA/GMO/NL/2008/52, 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 and 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

Particle bombardment was used to transform embryo shoot apices derived from the soybean cultivar A5547 to generate the glufosinate-ammonium tolerant event A5547-127. DNA fragments of the plasmid pB2/35SAcK were used in the transformation. Initial selection was performed by treating new plantlets with glufosinate-ammonium and transferring tolerant plants to a greenhouse for further assessments and development.

2.1.2 Nature and source of vector used for the transformation

The plasmid pB2/35SAcK (~ 4kb) is based on the vector pUC19. It contains a Right Border fragment (RB) from the *Agrobacterium tumefaciens* Ti plasmid pTiAch5 and a synthetic *pat* gene fused to the 35S-promotor (P35S) and 35S-terminator (T35S) from Cauliflower Mosaic Virus (CaMV) (Berghman & De Beuckeleer, 2002a).

The plasmid backbone contains the *β-lactamase* (*bla*) gene which confers resistance to the antibiotic ampicillin, and the bacterial origin of replication (*ori*) from vector pUC19. Prior to transformation, plasmid pB2/35SAcK was digested with the restriction enzyme *Pvu*I, which has two restriction sites within the plasmid. One of these sites lies within the coding sequence of the *bla* gene, and thereby disrupts the gene. The *Pvu*I digestion resulted in one 3119bp plasmid-fragment and one 957bp fragment. A plasmid map of pB2/35SAcK is shown in Figure 2.1.2-1, and an overview including the relative position and function of the genetic elements in the plasmid is given in Table 2.1.2-1.

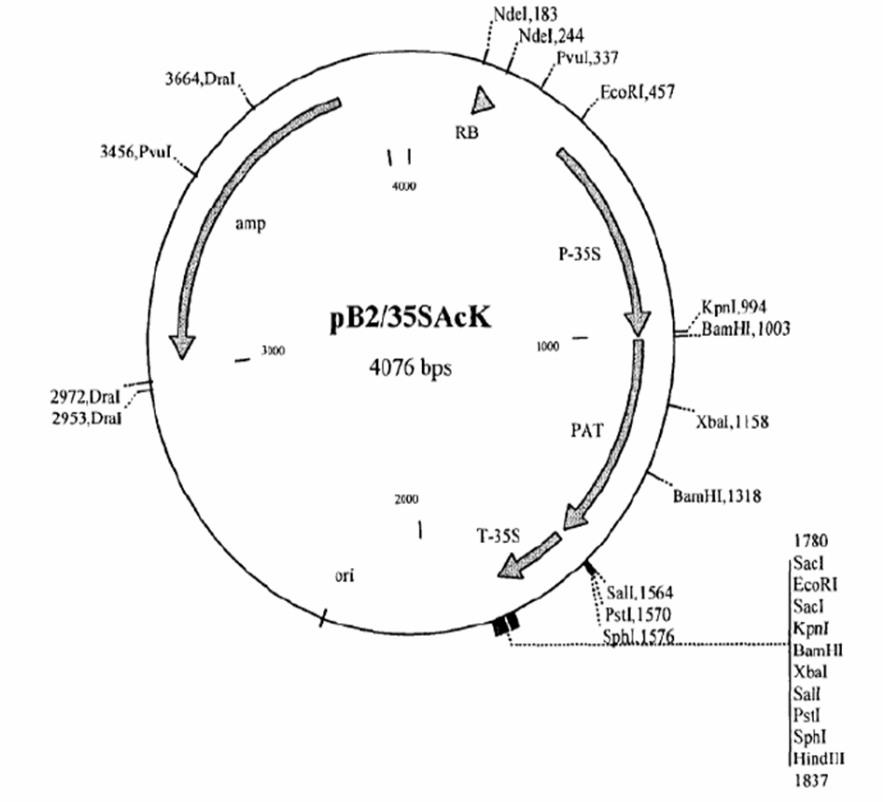

Figure 2.1.2-1. Plasmid map of pB2/35SAcK (Figure 5 in Technical dossier)

Table 2.1.2-1. Genetic elements of the plasmid pB2/35SAcK (Table 3 in Technical dossier)

Position in vector	Genetic element
0001-0188	Sequence of the vector pUC19 (Yanisch-Perron *et al.*, 1985)
0189-0243	**RB: Right Border** fragment of octopine plasmid TiAch5 (Gielen *et al.*, 1984)
0244-0460	Sequence of the vector pUC19 (Yanisch-Perron *et al.*, 1985)
0461-1003	**P35S: promoter** from Cauliflower mosaic virus from the vector PDH51 (Pietrzak *et al.*, 1986)
1004-1011	Synthetic polylinker derived sequences
1012-1563	*pat:* Synthetic *pat* gene (amino acid sequence from *Streptomyces viridochromogenes*) (Strauch *et al.*, 1993)
1564-1581	Synthetic polylinker derived sequences
1582-1784	**T35S: terminator** from Cauliflower Mosaic Virus from the vector pDH51 (Pietrzak *et al.*, 1986)
1785-4076	Sequence of the vector pUC19, including the polylinker (pos. 185-1843), the **ori** (origin of replication) at position 2257 and the ***β-lactamase* (*bla*) gene** (pos. 3876-3016) (Yanisch-Perron *et al.*, 1985)

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

The right border repeat, RB, is a fragment of the octopine plasmid TiAch5 and facilitates the incorporation of the T-DNA to the receiving genome. The modified *pat* gene is derived from the bacterium *Streptomyces viridochromogenes*, a gram positive sporulating soil bacterium. The modified *pat* gene encodes the enzyme phosphinothricin acetyl transferase (PAT) which confers tolerance to glufosinate-ammonium based herbicides by acetylating glufosinate into a non-phytotoxic metabolite. The 35S promoter and 35S terminator from CaMV are derived from the vector PDH51, and direct constitutive expression of the *pat* gene and termination of transcripts, respectively. *β-lactamase* (*bla*) is an antibiotic resistance gene used as a bacterial marker during cloning. Due to cleavage by the restriction enzyme *Pvu*I in the coding sequence of the *bla* gene, it is not functional in soybean A5547-127. The genetic elements are summarised in Table 2.1.3-1.

Table 2.1.3-1. Genetic elements of Plasmid pB2/35SAcK inserted into the plant genome (Table 4 in Technical dossier)

Symbol	Definition	Source	Size (bp)	Reference	Function
	Sequence of the vector pUC19		188	(Yanisch-Perron *et al.*, 1985 M-141342-01-1)	Vector backbone
RB	Right border repeat	Fragment of octopine plasmid TiAch5	55	(Gielen *et al.*, 1984 M-147629-01-1)	*Cis*-acting element for T-DNA transfer
	Sequence of the vector pUC19		217	(Yanisch-Perron *et al.*, 1985 M-141342-01-1)	Vector backbone
P35S	Promoter	Cauliflower mosaic virus from the vector PDH51	543	(Pietrzak *et al.*, 1986 M-131628-01-1)	High level constitutive expression
	Polylinker sequence	Synthetic	8		Plasmid cloning site
pat	Synthetic *pat* gene	Synthetic (amino acid sequence from *Streptomyces viridochromogenes*)	552	(Strauch *et al.*, 1993 M-209751-01-1)	Herbicide tolerance and selectable marker Stop signal
	Polylinker sequence	Synthetic	18		Plasmid cloning site
T35S	Terminator	Cauliflower Mosaic Virus from the vector pDH51	203	(Pietrzak *et al.*, 1986 M-131628-01-1)	Stop signal
ori and *amp* (=*bla*)	Sequence of the vector pUC19, including the polylinker (pos. 185-1843), the ori (origin of replication) at position 2257 and the ***β-lactamase* (*bla*) gene** (pos. 3876-3016)		2292	(Yanisch-Perron *et al.*, 1985 M-141342-01-1)	Bacterial origin of replication and bacterial selection marker

2.2 Information relating to the GM plant

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

Soybean A5547-127 contains one functional copy only of the *pat* gene cassette, at a single integration site (single locus). The *pat* gene encodes the enzyme phosphinothricin acetyl-transferase (PAT) which metabolises glufosinate to an inactive, acetylated derivative, thereby conferring tolerance to glufosinate-ammonium herbicides (Freyssinet, 2002a).

The native bacterial *pat* gene has a high G:C content, which is not typical of plant genes. To improve expression of *pat* in soybean A5547-127, a synthetic version with a lower G:C content was therefore constructed for the development of A5547-127. This modified *pat* gene has approximately 70% DNA sequence identity with the native *pat*. According to the applicant the modification did not alter the encoded amino acid sequence of the PAT protein.

2.2.2 Information on the sequences actually inserted or deleted

Molecular analyses were conducted to determine the nature, number, integrity and stability of the DNA insert in soybean A5547-127. Genomic DNA was analysed 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 one integration site/locus). The DNA insert in A5547-127 has a length of 3436 bp and its sequence is described in its entirety in Berghman & De Beuckeleer (2002b; updated in 2009).

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

Determination of the inserted sequences in soybean A5547-127, showed the presence of one copy of the *pat* gene cassette, as well as truncated parts of the *bla* gene at the 5' and 3' ends of the insert. According to the applicant, the integration of the insert has occurred at a single locus only. This is supported by the inheritance patterns of soybean A5547-127, described in section 2.2.4.

The Southern blot analyses were conducted on isolated genomic DNA from leaf tissues of soybean A5547-127 and control plants, digested with the restriction enzymes *EcoR*I, *Sph*I, *Nco*I/*Hind*III, *Hind*III, *BamH*I, *EcoRV*, and *Dra*I. Genomic DNA from the nontransgenic parent cultivar A5547, and A5547 + plasmid pB2/35SAcK, were used as negative and positive controls, respectively. Radioactive labelled *pat* and *bla* probes were used in the analyses (De Beuckeleer and Botterman, 1997a). Further Southern blot analyses were performed by the applicant in 2009 (upon request from EFSA) with additional probes in order to cover the entire sequence of plasmid pB2/35SAcK. The new additional probes were: 1) a probe between the *bla* and P35S sequences and 2) a probe between T35S and *bla* (De Pestel, 2009c).

According to the applicant the analyses show that only one copy of the *pat* gene is integrated into the plant genomic DNA, in addition to the two truncated parts of the *bla* gene, one on each side of the *pat* gene cassette. The two *bla* fragments are integrated in the same orientation, but reverse to the orientation of the *pat* gene cassette, and do not constitute an intact *bla* gene (Figure 2.2.2.1-2). A more detailed description of the results can be found in the Technical dossier, De Beuckeleer & Botterman (1997a), Berghman & De Beuckeleer (2002b), and De Pestel (2009c).

Figure 2.2.2.1-1 shows a schematic representation of the insert in soybean A5547-127 including restriction sites and the original probes. Figure 2.2.2.1-2 shows the organisation and orientation of the *bla* fragments.

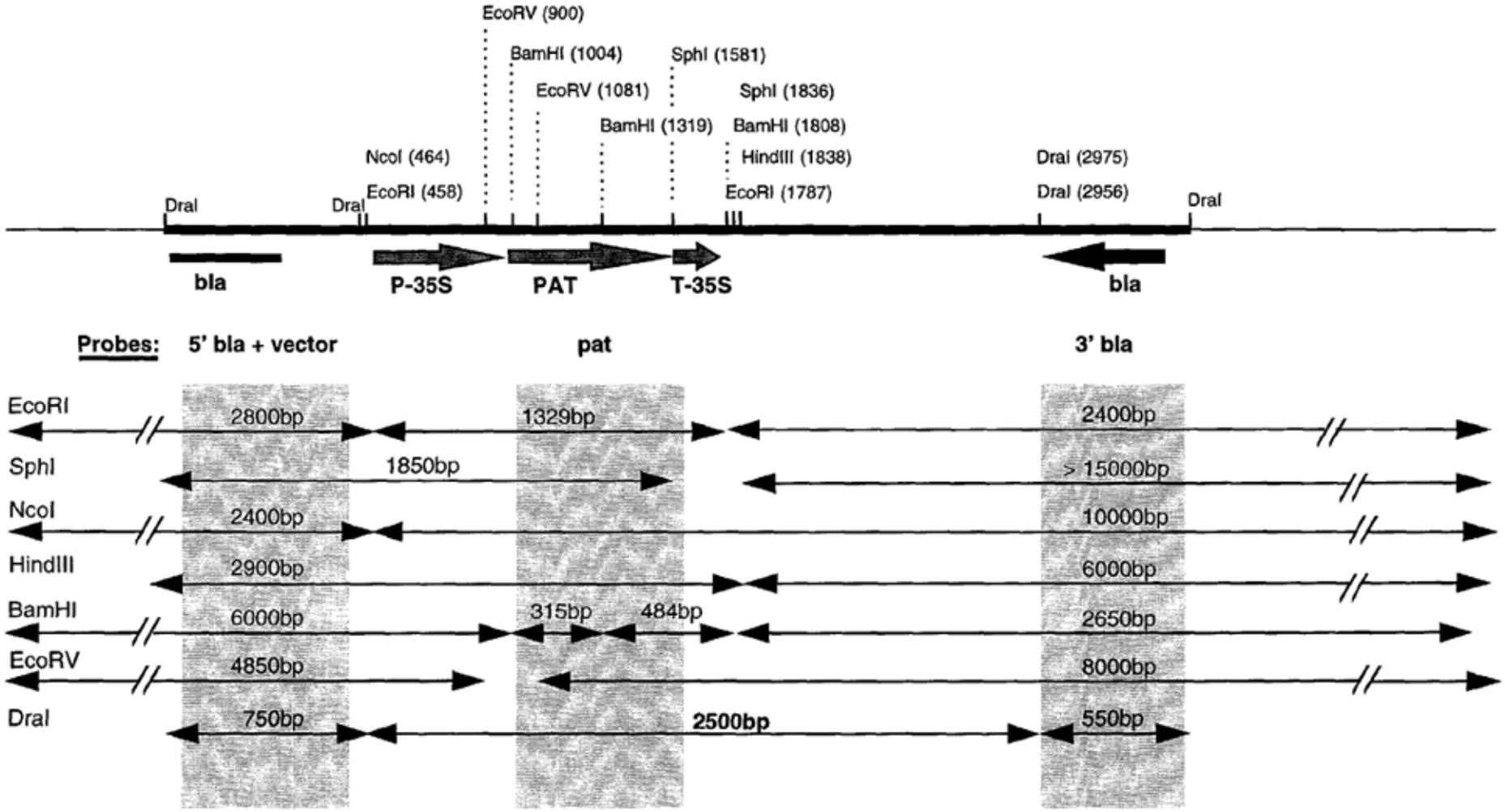

Figure 2.2.2.2-1. Schematic representation of the insert sequence in soybean A5547-127, with restriction sites used in the Southern blot analyses (Figure 11 in Technical Dossier).

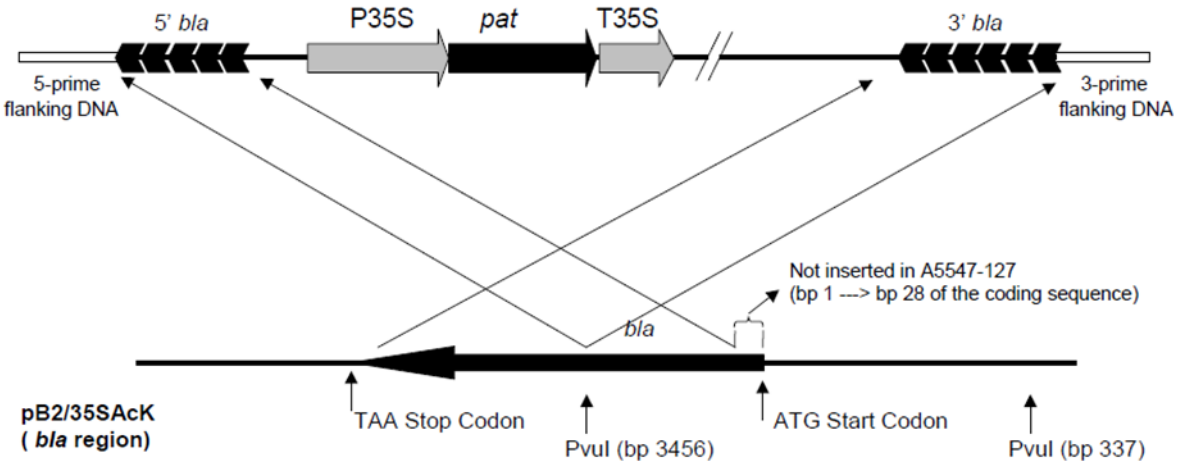

Figure 2.2.2.1-2. Schematic representation of the insert sequence in soybean A5547-127, showing the organisation of the *bla* sequences (Berghman & De Beuckeleer,. 2002b).

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

In the scientific review by EFSA of application EFSA-GMO-NL-2008-52, additional information was requested by the EFSA Molecular Characterisation Working Group on 5 December 2008. Updated information was submitted by the applicant on 15 April 2009.

This update included extended 5' flanking sequences, from 323 bp to 1049 bp. Analysis of the extended sequences showed that these were identical to the already known 323 bp, and to the extended flanking sequences determined at the pre-insertion locus (De Pestel 2009a).

2.2.2.3 Size and function of deleted region(s)

According to the applicant, analyses described in a study by Habex (2008) with the thermal asymmetric interlaced PCR method (TAIL-PCR), showed no unintended insertion or deletion at the integration site in soybean A5547-127.

2.2.3 Information on the expression of the inserted sequence

PAT protein levels have been measured in samples of leaves, stems, and roots of soybean A5547-127, from greenhouse trials conducted in Belgium (De Wulf and De Pestel, 2007). Plants were sprayed with glufosinate-ammonium herbicide (Liberty®) before sampling at two different growth stages V3 (three unfolded trifoliolate leaves) and V8 (eight unfolded trifoliolate leaves). Samples of the unsprayed A5547 parental line were also collected at these two stages. PAT protein was quantified by ELISA.

The average levels of PAT protein in the three tissues were on a fresh weight (fw) basis: 18.40 µg/g (V3) and 26.22 µg/g (V8) in leaf, 39.18 µg/g (V3) and 13.85 µg/g (V8) in stem, and 8.16 µg/g (V3) and 3.60 µg/g (V8) in root. The results show a significant difference in PAT levels between the different plant tissues, as shown in Table 2.2.3-1.

Table 2.2.3-1. Summary of PAT protein levels in leaves, stems, and roots of soybean A5547-127 (Table 10 in Technical dossier)

Matrix	PAT protein					
	PAT content (µg/g fresh weight) ± SD		TEP[a] contents (mg/g fresh weight) ± SD		PAT as % of TEP	
	V3[b]	V8[c]	V3	V8	V3	V8
Leaf	18.40 ± 6.50	26.22 ± 9.87	8.43 ± 1.40	3.65 ± 1.12	0.22	0.73
Stem	39.18 ± 3.04	13.85 ± 6.12	4.75 ± 0.35	1.84 ± 1.05	0.83	0.84
Root	8.16 ± 2.50	3.60 ± 0.42	2.53 ± 0.40	1.20 ± 0.38	0.32	0.32

a- Total Extractable Protein
b- Growth stage V3 three unfolded trifoliolate leaves
c- Growth stage V8 eight unfolded trifoliolate leaves

PAT levels have also been measured in grain samples of Soybean A5547-127 grown in field trials in USA in 1999. Soybean A5547-127 and the non-GM counterpart A5547 (negative control) were planted in a total of nine plots: three plots of A5547, and six plots of soybean A5547-127. Three out of the six plots of A5547-127 were sprayed twice with Liberty ® - herbicide, at a dose of 392 g of active ingredient per hectare (ai/ha) (Shillito, 2003). The levels of PAT protein in grain were 17.5 and 20.2 µg/g fw for sprayed and unsprayed soybean A5547-127, respectively. In hulls the corresponding levels were 9.5 and 11.4 µg/g. PAT protein was also detected in toasted and untoasted defatted meal and soybean isolate at 0.069, 0.013 and 0.081 µg/g, respectively from sprayed soybean A5547-127, and 0.105, 0.035 and 0.041 µg/g from unsprayed soybean A5547-127. No PAT protein (<4 ng/g) was detected in crude lecithin, refined oil, and refined bleached and deodorised oil. The PAT protein constitutes 0.0048% - 0.0056% of the crude protein in soybean grain, and 0.0037%-0.0048 % in hulls. To determine potential expression of the *bla* sequences in soybean A5547-127, the applicant has performed a Northern Blot analysis. Plant RNA was extracted from leaf, root and stem tissues, separated according to size and transferred to a membrane. The membrane was probed with radioactive labeled anti-sense *bla* RNA and measured by autoradiography. *In vitro* synthesised sense *bla* RNA served as the positive reference substance. The analysis detected no *bla* expression in the tested plant tissues (De Beuckeleer & Botterman, 1997b).

2.2.3.1 Part of the plant where the insert is expressed

Production of the PAT protein is expected to occur throughout the plant since the 35S promoter (P35S) from the CaMV is considered to drive constitutive expression. However, there is some natural variation in promoter activity between cell types, supported from the protein levels reported (see 2.2.3 and Table 2.2.3-1).

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

According to the applicant, three new junctions were created during the transformation of soybean A5547-127, two located at the flanking genomic host DNA, and one inside the insert. Analyses of these junction regions identified and characterised eight newly created open reading frames (ORFs 1-8) in 2007 (Vandermarliere and De Pestel, 2007). These analyses showed a theoretical possibility that ORF-5 could lead to the production of a newly created chimeric protein since it apparently has all the regulatory elements necessary to initiate transcription and translation. The other ORFs do not have all the regulatory elements, required for initiating transcription, and were therefore considered by the applicant as highly unlikely to produce any proteins or polypeptides. The putative ORF - translated amino acid sequences were compared with sequences of known toxins and allergens in the Uniprot-Swissprot, Uniprot-TrEMBL, PIR, DAD, NRL-3D, GenPept and Allergen databases, by using BLASTP or FindPatterns algorithms (Capt, 2007a). According to the applicant none of the eight sequences presented any significant sequence identity with known toxins or allergens. However, the ORF-7 sequence showed significant homologies to known antibiotic resistance protein fragments. The observed similarities were with beta-lactamase or beta-lactamase precursors from various origins. Beta-lactamase is the enzyme coded by the *bla* gene as previously described. The ORF-7 sequence showed significant homology with the 5' sequences of the *bla* fragment.

Upon request by the Molecular Characterisation Working Group in EFSA, the applicant provided an updated bioinformatics analysis on ORFs, their potential expression, and new homology searches with databases over known toxins and allergens in 2009. In this analysis, the ORFs were defined as regions between start and stop and between two stop codons with a minimum size of three amino acids, not limiting their length, spanning the three junction regions formed in soybean A5547-127 (De Pestel, 2009b). The new analyses covered a total of 23 ORFs (Figure 1, Appendix I). The deduced amino acid sequences of these ORFs were compared to sequences of known toxins and allergens contained in the Uniprot-Swissprot, Uniprot-TrEMBL, DAD, PDB, GenPept and Allergen databases, by using BLASTP or FindPatterns algorithms (Capt, 2009).

According to the applicant, the results show that the potential of producing novel chimeric proteins from the newly created ORFs is very low. None of the ORFs' amino acid sequences showed similarity to known toxins or allergens in the tested databases.

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

The applicant has conducted Southern blot and segregation analyses over different generations to assess the genetic and phenotypic stability of soybean A5547-127.

2.2.4.1 Genetic stability of the insert in soybean A5547-127

In a study by De Beuckeleer (1998), Southern blot analysis was used on DNA extracts from leaf samples of three successive generations (R3, R4 and R5) of soybean derived from A5547-127. Genomic DNA from non-transgenic soybean A5547, and plasmid pB2/35SAcK were used as controls. The DNA samples were subjected to digestion with *Hind*III and *Nco*I, both enzymes having one restriction site within the transforming plasmid. The *pat* cassette sequence (*EcoR*I fragment of 1329 bp of plasmid pB2/35SAcK) was used as probe in the analysis. The probe hybridised with the plasmid and upstream plant DNA sequences when the samples were digested with *Hind*III, and with plasmid and downstream plant DNA sequences when digested with *Nco*I. The results of the analysis showed no differences in banding patterns between the samples, indicating genetic stability of the insert over at least three generations of soybean A5547-127.

2.2.4.2 Phenotypic stability of the glufosinate-ammonium tolerant trait in A5547-127

The applicant has assessed the phenotypic stability of soybeans derived from event A5547-127 by evaluating the inheritance patterns of glufosinate-ammonium tolerance through successive generations (Van Wert,. 1998). The original (R0) hemizygous (*pat*/-) transformant plant was first self-pollinated to produce R1 progeny seeds, consisting of homozygous (*pat*/*pat*), heterozygous (*pat*/-) and homozygous non-transgenic (-/-) seeds (expected ratio of 1:2:1, respectively). The R1 progeny seeds were subsequently planted and the R2 plants sprayed with glufosinate-ammonium.

R2 seeds from the R1 plants tolerant to glufosinate-ammonium (pat/pat or pat/- plants) were retained and planted in a plant to row fashion (i.e. rows were planted with seeds taken from only one plant).

If the *pat* gene was inherited as a single dominant gene, it would be expected that the R2 plants in 1/3 of the rows would be fully tolerant to glufosinate-ammonium (*pat*/*pat*) and 2/3 of the rows would consist of both fully tolerant and partially tolerant plants, as well as plants sensitive to glufosinate-ammonium (*pat*/*pat*, *pat*/-, and -/-). The results show that 10 rows were fully tolerant and 21 rows were partially tolerant to glufosinate-ammonium (Table 1, Appendix I). Of the plants in the partially tolerant rows, it would be expected that ¾ of the plants would be tolerant to glufosinate ammonium and ¼ would not. Progeny (R3) from most rows segregated in a 3:1 fashion with respect to glufosinate- ammonium tolerance. According to the applicant these results were somewhat affected by an infestation with white grubs (*Phyllophaga* spp.) as indicated by deviations in rows 9, 14, 25, 29 (Table 1, Appendix I). However, the overall results show an expected distribution of glufosinate-ammonium tolerant to sensitive plants according to Mendelian laws of inheritance, consistent with a single dominant *pat* locus.

2.3 Conclusion

The applicant has provided sufficient analyses to characterise the DNA inserts, number of inserts, integration sites and flanking sequences in the soybean genome. The results show that one full length functional copy of the *pat* gene is present in the soybean A5547-127 genome. Similarity searches in 2007 and 2009, with databases of known toxins and allergens did not indicate any 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 several generations, and consistent with the observed phenotypic characteristics of soybean A5547-127. The VKM GMO Panel concludes that the molecular characterisation of soybean A5547-127 does not indicate a safety concern.

3 Comparative assessments

3.1 Production of material for comparative assessment

For compositional studies, A5547-127 soybean was compared to the commercial non-transgenic parental variety A5547 (conventional counterpart; control), which is grown in the US because of its desirable agronomic performance. Compositional and nutritional analyses were performed on the raw agricultural commodity soybean seed. The seeds were grown at 16 field trial sites in the USA in the years 1999, 2000 and 2006 (Table AII-1).

The plants in this study were grown in a randomised block design. The growth conditions were typical of production practices for cultivars of an intermediate plant type (belongs to maturity group V), which are well suited for growing in the geographic area generally encompassing the Mid-southern growing regions of the United States (North Carolina, Florida, Georgia, Mississippi and Arkansas). The A5547 background combines high yield, good standability, excellent emergence and tolerance to some of the leaf and stem diseases. The transgenic A5547-127 was in addition to A5547, also compared to unrelated non-transgenic soybeans for which information was available in the literature (Table AII-3).

At each test site transgenic soybean A5547-127 was grown at six plots and non-transgenic soybean A5547 was grown at three plots. Three of the transgenic A5547-127 plots were sprayed two times with glufosinate ammonium herbicide (Liberty Link), while the other three plots were untreated. The fields were sprayed twice at the equivalent of 500 g of active ingredient (ai) per Ha.

The soybean seeds that were processed to various soybean products were grown at site 402 in Arkansas, USA in year 2006 (Technical dossier, Öberdoerfer, 2008). Material from two test plots established in this field trial was used for the processing experiment. One plot was planted with the non-transgenic counterpart soybean variety A5547. On the other plot the transgenic soybean event A5547-127 was grown and sprayed two times with glufosinate ammonium herbicide. The two soybean seed samples were processed into hulls, untoasted meal, toasted meal, protein isolate, crude oil, refined oil, refined, bleached and deodorized oil (food grade oil) and crude lecithin. The processed samples were analyzed for some nutrients (Table AII-2). Soybean protein concentrate, used extensively in formulated feeds for Norwegian salmonid aquaculture, was apparently not analysed.

Statistical analysis

All statistical analysis was performed with SAS version 8.2 (WINDOWS XP). In total 143 soybean seed samples from 16 sites were analysed for 84 components.

Analysis of differences was done by descriptive statistics: For each component mean values, standard deviations, minimum and maximum were calculated by site, by year and overall. A

by-site (or site by site) analysis of differences was performed for each component: analysis of variance (ANOVA) with 95% confidence level. The means have been compared with a t test, at 95% confidence level.

Overall analysis: Field trials are performed at 3 comparable sites in 1999 and 2000 (sites 201-99 and 201-00 (North Carolina), 301-99 and 302-00 (Florida) as well as sites 402-99 and 403-00 (Arkansas) and at 3 comparable sites in 2000 and 2006 (sites 403-00 and 402-06 (Arkansas), 404-00 and 404-06 (Arkansas) as well as sites 405-00 and 407-06 (Louisiana) (Table AII-1). The data from these locations were analysed separately for field trials performed in the two years 1999/2000 and 2000/2006 with analysis of variance (ANOVA), 95% confidence level, and a mixed model with the factors TREAT (for treatment) and YEAR treated as fixed effects and SITE (for location) as a random effect omitting interaction terms. Based on the ANOVA model treatment, the significance of differences between A (control) versus B (transgenic not sprayed) and A versus C (transgenic sprayed with glufosinate) were estimated (Technical dossier, Rattemeyer-Matschurat, 2008a). B versus C was apparently not analysed. None of the studies were performed according to EFSA's most recent guidelines (EFSA, 2011) however the studies were carried out prior to the publication of these guidelines.

3.2 Compositional analysis

3.2.1 Field trials performed in 1999, 2000 and 2006

Soybean seeds were collected for compositional analysis. The following components were analysed, the proximate and fibre compounds, the total amino acids, the total fatty acids, the micro-nutrients, such as minerals and vitamins, the isoflavones, and the anti-nutrients raffinose, stachyose, phytic acid, trypsin inhibitors, and lectins,. Table AII-2 lists the components analysed in the raw agriculture commodity seeds, as well as other important components analysed in the processed soybean products. The compounds analysed were those suggested relevant according to the recommendation by OECD (OECD, 2001).

The applicant has compared the compositional data in soybean A5547-127 and A5547 with standard composition data taken from the sources presented in Table AII-3.

Proximate and fiber composition of harvested seeds
In Table AII-4, the mean values from all sites of proximates in seeds are presented, together with standard composition data for soybean. All values are within the reference ranges found in the literature, except for moisture. The moisture content of seeds depends on the post-harvest treatment like drying or storing, which can be carried out under varying conditions. To enable and facilitate the comparison of analytical results, the fresh weight values are always transformed into dry matter values. The by-site analysis of all proximates except moisture resulted in statistically significant differences ($p < 0.05$) between treatments for up to six of 16 sites. For moisture, comparing treatment A (control) versus B (transgenic not sprayed) indicated statistically significant differences for a total of eight of the 16 sites.

When A (control) versus C (transgenic sprayed with glufosinate) were compared, a total of seven out of 16 sites were significantly different (Table AII-5). All values were, however, within the range of values reported for soybeans.

Amino acid composition of harvested seeds

Soybean is considered a good protein source, but compared to other plant protein sources it contains a lower level of the essential amino acid methionine. The measured average levels of amino acids, including methionine, from all sites were well within the range of values reported in the literature (Table AII-6). The results of the by-site analysis are shown in Table AII-7. At maximum six of 16 sites, significant differences between sites were found ($p<0.05$; Table AII-7). However, the applicant has not evaluated/provided data regarding the concentration differences of essential and/or limiting amino acids between sites.

Fatty acid composition of harvested seeds

Soybean belongs to the oleic- (C18:1) and linoleic acid (C18:2) rich seed oils. Other main fatty acids in soybean oil are palmitic acid (C16:0), stearic acid (C18:0) and linolenic acid (C18:3). Both linoleic- and linolenic acids are essential fatty acids for humans and mammals. The mean fatty acid contents over all sites are presented together with the literature values in Table AII-8. The measured levels are in good compliance with the composition ranges reported in the literature.

In the by-site analysis, more significant differences between treatments A versus C were found than between A versus B among the sites (Table AII-9). For oleic acid (C18:1), the majority of sites (nine of 16) in the by site analyses of A versus C resulted in statistically significant differences ($p<0.05$), in the comparision of A versus B three of 16 sites were significantly diffrerent. For palmitic (C16:0) and linoleic (C18:2) acids half of the sites differed significantly between the treatments A and C. In the comparison between the treatments A and B, seven of the 16 sites were significantly different for palmitic acid, while for linoleic acid four of 16 sites were significantly different. An overall observation is that a higher number of sites are significantly different when comparing control (A) with genetically modified and glufosinate treated soybeans (C), than when comparing control (A) with genetically modified soybeans not treated with glufosinate (B).

The overall analyses including the year effects confirmed this difference for the respective subsets of data. The estimated differences between the oleic acid mean values for the non-transgenic and the transgenic groups are all lower than 1.5%. Oleic acid does not belong to the essential, poly-unsaturated fatty acids and can be synthesised in mammals. The difference observed is therefore not regarded as having any biological relevance or nutritional impact on human or animal diet. The mean content of palmitic acid overall sites was for treatment A 11.4% and for treatment C 11.6% ((Table II-8). For the essential linoleic acid the values were 51.8% and 50.9% for treatment A and C, respectively. However, the applicant has not evaluated/provided data regarding the concentration differences of essential and/or limiting fatty acids between sites.

Minerals and vitamins in harvested seeds

The overall site averages for mineral and vitamin contents are presented together with the literature values in table AII-10. The measured levels are in good compliance with the composition ranges reported in the literature.

In Table AII-11, the by-site analysis of minerals and vitamins are presented. For a majority of the sites no statistical significant differences between the two treatments of A5547-127 compared to the control A5547 were detected. However, the applicant has not evaluated/provided data regarding the concentration differences of essential and/or limiting minerals or vitamins between sites.

Antinutrients in harvested seeds

Overall mean antinutrient levels across sites for each treatment groups are given in Table AII-12. The two transgenic treatment groups had slightly lower raffinose mean values but slightly higher phytic acid and trypsin inhibitor values than the non-transgenic control group. For raffinose, the estimated difference between treatment means for A5547-127 and its nontransgenic counterpart A5547 was less than 0.1% dry matter on the basis of a 95% confidence interval (results not shown; Technical dossier, Öberdoerfer, 2008). These findings were not confirmed by the comparison between the non-transgenic control (A) and the transgenic seed group obtained from untreated plants (B), nor by the overall analyses including the year effects. The raffinose, phytic acid and trypsin inhibitor mean values for all three treatment groups were within the reference ranges.

For all anti-nutrient compounds except raffinose, few significant differences were identified in the by-site analysis, although more were seen in A vs. C than A vs. B. For the anti-nutrient raffinose, the statistical evaluation resulted in findings of significant differences in the comparison between the A and C treatment groups at the majority of test sites (Table AII-13). For nine out of sixteen sites, the t-tests resulted in significant differences ($p<0.05$) between treatment A and C, with slightly lower values of raffinose in the the GM A5547-127 than in the control. However, this was not confirmed by the comparison between raffinose data obtained from treatment A and B seed samples. Slightly higher mean phytic acid and trypsin inhibitor values were observed for transgenic groups B and C. However, soybean seeds are soaked, heat-treated, and/or fermented before consumed by humans or animals. This lowers the contents of many antinutrients to levels that do not cause discomfort or alter nutrient digestibility. The practical importance for production animals may be more relevant, and should be evaluated on a case-by-case basis.

Isoflavones in harvested seeds

Soybeans contain a number of isoflavone compounds, reported to have both beneficial and adverse biological effects at higher intake levels. In the plant, they occur as glucosides, acetylglucosides, or malonylglucosides, but they are commonly reported as aglycones. The over all site average contents of isoflavones are presented together with the literature values in table AII-14. Whereas no statistically significant difference between soybean A5547-127 (sprayed or non-sprayed with glufosinate-ammonium containing herbicides) and the conventional counterpart was found for glucosides, mean levels of the daidzein, genistein,

and glycitein aglycone equivalents were reduced in soybean A5547-127 compared to the control A5547. The measured levels are in good compliance with the composition ranges reported in the literature (Table AII-14).

In Table AII-15, the by-site analysis is presented. Statistically significant differences ($p<0.05$) between treatments was observed at maximum five of 16 sites. For a majority of the sites, no statistical significant differences between the two treatments of A5547-127 compared to the control A5547 were detected.

3.2.2 Processed soybean product compositions from seeds harvested in the field trials from 2006

In addition to the compositional studies on soybean seeds presented above, the applicant compared the composition of processed products of GM soybean A5547-127 (unsprayed and sprayed with glufosinate-ammonium containing herbicides) with that of the conventional counterpart (Technical dossier, Öberdoerfer, 2008). Products analysed were hulls, untoasted defatted meal, toasted defatted meal, refined bleached and deodorised oil, and soy isolate (Table AII-2). All soybean products were analysed for proximates except the oil and lecithin samples. Hulls were analysed for proximates only. Untoasted and toasted meals were analysed for proximates, isoflavones, anti-nutrients, amino acids and fatty acids. Protein isolate was analysed for proximates, anti-nutrients and fatty acids. Crude and food grade refined oil were both analysed for tocopherols and fatty acids (Table AII-2).

The proximate, fibre and total amino acid contents of the meal derived from A5547-127 are comparable to the ones determined in the A5547 meal sample. In general, the analysed samples show the same values as commercial soybean meal. Only the crude fat contents fell short of the reported range. The results from the anti-nutrient and isoflavone analyses (see Tables AII-12 and AII-14) indicated no major differences except for daidzin, glycitin, genistin and total isoflavones and all isoflavone contents were within the ranges reported in the literature.

Although some data from products processed from soybean A5547-127 and the conventional counterpart did not fall within the range of values reported in the literature, those literature data could have been obtained from products produced by processing technologies that differed somewhat from those used in the above-mentioned study. The composition of the processed products did not raise any safety concerns.

3.3 Agronomic traits and GM phenotype

The application EFSA/GMO/NL/2008/52, covering authorisation of soybean A5547-127 for all food and feed uses, include data on agronomic and morphological characteristics from field trials in the USA in 1996, 1997, 2006 and 2007. The field trials in the 1990´s (19+48 sites) were made as part of the event evaluation process, and the characteristics evaluated included primarily those important for varietal registration (Van Wert, 1998). A two year field

trial performed on 8 sites in 2006 and 2007, respectively, were conducted to demonstrate the morphological and agronomic equivalence of soybean A5547-127 to the corresponding near-isogenic cultivar A5547 (Kowite, 2007, 2008). The trials were comparable and allowed a by-year analysis of the obtained data.

At each field trial site, soybean A5547-127 and the conventional counterpart (A5547) were planted following a randomised complete block design with three replicates per site. The conventional counterpart was compared with soybean A5547-127 not treated with glufosinate-ammonium containing herbicides and soybean A5547-127 treated two times with the herbicide.

No commercially available soybean varieties were included in the field trials. According to the updated EFSA guidance on risk assessment of food and feed from genetically modified plants (EFSA 2011a), there should be at least three appropriate non-GM reference varieties of the crop that have a known history of safe use at each site. The test of equivalence is used to verify whether the agronomic, phenotypic and compositional characteristics of the GM plant fall within the normal range of natural variation. Such a range of natural variation is estimated from a set of non-GM reference varieties with a history of safe use (EFSA 2011b) and therefore allows comparisons of the GM plant with a similar plant produced without the help of genetic modification and for which there is a well-established history of safe use. These requirements were, however, not in place at the time of submission.

Extensive data on qualitative and quantitative characteristics were evaluated in these field trials; emergence, stand count, plant vigour and health rating, days to 50% flowering (flowering date), flower morphology and colour, pubescence colour, pod colour, hilum colour and shape, canopy architecture, leaf shape, plant height, susceptibility to pests and diseases, pollen viability and germination, and yield. A summary of the mean values and by-site analysis of the morphological and agronomic data are given in Tables AII-16 and AII-17, respectively. Due to lack of variation, analysis of variance methods were not appropriate for analysing the characteristics related to plant health.

Statistical analysis across all locations revealed no statistically significant differences in the quantitative traits 50% plant emergence, stand count, plant vigour and yield. There were no significant differences in the parameters flowering date, plant height and days to maturity between soybean A5547-127 and A5547 at the majority of the field trial sites. Similarly, no overall difference between these two soybean varieties was found in the qualitative characteristics flower colour, pubescence colour, pod colour, hilum colour, canopy architecture, leaf shape and susceptibility to pests and diseases.

For stand count, a statistical difference between the soybean A5547-127 and its comparator was observed when GM soybean was sprayed with glufosinate-ammonium containing herbicides. The differences were detected at two of the sixteen individual field trial sites only. No consistent trend was observed across locations and years. For the other parameters observed, no effects of the herbicide regimes were revealed.

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 significant differences exist between soybean A5547-127 and its corresponding control A5547 in the analysis of seeds and various processed food and feed commodities. Any differences observed were within the range of values reported for conventional soybean varietals in the literature. The few observed statistical differences between A5547-127 and A5547 are likely to reflect the natural variability of the analytes since they were within the range of values reported in the literature for conventional soybean varietals. 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 A5547-127 is compositionally, agronomically and morphologically equivalent to its conventional counterpart and other conventional soybean varieties.

4 Food and feed safety assessment

4.1 Previous evaluation by the VKM GMO Panel

In an earlier risk assessment of soybean A5547-127 the VKM GMO Panel concluded that the soybean A5547-127 was nutritionally equivalent to and as safe as conventional soybean varieties (VKM 2008).

4.2 Product description and intended uses

Soybean A5547-127 was first cultivated in the USA in 1998 and Canada in 1999, and subsequently cultivated in Japan in 2006, Brazil in 2010, Argentina in 2011, and Uruguay in 2012. Soybean A5547-127 was commercialised for food and/or feed use in Argentina (2011), Australia/New Zealand (food 2004), Brazil (2010), Canada (2000), Colombia (feed 2012), EU (2012), India (2014), Japan (food 2001, feed 2003), Malaysia (2014), Mexico (2003), Phillippines (2011), Russian Federation (food, 2008, feed, 2007), South Korea (2001) and Taiwan (food 2010).

Soybean A5547-127 has been used in food and feed since 1998. According to the applicant the commercial experience since then has confirmed that the post-harvest production and processing of the genetically modified A5547-127 does not differ from the production and processing of the equivalent foods and feeds originating from traditional soybean. The major soybean commodity products are seeds, oil, meal and protein concentrates/isolates. Soybean protein concentrate is a commonly used feed ingredient in Norwegian salmon feeds (www.mattilsynet.no). Since 2008, NFSA has given four fish feed producers in Norway extended exemption from seeking approval for inclusion of GM products in fish feeds. The exemption applies to processed, non-viable feed products from 19 different GM varieties. In October 2014, this exemption was not extended.

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 used as feed to a limited extent, with forage, hay and hulls primarily used 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 soybean A5547-127 and all food, feed and processed products derived thereof are expected to replace a portion of similar products from traditionally bred/selected soybean.

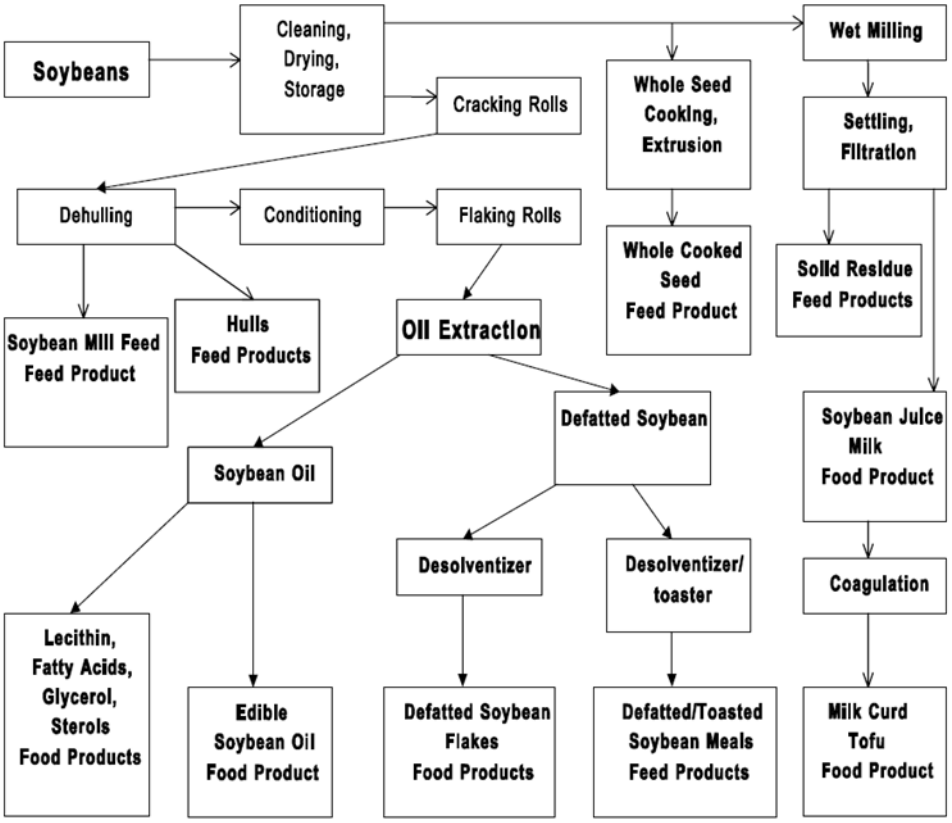

Figure 4.2-1. Processing of soybean, adapted from Waggle and Kolar, 1997, Technical dossier

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. The first step in processing most soybeans is to separate the oil, either by solvent extraction or by expelling. For this, the soybeans are first cracked and de-hulled, then heated to approximately 60 degrees, ground to flakes using rollers, and are then treated with solvent (e.g. hexane) 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 can lead to denaturation and loss of protein function. Heat stability study of soybean A5547-127 performed by the applicant (ref. Eisdail 2002c; Rascle 2009) showed that the PAT-protein was heat stable when incubated up to 30 minutes at 90°C , and slightly degraded when incubated 60 minutes at 90°C.

4.4 Toxicological assessment of soybean A5547-127

The potential toxicity of genetically modified soybean A5547-127 has been studied in animal studies by the applicant. There have been different formulations of the test material. Some tests have been performed with the gene product of the PAT gene, produced in another

organism (E.coli) and purified before use. Other studies have been conducted with the complete GM food/feed. Both formulations are considered relevant for assessing the safety of soybean A5547-127.

In addition to the safety testing conducted by the applicant, a safety testing programme has been conducted on soybean A5547-127 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 A5547-127. 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.

Submitted data by the applicant demonstrated a low expression of the transgenic PAT protein in soybean A5547-127 (approx. 11 µg PAT/g dry matter (dm) in seeds, approx. 77 ng/g in meal (dm) and 14 ng/g toasted meal (dm)). The protein was not detectable in soybean oil (applicant dossier) and showed no meaningful amino acid sequence homology to known toxic proteins (Herouet et al 2005). Also *in vitro* digestion studies using simulated gastric fluid (SGF) and simulated intestinal fluid (SIF), demonstrated that PAT is rapidly degraded under conditions mimicking the stomach and intestine (Esdaile 2002, 2004; Applicant documents). Rapid degradation of the PAT-protein was also observed in pig stomach fluid (pH 1.7 to pH 4.0) and bovine rennet-bag (pH 1.28 to pH 3.18) (Schultz 1993, Applicant document). In bovine paunch (rumen) fluid (pH 7.11) total degradation of PAT-protein was observed after 30 min (Schultz, 1993, Applicant document). Digestion of PAT-protein in ground leaves from transgenic zoysiagrass (Z. japonica Steud.) and in total soluble proteins extracted from zoysiagrass has also been confirmed in studies using SGF digestion assays (Sun et al 2010).

The toxicological assessment is further based on results available from testing in mice, rats and broiler chickens. Studies are grouped according to their objective; acute, repeated dosing, reproductive function, immunotoxicity etc.

Animal studies have utilised various formulations of soybean A5547-127, such as purified PAT protein, protein concentrate from soybean A5547-127 or whole GM food/feed. Protein concentrate is about 70% soy protein and is basically defatted soy flour without the water-soluble carbohydrates and ethanol-soluble antinutritional factors. Isolated soy proteins are obtained by extracting the soluble proteins with water at pH 8-9, precipitating at pH 4.5, centrifugation, washing, redispersing, and drying. Concentrates and isolates are widely used as functional or nutritional ingredients in a wide variety of food products, mainly in baked foods, breakfast cereals, and in some meat products.

The formulations used are indicated under each study presentation.

4.4.1 Acute toxicity testing

An acute intravenous study with the PAT protein has been performed by the applicant to assess potential toxicity in mice (Kennel, 2003, Technical dossier). The PAT protein was produced in *E. coli*, purified (>90 %), and administered i.v. to mice in a single dose. The OECD TG 420 fixed dose guidance document (OECD 2001) was used as a basis for assessing their potential acute toxicity. The number of animals, the use of a single sex and the general protocol details complied with this OECD guidance document. Three groups of mice (each 5 females) were intravenously injected with 1 or 10 mg per kg body weight with either PAT protein, aprotinin or melittin, respectively. All animals were observed for clinical signs daily for 15 days after dosing. Microscopic examination of internal organs was carried out at necropsy. No treatment-related adverse effects were observed in mice administered the PAT protein at the highest dose tested, i.e. 10 mg/kg bw.

The results showed that mice treated i.v. with PAT protein or apoprotein at 10 mg/kg body weight showed no signs of systemic toxicity, whereas all mice treated i.v. with melittin at the same dose died within 5 minutes.

Acute toxicity testing following i.v. application 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 now discourages the use of acute toxicity studies in risk assessments of GMOs (EFSA 2011).

4.4.2 Repeated dose toxicity testing

The potential toxicity of the PAT-protein expressed in soybean A5547-127 has been assessed in a repeated dose toxicity study (feeding trial) in rats (Pfister et al. 1999).

The applicant provided a 14-day repeated dose feeding study in which groups of 5 Wistar rats (HanIbm:WIST) of each sex were given a low protein diet containing 0, 0.5 or 5.0% (w/w) (group 1, 2 and 3, respectively) of a lyophilized powder of the PAT protein (Pfister et al., 1999). Group 1 were fed standard rat diet, group 4 were fed non-GM soybean protein. The highest dietary inclusion resulted in a daily dose of ca. 7.6 and 7.9 g/kg body weight for males and females, respectively. The total protein levels in the diets for the control and low-dose groups were adjusted with soya protein from commercial non-GM soybeans to reach a level comparable to that in the diet for the high-dose group. An additional group was fed a standard rodent diet. There were no mortalities, and no relevant influence on food consumption and body weight development induced by the treatments. According to the applicant there were no adverse effects except for statistically significant increases in blood cholesterol levels (males of groups 2 (0.5% PAT), 3 (5% PAT) and group 4 (without PAT)) and phospholipid levels (females of group 3 and males of groups 2 and 3).

At the end of the treatment period haematology, clinical chemistry and urine analysis were performed, organ weights determined, and macroscopic and histopathology examinations of selected organs and tissues were carried out.

This repeated dose toxicity study in rats gave no indications of adverse effects attributable to the PAT protein up to the highest dose tested, which was 5% in the diet, corresponding to 7.9 g/kg bw.

The VKM GMO-Panel notes that this repeated dose feeding study is performed with only 14 days of exposure contrary to the recommended 28 days.

4.4.3 Studies on Allergenicity

4.4.3.1 Assessment of allergenicity of the newly expressed protein

The applicant has assessed the allergenic potential of the PAT protein by bioinformatic comparison of the amino acid sequence of the PAT protein produced in A5547-127 with known database sequences of IgE-dependent allergens, as well as the evaluation of the stability of the protein in an in vitro gastric digestion model.

The *pat* gene originates from *Streptomyces viridochromogenes*, a soil microrganism that is not known to be allergenic. The bioinformatic analyses were conducted to assess the potential for allergenicity of the PAT protein sequence (Hérouet, 2004b, applicant dossier). The total amino acid sequence of PAT was compared with epitopes of known IgE-dependent allergens. In addition search was performed of potential N-glycosylation sites with known consensus sequences that may be found in allergenic proteins. A search was also performed on all protein sequences presented in reference databases, i.e. allergen, gliadin and glutenin sequences database (AD4) assembled from publicly available databases (GenBank, EMBL, PIR, NRL3D version of RCSB PDB and SwissProt) and from current literature (Uniprot-TrEMBL, Uniprot-SwissProt, PIR, NRL_3D, DAD and GenPept).

The applicant has also carried out an amino acid sequence homology study of the complete amino acid sequence of PAT with protein sequences of known toxins and allergens in the databases NRL-3D, PIR, DAD, GenPept, Uniprot_TrEMBL and Uniprot-SwissProt.

The amino acid sequence of the PAT protein was compared to all sequences in the databases with the FASTA sequence alignment tool. The extent of each similarity was evaluated by visual inspection of the alignment, the calculated percent identity and the E score for that alignment. Additionally, the PAT amino acid sequence was also screened against the allergen database with an algorithm that scans for a window of eight linearly contiguous amino acids. Such identities might indicate the presence of potentially cross-reactive allergenic epitopes. The results of this bioinformatics search indicate that the PAT protein shares no structurally significant sequence similarity to sequences within the allergen

databases and no immunologically significant sequence similarity to protein associated with IgE-mediated allergies or to proteins associated with coeliac disease.

European and Asian patients allergic to soybean and/or other foods do not express IgE that specifically bind the purified PAT protein (Chang et al., 2003; Batista et al., 2005; Kim et al., 2006a, 2006b; Hoff et al., 2007). The purified PAT enzyme also did not result in pronounced change in histamine release or cytokine production in sensitised peritoneal mast cells or unsensitised but antisera-labelled mast cells cultivated *in vitro* (Chang et al., 2003). It is considered that these studies further confirm that the expressed PAT protein in A5547-127 is unlikely to be allergenic.

4.4.3.2 Assessment of the allergenicity of the whole GM plant

In the submitted dossier the applicant has assessed the allergenicity of the whole GM plant as follows: Soybean is known to cause food allergies in certain individuals (Lehrer, 1997, applicant dossier). Therefore, an assessment of the endogenous IgE-dependent allergens in A5547-127 and traditional soybean has been conducted with Radioallergosorbent Test (RAST). The applicant screened soybean seed extracts from A5547-127 as well as from the non-transgenic counterparts against a panel of sera from 16 patients sensitive to soybean protein (Lehrer, 1997). The purpose of the study was to qualitatively and quantitatively compare the endogenous allergens in A5547-127 and the traditional soybean A5547 with the same genetic background as A5547-127. The analysis of the protein extracts prepared from A5547-127 revealed that both the composition and the quantity of proteins detected by RAST were indistinguishable from the results produced with A5547 (the control), demonstrating that the production of the PAT protein in A5547-127 does not cause any change in the composition of the allergenic proteins endogenous to soybean.

The Food/Feed Safety Working Group in EFSA requested additional clarification and information from Bayer regarding the testing of seed extracts from A5547-127 in the experiments described above. The response from Bayer (Bayer Cropscience Response, 2010) provided clinical characteristics relevant for the selection of the 16 patients whose sera were used for the RAST experiments, along with the results of RAST tests performed on individual patient's sera in terms of specific IgE values and not as percentage of binding as presented by Lehrer (1997). EFSA concludes that whole-product testing on sera from soybean-allergic patients showed that the overall allergenicity of soybean A5547-127 is not different from that of commercial soybeans.

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 reported 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 A5547-127.

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 suggest 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 PAT-protein has not been reported to have adjuvant properties.

4.5 Nutritional assessment of GM food and feed

Compositional analyses of soybean A5547-127 indicate nutritional equivalence to the non-GM control soybean with comparable genetic background and to the published range of values in the literature (see 3.2). The nutritional equivalence between soybean and non-GM control soybean has been further shown by the results from feeding studies with broiler chickens (see chapter 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 analysis (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 A5547-127, the estimated average exposure of the European consumer to products of soybean A5547-127 would be approximately 3.4-3.7 g/person/ day (Technical dossier).

In Table 4.5.1-1 the mean intake of soy protein/day for an adult person 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 where milk products were replaced with soy products was composed. 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 A5547-127 meal in the EU would be 21% for broiler chickens, 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 A5547-127, the estimated average exposure of Atlantic salmon (post smolt, 200 g) to products of soybean A5547-127 would be approximately 2 g/fish/day (assuming 3% growth per day and feed conversion ratio of 1).

Norwegian surveillance data show that imported SPC intended for feed production only contains trace amounts of GMO (*e.g.* below 0.9%) (Spilsberg et al., 2014). The DNA specific

targets that are included in the GMO methodology are 35S promoter (p35S), *Agrobacterium* nopalin synthase terminator (tNOS), *ctp2-cp4epsps*, the *bar* gene from *Streptomyces hygroscopicus* and the *pat* gene from *Streptomyces viridichromogenes*.

4.5.2 Nutritional assessment of feed derived from the GM plant

4.5.2.1 Applicant's data for nutritional assessment

Broiler feeding study

The applicant provided a 42-day broiler (Commercial broilers species not given) feeding study using a non-defined commercial strain of broilers and two types of diets (Leeson, 1998). The rapidly growing broiler is considered to be sensitive to changes in nutrient quality in diets, and therefore is often used as a model to assess the wholesomeness of feed.

The study was done to compare the wholesomeness of transgenic soybean line A5547-127 compared to the non-transformed parental soybean lines when fed to rapidly growing broilers. Each treatment consisted of 120 female broilers (6 replicates of 20 birds; replicates in a randomised complete block design). One group of broilers received diets with seeds of heat-treated soybean A5547-127, another group received diets with heat-treated seeds of the conventional counterpart. The starter diets (day 1- 17) contained 18.24%, the grower diets (day 17-31) 14.94% and the finisher diets (day 31-42) 11.69% soybean meal. The crude protein content during the study varied from 22.0% during the starter period, to 20.5% during the grower period, and 19.1% during the finisher period. To achieve this protein concentration, the diet also contained conventional corn and barley.

There was no statistically significant effect (calculated by a T-test) of the test diet containing soybean A5547-127 on body weight (42-day final weight: 2132 g and 2144 g for broilers receiving the conventional counterpart and A5547-127, respectively), body weight gain, feed intake, feed intake/body weight gain or percent mortality. After 42 days, 8 birds were randomly selected from each pen (altogether 48 broilers per treatment) and slaughtered. Birds fed diets containing soybean A5547-127 showed no biologically relevant differences in carcass characteristics (carcass weight, abdominal fat pad weight, deboned breast meat weight, abdominal fat pad weight/carcass weight, and deboned breast meat yield/carcass weight) compared with chickens receiving diets containing seeds of the conventional counterpart (A5547).

These data indicate that soybean line A5547-127 is equivalent to conventional soybean lines in terms of their ability to support the rapid growth of broilers and confirm the results of the compositional analyses.

4.6 Conclusion

A 14-day repeated dose toxicity study with rats fed PAT protein, as well as a nutritional assessment trial with broilers fed diets containing soybean A5547-127 did not indicate any adverse effects. The PAT protein in A5547-127 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 A5547-127 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/2008/52, the environmental risk assessment is concerned with the accidental release into the environment of viable soybean A5547-127 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 contents 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 A5547-127 will increase the potential to establish feral populations. A series of field trials with soybean A5547-127 was conducted by the applicant at several locations in the USA in 1996, 1997, 2006 and 2007 to compare the agronomic performance and field characteristics

of soybean A5547-127 with its comparators (see section 3.3). With the exception of targeted responses to the presence of glufosinate herbicides, the agronomic and morphological field trial data did not show major changes in plant characteristics indicating altered fitness, persistence and invasiveness of soybean A5547-127 plants compared to its conventional counterpart.

In addition to the data presented by the applicant, the VKM GMO Panel is not aware of any scientific reports indicative of increased establishment or spread of soybean A5547-127, or changes to its survivability (including over-wintering), persistence or invasive capacity. Because the general characteristics of soybean A5547-127 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 A5547-127 will not differ from that of conventional soybean varieties.

5.2 Potential for gene transfer

A prerequisite for any 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 A5547-127. 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 A5547-127 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 A5547-127 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 *pat* gene from A5547-127 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, 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 A5547-127 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 A5547-127, 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 A5547-127, 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 A5547-127, 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 A5547-127, 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 A5547-127.

Soybean A5547-127 has no altered survival, multiplication or dissemination characteristics compared to conventional soybean, and there are no indications of an increased likelihood of spread to or establishment of feral soybean plants in the case of accidental release of seeds from soybean A5547-127 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.

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 any possible effects that were not anticipated in the ERA, or that are long-term or cumulative.

No specific environmental impact of genetically modified soybean A5547-127 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 A5547-127.

7 Conclusions

Molecular characterisation

The applicant has provided sufficient analyses to characterise the DNA inserts, number of inserts, integration sites and flanking sequences in the soybean genome. The results show that one full length functional copy of the *pat* gene is present in the soybean A5547-127 genome. Similarity searches in 2007 and 2009, with databases of known toxins and allergens did not indicate any 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 several generations, and consistent with the observed phenotypic characteristics of soybean A5547-127. The VKM GMO Panel concludes that the molecular characterisation of soybean A5547-127 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 significant differences exist between soybean A5547-127 and its corresponding control A5547 in the analysis of seeds and processed food and feed commodities. Any differences observed were within the range of values reported for conventional soybean varietals in the literature. The few observed statistical differences between A5547-127 and A5547 are likely to reflect the natural variability of the analytes since they were within the range of values reported in the literature for conventional soybean varietals. 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 A5547-127 is compositionally, agronomically and morphologically equivalent to its conventional counterpart and other conventional soybean varieties.

Food and feed risk assessment

A 14-day repeated dose toxicity study with rats fed PAT protein, as well as a nutritional assessment trial with broilers fed diets containing soybean A5547-127 did not indicate any adverse effects. The PAT protein in A5547-127 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 A5547-127 is nutritionally equivalent to and as safe as its conventional counterpart and other conventional soybean varieties.

Environmental assessment

Considering the intended uses of soybean A5547-127, 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 A5547-127. Soybean A5547-127 has no altered survival, multiplication or dissemination characteristics compared to conventional soybean, and there are no indications of an increased likelihood of spread to or establishment of feral soybean plants in the case of accidental release of seeds from soybean A5547-127 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 A5547-127 is as safe as its conventional counterpart and other commercial soybean varieties. With the exception of the introduced trait, soybean A5547-127 is nutritionally, morphologically and agronomically equivalent to conventional soybean varieties.

Likewise, the VKM GMO Panel concludes that soybean A5547-127 does not represent a discernible 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 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 herbicide 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.

At present, the potential changes related to herbicide residues of genetically modified plants as a result of the application of plant protection products fall outside the remit of the Norwegian Scientific Committee for Food Safety.

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Appendix I

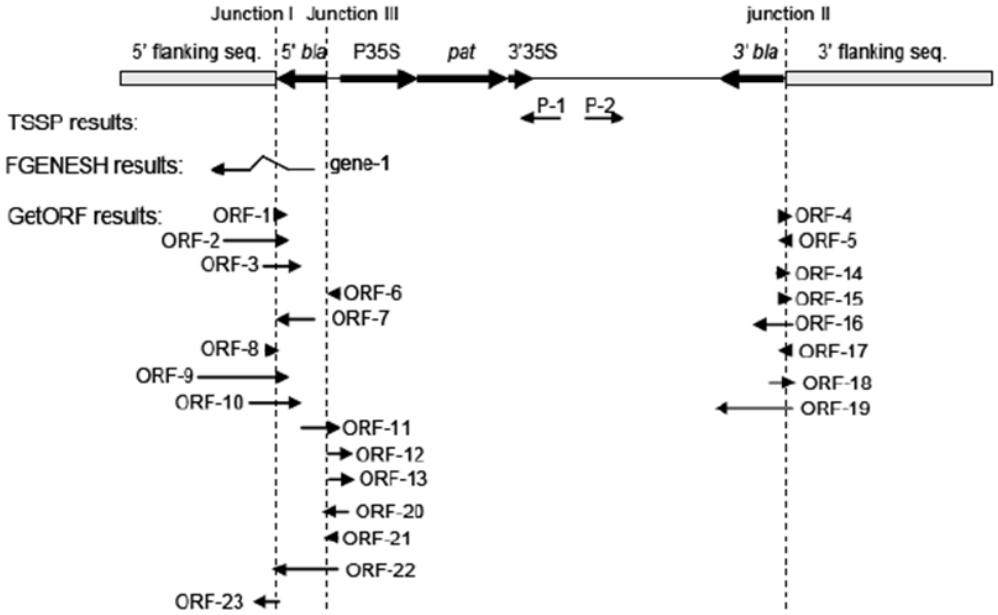

Figure 1. Schematic overview of the newly created ORFs and genes in soybean event A5547-127 (figure 1 in the BayerCropScience response to EFSA, 15 april 2009)

Table AI-1. Segregation data for individuals and rows of progeny of self-pollinated event A5547-127 (Table 14 in Technical dossier).

Comparison	Progeny	Fully Resistant Rows	Partially Resistant Rows	Expected Ratio	$\chi 2^{c}$
all rows	R2[a]	10	21	1:2	0.0

Comparison	Progeny	Resistant Plants	Sensitive Plants	Expected Ratio	χ^{2c}
row 1	R3[b]	66	16	3:1	1.52
row 2	R3	141	36	3:1	1.97
row 3	R3	35	7	3:1	1.84
row 4	R3	50	11	3:1	1.46
row 7	R3	40	7	3:1	2.72
row 9	R3	73	13	3:1	4.8*
row 10	R3	43	8	3:1	2.51
row 11	R3	34	6	3:1	2.13
row 13	R3	34	8	3:1	1.02
row 14	R3	32	3	3:1	5.26*
row 16	R3	41	10	3:1	0.89
row 17	R3	36	10	3:1	0.40
row 21	R3	76	21	3:1	0.52
row 22	R3	47	8	3:1	3.37
row 23	R3	120	31	3:1	1.69
row 25	R3	141	22	3:1	11.68*
row 26	R3	143	53	3:1	0.44
row 27	R3	80	16	3:1	3.56
row 29	R3	119	25	3:1	4.48*
row 30	R3	148	46	3:1	0.23
row 31	R3	34	8	3:1	1.02

[a] entire rows of seeds from R1 plant
[b] individual progeny from heterozygous, partially resistant 21 R2 rows.
[c] No significant difference (p=0.05) for the Chi square goodness-of-fit test for hypothesis of either 1:2 or 3:1 segregation. (Significance at p=0.05 for $\chi 2 > 3.84$, df=1).
* Significant difference found.

Appendix II

Table AII-1. Summary of the field trials performed for compositional and agronomic comparative assessment studies (Technical dossier)

	Growing seasons State / Location / Site nr.			
	1999	2000	2006	2007
Composition	NC/ Pikeville/ 201[a] FL /Molino/ 301[b] MS,/Greenville/ 402[c] AR / West Memphis/401	NC/Pikeville/ 201[a] FL,/Molino/ 302[b] AR/West Memphis/ 403 [c,d] AR/ Newport/ 404 [e] LA/ Rosa/ 405 [f]	GA/ Chula/ 201 AR/ Tillar / 403 AR/ Proctor/ 402 [d -1] AR/ Newport/ 404 [e] LA/ Washington/ 407 [f] MS/ Senatobia/ 405 MS/ Leland/ 406	
Agronomic traits			GA/ Chula/ 201[g] AR/ Proctor/ 402[h] AR/ Tillar / 403[i] AR/ Newport/ 404[j] MS/ Coldwater/ 405[k] MS/ Leland/ 406[l] LA/ Washington/ 407[m] TX/ East Bernard/ 608[n]	GA/ Chula/ 201[g] AR/ Proctor/ 402[h] AR/ Tillar / 403[i] AR/ Newport/ 404[j] MS/ Coldwater/ 405[k] MS/ Leland/ 406[l] LA/ Washington/ 407[m] TX/ East Bernard/ 608[n]

NC-North Carolina; FL-Florida; MS- Mississippi; AR-Arkansas, LA- Louisiana, GA-Georgia; TX- Texas

[-1] Soybean seed production for processed product analysis

Site numbers followed by the same letters indicate sites compared in 1999 and 2000, 2000 and 2006 and 2006 and 2007

Table AII-2. Components analysed in Raw Soybean Seeds and Products derived from Soybeans (Technical Dossier, Oberdoerfer, 2008)

Compound	Raw Seed	Hulls	Meal	Toasted Meal	Protein Isolate	Crude Oil	Food Grade, Refined Oil	Crude Lecithin
Proximate and fibre compounds [a]	X	X	X	X	X [b]			
Ca, P, K, Mg, Na, Fe	X							
Vitamin B1, B2 E, Folic acid	X							
Tocopherols	X					X	X	
Isoflavones	X		X	X				
Stachyose	X		X	X				
Raffinose	X		X	X				
Phytic acid	X		X	X				
Trypsin Inhibitors	X		X	X	X			
Lectins			X	X	X			
Total Amino Acids	X		X	X	X			
Total Fatty Acids	X					X	X	
Phosphatides								X

a Proximates comprise moisture, crude protein, crude fat, ash, total carbohydrates (calculated), acid detergent fibre and neutral detergent fibre.

b Protein isolate was only analysed for crude protein content.

Table AII-3. Sources of Standard Composition Data (from Technical dossier, Oberdoerfer, 2008)

Abbreviation	Source
BG	Belitz, Grosch (1985); Lehrbuch der Lebensmittelchemie; 2nd ed.; Springer Verlag; Berlin, pp 559-572.
Codex	FAO/WHO Food Standards. Codex Alimentarius. 2001. Vol 8. Named Vegetable Oils. Codex Stan 210 from http://codexalimentarius.net/standard-list accessed December 5, 2002.
CRC (1983)	CRC (1983) Handbook of Processing and Utilization in Agriculture, Volume 2, Part 2 - Plant Products. pp 27-32, 39, 41-45, 50, 57-59, 83-89.
CRC (1989)	CRC. 1989. Vaidehi M.P., Kadam S.S. Soybean In: Handbook of World Food Legumes. Vol III. CRC Press Inc. Boca Raton, Florida, USA. Pp 1-21.
Ensminger	Ensminger M.E., et al. (1990) Feeds and Nutrition, 2nd edition, Ensminger Publishing Co.
FSK	Scherz H. and Senser F.; (1994); Food Composition and Nutrition Tables; 5th ed.; CRC Press Boca Raton; Florida; USA.
ILSI	ILSI. 2007. International Life Science Institute - Crop Composition Database - search results version 3.0; Average, minimum and maximum nutrient and anti-nutrient content in soybean seeds. ILSI Crop Composition database. http://www.cropcomposition.org/cgi-perl/search_ora.cgi accessed September 7, 2007
Hui	Hui Y.H., (1992), Encyclopedia of food science and technology, p2389-2396, Vol4, John Wiley & sons Inc. New York
Kakade	Kakade M.L. et al., (1972), Biochemical and nutritional assessment of different varieties of soybeans. J. Agr. Food Chem., p. 87-90, 20
Kellems	Kellems, R.O., Church, D.C. (1998); Livestock Feeds and Feeding; 4th ed.; Prentice Hall; New Jersey.
Liener	Liener I.E., 1994, Implications of Antinutritional Components in Soybean Foods. Critical Reviews in Food Science and Nutrition, 34(I): 31-67.
Macgregor	Macgregor C.A. (1994); Directory of Feeds & Feed Ingredients; 2nd ed.; W.D. Hoard & Sons Company, Fort Atkinson, Wiskonsin, USA
Nasner	Nasner, A. (1985), Die antioxidativen Eigenschaften von Lecithin, Fette, Seifen Anstrichmittel, p477-481, Jg. 87, Nr. 12,
NCDA	North Carolina Feed Report (1984-1985) Bulletin of the NC Dept. Agriculture, Raleigh, NC. Number 261.
Novak + Haslberger	Novak and Haslberger, 2000; Substantial Equivalence of Antinutrients and Inherent Plant Toxins in Genetically Modifed Novel Foods; Food and Chem. Tox. 38 (2000) 473-483.
Nut Beef	Nutrient Requirements of Beef Cattle, (1984) 6th edition, National Academy Press, Washington D.C.
Nut Cow	Nutrient Requirements of Dairy Cattle, (1978) 5th edition, National Academy Press, Washington D.C.
OECD	OECD. 2001. Consensus Document on Compositional Considerations for New Varieties of Soybean: Key Food and Feed Nutrients and Anti-nutrients. ENV/JM/MONO(2001)15.
Pardun	Pardun H., (1989), Pflanzenlecithine- wertvolle Hilfs- und Wirkstoffe?, Fat Sci Technol. P45-58, Jg. 91, Nr. 2
Red Book	Feed Industry Red Book 1995, Reference and Buyers' Guide. Communications Marketing, Inc., Eden Prairie, Minnesota.

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APPENDIX A Table1: Sources of Reference Composition Data (continued)

Sotirhos	Sotirhos N. *et al.*, High Performance Liquid Chromatographic Analysis of Soybean Phospholipids, (1986), Fette, Seifen Anstrichmittel Jg. 88, Nr. 1
TAS	Douglas J.S., (1996), Recommended Compositional and nutritional parameters to test in soybean, TAS Inc. Washington, USA
USCA	United States – Canadian Tables of Feed Composition, (1982) 3rd Revision National Academy Press, Washington D.C.
USDA	U.S. Department of Agriculture, Agricultural Research Service. USDA Nutrient Database for Standard Reference, Release 13, Nutrient Data Laboratory, Home Page, http://www.nal.usda.gov/fnic/foodcomp accessed May 15, 2001.
USDA-IOWA	U.S. Department of Agriculture, Agricultural Research Service. USDA-IOWA State University Database on the Isoflavone content of Foods, Release 1.2-2000, Home Page, http://www.nal.usda.gov/fnic/foodcomp accessed May 15, 2001.

Table AII-4. Mean values and standard deviations for the content of proximates in seeds over all sites, listed separately for the three treatments: A: nontransgenic A5547, B: transgenic A 5547-127 not sprayed, C: transgenic A 5547-127 sprayed with glufosinate. Standard values from the literature is included (Technical dossier).

Parameter	Comparator (A)	A5547-127 Not sprayed (B)	A5547-127 Sprayed (C)	Reference ranges [a]
	Mean ± SD	Mean ± SD	Mean ± SD	
Moisture % fw	14.83 ± 2.60	14.40 ± 2.42	14.04 ± 2.72	5.6 - 12
Protein % dm	40.33 ± 1.25	40.51 ± 1.49	40.91 ± 1.24	32 - 45.5
Fat % dm	21.50 ± 1.30	21.36 ± 1.46	21.51 ± 1.08	8.1 - 24.7
Ash % dm	5.84 ± 0.37	5.95 ± 0.34	5.97 ± 0.38	3.9 - 7.0
Total Carbohydrates % dm [b]	32.47 ± 1.32	32.47 ± 1.41	31.60 ± 1.08	29.6 - 50.2
ADF % dm	8.60 ± 2.63	8.57 ± 2.58	8.22 ± 2.45	7.8 - 18.6
NDF % dm	12.54 ± 4.29	12.26 ± 3.93	12.01 ± 3.99	5.0 - 21.3

SD: standard deviation

[a] Standard values from Table 2 of appendix A in Oberdoerfer, 2008 M-293249-02-1

[b] Total Carbohydrates calculated as 100% - (protein %dm + fat %dm + ash %dm)

Table AII-5. Summary of the by site analysis of the proximate data of seeds for the A: nontransgenic A5547, B: transgenic A 5547-127 not sprayed, C: transgenic A 5547-127 sprayed with glufosinate (Technical dossier, Öberdoerfer, 2008).

Summary t-test procedures *)	A vs B		A vs C	
	significant	not significant	significant	not significant
Moisture	8	8	7	9
Protein	2	14	5	11
Crude Fat	3	13	2	14
Ash	5	11	5	11
Total Carbohydrate	4	12	6	10
Acid Detergent Fibre	1	15	1	15
Neutral Detergent Fibre	2	14	1	15

*) N of sites with significant ($p < 0.05$) and not significant ($p \geq 0.05$) treatment differences

A = non-transgenic, control samples

B = transgenic, not Liberty sprayed samples

C = transgenic, Liberty sprayed samples

Table AII-6. Mean levels and standard deviations for Amino Acids in Seeds of A: nontransgenic A5547, B: transgenic A 5547-127 not sprayed, C: transgenic A 5547-127 sprayed with glufosinate, compared to Commercial Soybean Varieties. (Technical dossier).

Total Amino Acid	% Dry matter			
	Comparator (A)	A5547-127 Not sprayed (B)	A5547-127 Sprayed (C)	Reference Range [a]
	Mean ± SD	Mean ± SD	Mean ± SD	
Alanine	1.73 ± 0.15	1.73 ± 0.16	1.76 ± 0.14	1.51 - 2.10
Arginine	2.94 ± 0.22	2.95 ± 0.24	3.01 ± 0.25	2.17 - 3.40
Aspartic acid	4.74 ± 0.38	4.74 ± 0.37	4.75 ± 0.47	3.81 - 5.12
Cystine	0.64 ± 0.05	0.64 ± 0.06	0.65 ± 0.04	0.37 - 0.81
Glutamic acid	7.34 ± 0.47	7.33 ± 0.47	7.42 ± 0.48	5.84 - 8.20
Glycine	1.76 ± 0.19	1.75 ± 0.18	1.75 ± 0.14	1.46 - 2.27
Histidine	1.07 ± 0.05	1.07 ± 0.07	1.08 ± 0.05	0.84 - 1.22
Isoleucine	1.68 ± 0.18	1.69 ± 0.21	1.70 ± 0.18	1.54 - 2.32
Leucine	2.97 ± 0.14	2.98 ± 0.15	3.00 ± 0.14	2.2 - 4.0
Lysine	2.47 ± 0.11	2.48 ± 0.12	2.50 ± 0.10	1.55 - 2.84
Methionine	0.55 ± 0.04	0.54 ± 0.04	0.54 ± 0.04	0.43 - 0.76
Phenylalanine	2.00 ± 0.12	2.01 ± 0.13	2.04 ± 0.12	1.60 - 2.39
Proline	2.11 ± 0.13	2.10 ± 0.13	2.14 ± 0.12	1.69 - 2.33
Serine	2.15 ± 0.16	2.09 ± 0.17	2.11 ± 0.17	1.11 - 2.48
Threonine	1.61 ± 0.11	1.59 ± 0.10	1.60 ± 0.13	1.14 - 1.89
Tryptophan	0.62 ± 0.06	0.62 ± 0.06	0.63 ± 0.06	0.36- 0.67
Tyrosine	1.29 ± 0.17	1.28 ± 0.18	1.30 ± 0.18	0.10 - 1.61
Valine	1.78 ± 0.18	1.79 ± 0.22	1.81 ± 0.21	1.50 - 2.44

SD: standard deviation

[a] Reference ranges from table 4 of appendix A in Oberdoerfer, 2008 M-293249-02-1

Table AII-7. Summary of the by site analysis of Amino Acids in seeds (Technical dossier).

Summary t-test *)	A vs B		A vs C	
	significant	not significant	significant	not significant
Alanine	2	14	2	14
Arginine	1	15	2	14
Aspartic acid	2	14	1	15
Cystine	4	12	6	10
Glutamic acid	1	15	2	14
Glycine	1	15	-	16
Histidine	1	15	2	14
Isoleucine	4	12	4	12
Leucine	2	14	3	13
Lysine	3	13	2	14
Methionine	2	14	-	16
Phenylalanine	2	14	3	13
Proline	1	15	1	15
Serine	3	13	2	14
Threonine	2	14	-	16
Tryptophan	2	14	3	13
Tyrosine	2	14	3	13
Valine	3	13	3	13

*) N of sites with significant ($p < 0.05$) and not significant ($p \geq 0.05$) treatment differences
A = non-transgenic, control samples
B = transgenic, not Liberty sprayed samples
C = transgenic, Liberty sprayed samples

Table AII-8. Mean levels and standard deviations for Fatty Acids in Seeds of A: nontransgenic A5547, B: transgenic A 5547-127 not sprayed, C: transgenic A 5547-127 sprayed with glufosinate, over all sites, compared to Commercial Soybean Varieties (table from Technical dossier).

Total fatty acids	% Relative			
	Comparator (A)	A5547-127 Not sprayed	A5547-127 Sprayed (C)	Reference ranges [a]
	Mea ± SD	Mea ± SD	Mea ± SD	
Saturated				
Myristic C14:0	< 0.10 - 0.14	< 0.10 - 0.14	< 0.10 - 0.12	< 0.10 - 0.27
Palmitic C16:0	11.4 ± 0.38	11.6 ± 0.36	11.6 ± 0.35	7 - 16
Margaric C17:0	< 0.10 - 0.10	< 0.10 - 0.11	< 0.10 - 0.10	< 0.10 - 0.15
Stearic C18:0	4.38 ± 0.45	4.50 ± 0.53	4.47 ± 0.48	2 - 5.9
Arachidic C20:0	0.47 ± 0.06	0.49 ± 0.08	0.48 ± 0.07	< 0.10 - 0.48
Behenic C22:0	0.58 ± 0.07	0.58 ± 0.09	0.58 ± 0.08	0.28 - 0.60
Lignoceric C24:0	**0.21 ± 0.05**	**0.21 ± 0.06**	**0.21 ± 0.05**	0.15
Sum saturated	17.12 - 17.36	17.44 - 17.69	17.36 - 17.58	9.43 - 23.55
Mono.unsaturated				
Palmitoleic C16:1	< 0.10 - 0.19	< 0.10 - 0.19	< 0.10 - 0.17	< 0.10 - 0.48
Oleic C18:1	22.7 ± 2.30	23.0 ± 3.28	23.6 ± 3.70	14 - 34
Gadoleic C20:1	0.28 ± 0.04	0.28 ± 0.04	0.28 ± 0.04	0.14 - 0.35
Erucic C22:1	< 0.10 - 0.29	< 0.10 - 0.35	< 0.10 - 0.34	ND
Sum mono-unsaturated	23.05 - 23.53	23.31 - 23.85	23.9 - 24.41	14.14 - 34.83
Poly-unsaturated				
Linoleic C18:2	51.8 ± 1.83	51.3 ± 2.86	50.9 ± 2.99	48 - 60
Linolenic C18:3	7.35 ± 0.97	7.25 ± 1.05	7.22 ± 1.08	2 - 10
Sum poly-unsaturated	59.23	58.63	58.16	50 - 70
Sum of total fatty acids	99.4 - 100.12	99.38 -	99.42 -	-

SD: standard deviation
ND No data
[a] Reference ranges from table 5 of appendix A

Table AII-9. Summary of the by site analysis of Fatty Acids. (from Technical Dossier).

Summary t-test *)	A vs B		A vs C	
	significant	not significant	significant	not significant
C14:0 Tetradecanoic (Myristic)	1	3	2	2
C14:0 Tetradecanoic (Myristic) [a]	1	13	2	12
C16:0 Hexadecanoic (Palmitic)	7	9	8	8
C16:1 Hexadecenoic (Palmitoleic)	-	12	3	9
C16:1 Hexadecenoic (Palmitoleic) [a]	-	15	3	12
C17:0 Heptadecanoic (Margaric)	-	1	-	1
C17:0 Heptadecanoic (Margaric) [a]	-	13	-	13
C18:0 Octadecanoic (Stearic)	6	10	7	9
C18:1 Octadecenoic (Oleic)	3	13	**9**	**7**
C18:2 Octadecadienoic (Linoleic)	4	12	8	8
C18:3 Octadecatrienoic (Linolenic)	3	13	6	10
C20:0 Eicosanoic (Arachidic)	3	13	6	10
C20:1 Eicosenoic (Gadoleic)	2	14	3	13
C22:0 Docosanoic (Behenic)	4	12	4	12
C22:1 Docosenoic (Erucic)	-	3	-	3
C22:1 Docosenoic (Erucic) [a]	-	14	-	15
C24:0 Tetracosanoic (lignoceric)	3	13	2	14

*) N of sites with significant ($p < 0.05$) and not significant ($p \geq 0.05$) treatment differences
A = non-transgenic, control samples
B = transgenic, not Liberty® sprayed samples
C = transgenic, Liberty® sprayed samples
[a] 'not significant' was also assumed if all samples of a site were equal or below the limit of quantification for the two respective treatments

Table AII-10. Mean levels and standard deviations for minerals and vitamins in Seeds of A: nontransgenic A5547, B: transgenic A 5547-127 not sprayed, C: transgenic A 5547-127 sprayed with glufosinate, over all sites, compared to Commercial Soybean Varieties (Technical dossier).

Parameter	Based on dry matter			
	Comparator (A)	A5547-127 Not sprayed (B)	A5547-127 Sprayed (C)	Reference ranges [a]
	Mean ± SD	Mean ± SD	Mean ± SD	
Calcium %	0.31 ± 0.03	0.33 ± 0.03	0.33 ± 0.04	0.21 - 0.34
Phosphorus %	0.68 ± 0.07	0.71 ± 0.07	0.71 ± 0.07	0.49 - 0.94
Potassium %	2.17 ± 0.14	2.19 ± 0.12	2.21 ± 0.09	1.4 - 2.3
Magnesium %	0.27 ± 0.03	0.28 ± 0.03	0.28 ± 0.03	0.21 - 0.32
Sodium (1999 and 2000) %	0.0230 ± 0.0075	0.0292 ± 0.0060	0.0236 ± 0.0072	0.002 - 0.02
Sodium (2006) %	0.0063 ± 0.0038	0.0064 ± 0.0042	0.0067 ± 0.0047	0.002 - 0.02
Iron %	137 ± 343	87 ± 18	90 ± 23	55.4 - 172
Iron % [b]	87 ± 17	87 ± 18	90 ± 23	55.4 - 172
Vitamin B1 mg/kg dm	11.77 ± 2.15	12.49 ± 1.81	12.69 ± 1.62	1.01 - 16.02
Vitamin B2 mg/kg dm	4.27 ± 0.94	4.19 ± 0.78	4.20 ± 0.89	1.9 - 14.5
Folic Acid mg/kg dm	2.73 ± 0.78	2.67 ± 0.70	2.64 ± 0.75	2.4 - 4.7
Vitamin E IU/kg dm [c]	67.34 ± 44.84	90.88 ± 45.71	106.84 ± 60.92	2.8 – 91.9

SD: standard deviation

[a] Reference ranges from table 3 of appendix A in Oberdoerfer 2008 M-293249-02-1

[b] omitting an individual value of 2435 mg/kg dm

[c] Vitamin E as the sum of all tocopherols in IU/kg dm

Table AII-11. Summary of the by site analysis of minerals and vitamins. (Technical dossier).

Summary t-test *)	A vs B		A vs C	
	significant	not significant	significant	not significant
Calcium	6	10	6	10
Phosphorus	4	12	7	9
Potassium	-	16	1	15
Magnesium	3	13	5	11
Sodium	4	9	-	13
Sodium [a]	5	8	-	13
Iron	2	14	4	12
Iron [b]	3	13	4	12
Vitamin B1	4	12	5	11
Vitamin B2	1	15	1	15
Folic Acid	1	15	1	15
Total Vitamin E (Tocopherols)	6	10	8	8

*) N of sites with significant ($p < 0.05$) and not significant ($p \geq 0.05$) treatment differences
A = non-transgenic, control samples
B = transgenic, not Liberty sprayed samples
C = transgenic, Liberty sprayed samples
[a] 'not significant' was also assumed if all samples of a site were equal or below the limit of quantification for the two respective treatments
[b] omitting an individual value of 2435 mg/kg dm

Table AII-12. Mean levels and standard deviations over all sites for Anti-nutrients in Seeds of A: nontransgenic A5547, B: transgenic A 5547-127 not sprayed, C: transgenic A 5547-127 sprayed with glufosinate, compared to Commercial Soybean Varieties (Technical dossier, Öberdoerfer, 2008).

Parameter	Based on Dry matter			
	Non-Transgenic (A)	Transgenic Not sprayed (B)	Transgenic Sprayed (C)	Reference Range [a]
	Mean ± SD	Mean ± SD	Mean ± SD	
Raffinose (%)	1.02 ± 0.24	0.95 ± 0.22	0.95 ± 0.19	0.11 - 1.28
Stachyose (%)	4.19 ± 0.61	3.93 ± 0.62	4.03 ± 0.62	1.21 - 6.30
Phytic acid (%)	1.70 ± 0.18	1.75 ± 0.14	1.77 ± 0.19	0.63 - 2.74
Trypsin inhibition (TIU/g)	69 008 ± 8729	70 983 ± 8258	71 598 ± 8284	19 590 - 118 680
Lectin (HU/mg)	6.7 ± 3.0	6.6 ± 3.4	6.5 ± 3.3	0.11 - 129

[a] Reference ranges from tables 7 of appendix A

Table AII-13. Summary of the by site analysis of Anti-nutrients in Seeds of A: nontransgenic A5547, B: transgenic A 5547-127 not sprayed, C: transgenic A 5547-127 sprayed with glufosinate (Technical dossier, Öberdoerfer, 2008).

Summary t-test procedures *)	A vs B		A vs C	
	significant	not significant	significant	not significant
Raffinose	4	12	**9** [a]	**7** [a]
Stachyose	6	10	8	8
Phytic Acid	3	13	6	10
Trypsin inhibition	-	16	5	11
Lectin	2	13	2	13

*) N of sites with significant ($p < 0.05$) and not significant ($p \geq 0.05$) treatment differences
A = non-transgenic, control samples
B = transgenic, not Liberty sprayed samples
C = transgenic, Liberty sprayed samples
[a] Figures in bold indicate that at the majority of sites significant differences were found

AII-14. Mean levels and standard deviations for Isoflavones in Seeds of A: nontransgenic A5547, B: transgenic A 5547-127 not sprayed, C: transgenic A 5547-127 sprayed with glufosinate, over all sites, compared to Commercial Soybean Varieties (Figure from Technical dossier).

Parameter	Based on dry matter			
	Comparator (A)	A5547-127 Not sprayed (B)	A5547-127 Sprayed (C)	Reference ranges [a]
	Mean SD	Mean ± SD	Mean ± SD	
Daidzein (1999 and 2000) mg/kg	138 ± 101	91 ± 67	86 ± 77	5 - 35
Daidzein (2006) mg/kg	< 20 - 75	< 20 - 38	< 20 - 40	
Total Daidzin (1999 and 2000) mg/kg [b]	404 ± 152	392 ± 166	380 ± 161	60.0 - 2454 [c]
Total Daidzin (2006) mg/kg [b]	608 ± 108	600 ± 116	566 ± 111	
Genistein (1999 and 2000) mg/kg	136 ± 109	83 ± 68	78 ± 77	0.3 - 46
Genistein (2006) mg/kg	< 20 - 50	< 20 - 29	< 20 - 29	
Total Genistin (1999 and 2000) mg/kg [b]	475 ± 188	476 ± 197	444 ± 203	144 - 2837 [c]
Total Genistin (2006) mg/kg [b]	730 ± 165	724 ± 191	677 ± 150	
Glycitein (1999 and 2000) mg/kg	28 ± 30	15 ± 11	13 ± 11	1 - 80
Glycitein (2006) mg/kg	< 20	< 20	< 20	
Total Glycitin (1999 and 2000) mg/kg [b]	166 ± 31	178 ± 41	175 ± 41	15.3 - 1070
Total Glycitin (2006) mg/kg [b]	190 ± 26	213 ± 24	208 ± 31	
Total Isoflavones (1999 and 2000) mg/kg [b]	1044 ± 350	1049 ± 382	996 ± 384	679 - 3733
Total Isoflavones (2006) mg/kg [b]	1551 ± 244	1536 ± 310	1464 ± 280	

SD: standard deviation

[a] Reference ranges from tables 6 of appendix A in Oberdoerfer, 2008 M-293249-02-1

[b] Sum of isoflavone(s) glucosides and esters reported as aglycone(s) equivalent(s)

Table AII-15. Summary of the by site analysis of Isoflavones (from Technical dossier).

Summary t-test *)	A vs B		A vs C	
	significant	not significant	significant	not significant
Daidzein	4	8	4	8
Daidzein [a]	4	9	4	10
Total Daidzin (aglucon equivalent)	5	11	5	11
Genistein	2	8	4	6
Genistein [a]	2	10	4	8
Total Genistin (aglucon equivalent)	2	14	5	11
Glycitein	3	6	4	5
Glycitein [a]	3	13	4	12
Total Glycitin (aglucon equivalent)	5	11	4	12
Total Isoflavones (aglucon equivalents)	3	13	4	12

*) N of sites with significant ($p < 0.05$) and not significant ($p \geq 0.05$) treatment differences
A = non-transgenic, control samples
B = transgenic, not Liberty sprayed samples
C = transgenic, Liberty sprayed samples
[a] 'not significant' was also assumed if all samples of a site were equal or below the limit of quantification for the two respective treatments

Table AII-16. Agronomic and morphological characteristics evaluated in GM soybean A5547-127 and conventional control (A5547) in field trials in the USA in .

Agronomic traits	Comparator (A)			A5547-127 Not sprayed (B)			A5547-127 Sprayed (C)		
	Mean	±	SD	Mean	±	SD	Mean	±	SD
Emergence (50% of plants)[a]	5.7	±	2.1	5.8	±	2.1	5.4	±	1.0
Stand count (no. of plants)[b]	119.6	±	40.0	126.5	±	39.2	133.5	±	39.8
Stand count (% of planted seeds) [b]	71.8	±	15.1	75.8	±	14.5	79.3	±	10.9
Plant vigor [c]	8.3	±	1.2	8.3	±	1.1	8.4	±	0.8
Plant health at V4-5 [d]	5.0	±	0	5.0	±	0	4.9	±	0.3
Plant health at R1 [d]	5.0	±	0	5.0	±	0	5.0	±	0
Plant health at maturity [d]	5.0	±	0	5.0	±	0	5.0	±	0
Flowering date (days)[e]	47.1	±	4.6	46.4	±	4.7	46.5	±	4.8
Plant height (inches)[f]	25.5	±	4.7	24.3	±	4.2	24.3	±	3.8
Days to maturity (days) [g]	128.7	±	20.4	127.8	±	20.6	127.9	±	20.6
Yield (lb/acre)	2366	±	1182	2425	±	1124	2421	±	1157

A = non-transgenic, control samples, B= transgenic, not Liberty® sprayed samples, C= transgenic, Liberty® sprayed samples

a Emergence was defined to be when the cotyledons (2 leaves) have completely emerged from the soil.
b mean over 6 rows and adjusted to a standard of 20 row-feet
c rated between 1 (no emergence) and 9 (excellent emergence)
d rated between 0 (plant death) and 5 (no injury)
e days between planting and 50% flowering
f mean per plot
g days from planting to 90% of pods turning colors

Table AII-17. Results of the by-site t-test for agronomic and morphological characteristics.

Summary t-test for agronomic traits	A vs B		A vs C	
	significant	not significant (or equal [h])	significant	not significant (or equal [h])
Emergence (50% of plants) [a h]	-	4 (12)	-	4 (12)
Stand count (no. of plants) [b]	2	14	2	14
Stand count (% of planted seeds) [b]	2	14	2	14
Plant vigor [c,d]	-	5 (11)	-	5 (11)
Flowering date (days) [e,h]	4	1 (9)	3	2 (9)
Plant height (inches) [f]	3	13	4	12
Days to maturity (days) [g,h]	1	2 (11)	1	2 (11)
Yield (lb/acre)	1	15	2	14

*) N of sites with significant ($p < 0.05$) and not significant ($p \geq 0.05$) treatment differences

A = non-transgenic, control samples, B = transgenic, not Liberty® sprayed samples, C= transgenic, Liberty® sprayed samples

a Emergence was defined to be when the cotyledons (2 leaves) have completely emerged from the soil.

b mean over 6 rows and adjusted to a standard of 20 row-feet

c rated between 1 (no emergence) and 9 (excellent emergence)

d rated between 0 (plant death) and 5 (no injury)

e days between planting and 50% flowering

f mean per plot

g days from planting to 90% of pods turning colors

h "not significant" was also assumed if on all plots of a site results or ratings were equal for that characteristic for the two respective treatments (N of sites in brackets)

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). The goal was to examine how much soy protein a person can consume, realistically, by replacing meat and milk products with soy-products.

Reason for the choice of menus

The milk allergy menu
Milk allergy or intolerance are relatively common conditions. Persons with such conditions 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 wish 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 products, or eggs. In this scenario we envision a vegan who has previously eaten normal food and wishes 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 for drinking, in waffles, 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 10 MJ/day. We assume that in pure soy products (e.g. soy milk) all the protein comes 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 day 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 rather extensive safety testing programme has been conducted on soybean A5547-127 within the Russian Federation and summarised in "Tutelyan VA (2013) Genetically Modified Food Sources. Safety Assessment and Control. Amsterdam: Academic Press, Elsevier. DOI: 10.1016/B978-0-12-405878-1.00009-4". The research and testing is claimed compliant with 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 been difficult to assess for the VKM GMO panel. A brief summary of the testing is thus presented below.

Toxicological assessment of the whole GM food/feed

Potential genotoxicity of soybean A5547-127

The potential genotoxicity of soybean A5547-127 was investigated in an *in vivo* experiment in mice (Tutelyan, 2013). The study was conducted in accordance with the requirements of the Ministry of Health of the Russian Federation authorized for risk and safety assessment of food derived from GM sources (MUK 2.3.2.970-00, 2008). The genotoxicity studies were carried out on male C57Bl/6 and female CBA mice sensitive to mutagenesis. For 45 days, the mice weighing 20-25 g were fed diet with protein concentrate from soybean A5547-127 (test group n=15 mice) or its conventional counterpart (control group n=12 mice) with daily feed intake of 1.5 g/day/animal. These studies examined chromosomal aberrations in the cells of bone marrow and the dominant lethal mutations in the gametes of control and test mice. The cytogenetic analysis was carried out by metaphasic method (MUK 2.3.2.970-00, 2000). The mice of both groups were sacrificed 24 h after the last feeding. Two hours prior to termination of the experiment, the mice were intraperitoneally injected with colchicine to accumulate cells at the metaphasic stage of nuclear division. Bone marrow was isolated from both femoral bones. A total of 70–80 cells were taken for analysis from each mouse from the group of 2-month old male C57Bl/6 mice weighing 20–25 g. Genetic alterations in gametes were examined by assessing dominant lethal mutations.

After the 45-day feeding period, the C57Bl/6 test (n=15) and control (n=12) male mice were caged with virgin CBA female mice in a ratio of 1:2. The mating period of 3 weeks was sufficient to assess the effect of soybean diet on sex cells (spermatids and spermatozoa) during the postmeiotic period. Pregnant females were isolated and sacrificed on gestation days 15–17 by cervical dislocation. Numbers of corpus lutea and live and dead embryos were recorded. These data were used to calculate mutagenic parameters: pre-implantation, post-implantation, and inducible mortality.

Among various structural chromosomal abnormalities in animals of both groups, there were single segments, one circular chromosome (in a test mouse), and gaps. The number of cells

with such chromosomal abnormalities did not significantly differ in parental (F0) and first generation (F1) mice fed soy protein concentrate from A5547-127 or conventional counterpart soybeans.

To examine the dominant lethal mutations, 64 test and 56 control females were dissected to analyze embryos and corpus lutea. In the test group 393 embryous and 425 corpus lutea were analysed. The corresponding values in control mice were 350 embryos and 376 corpus lutea. The pre-implantation mortality was similar in the control and test groups.

At the stages of early and late spermatids or mature spermatozoa, the post-implantation embryonic mortality (the most reliable index of mutagenic activity of an examined substance) varied within equal range in the test and control groups. The induced mortality at these stages was in the range 0−1%, indicating the absence of mutagenic effect of transgenic soybean line A5547-127 on spermiogenesis in mice.

The Russian investigators concluded that glufosinate-tolerant soybean line A5547-127 produced no mutagenic effect in the described experiments.

Subchronic feeding studies in rats with soy protein concentrate derived from soybean A5547-127

A feeding study over 180 days with soy protein concentrate was conducted on male Wistar rats. Biochemical, hematological, and morphological parameters were monitored in accordance with the requirements of the Ministry of Health of the Russian Federation authorized for risk and safety assessment of food derived from GM sources (Tutelyan, 2013). Male Wistar rats (n = 60) with a body weight of 85–95 g were randomised into two groups. The test rats were provided daily with protein concentrate derived from the transgenic soybean line A5547-127. The control rats were provided with the same amount of protein concentrate prepared from the conventional counterpart. The amount of the protein concentrate in the semi-synthetic diets was 22.5 g per 100 g diet. Samples were collected on days 30 and 180 of the experiment.

During the entire length of the experiment, the general condition of the rats was similar in the control and test groups. No mortality was observed in either group. The absolute and relative weights and visual inspection of internal organs did not reveal any differences between the two groups. The histological assessments of internal organs (liver, kidneys, lung, spleen, small intestine, and testicle) revealed no differences between the control and test groups. The content of total protein, glucose, activity of alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase in blood serum, pH and the relative density of urine, urinary concentration of creatinine and its urinary excretion did not significantly differ between control and test rats at day 30 and 180. Hematological assays showed that feeding rats with protein concentrate derived from transgenic soybean line A5547-127 did not induce significant changes in concentration of hemoglobin, hematocrit, total erythrocyte count, MCH, MCHC, MCV, total leukocyte count, absolute and relative count

of eosinophils, neutrophils, and lymphocytes relative to the control values obtained at 30 and 180 days.

Assessment of allergenicity of proteins derived from the GM plant

Assessment of possible sensitivisation of A5547-127 on the immune response to endogenous metabolic products was carried out by testing sensitivity to histamine in mice (Tutelyan 2013). For 21 days, the control and test mice were fed diets with protein concentrate derived from conventional and transgenic soybean. Then the mice of both groups were injected intraperitoneally with 2.5 mg histamine hydrochloride dissolved in 0.5 mL physiological saline solution. Twenty-four hours post-injection, all mice were alive. The lifetime of the mice in the test group was somewhat longer than that of the control mice: the test mice infected with 105 or 104 microbial cells lived 4.2 and 6.2 days as compared with 1.2 and 2.2 days for the control mice. The smaller doses did not reveal any difference in the lifetime of mice in either groups. The values of LD_{50} were 256 and 175 bacterial cells per mouse in the test and control groups, respectively. The authors concluded that soybean A5547-127 does not contain any sensitising ingredient for mice.

9.1.1 Studies on Immunotoxicity

Potential effect on humoral component of immune system

Level of hemagglutination after injecting sheep erythrocytes
The immunomodulating effect of GM soybean on the humoral component of the immune system was examined by determining the level of hemagglutination after injecting sheep erythrocytes (SE) to mouse lines C57Bl/6 (low sensitivity to SE) and CBA (high sensitivity to SE) (Tutelyan, 2013). Soybean protein concentrate was fed to mice for 21 days. The control and test mice were fed a diet with conventional and transgenic soybean line A5547-127, respectively. On Day 21 the mice of both groups were intraperitoneally injected with 0.5 mL sheep erythrocytes (SE) (10 million cells). Blood was subsequently drawn on day 7, 14, and 21. Blood serum was titrated for reaction of hemagglutination by routine method. All mice demonstrated the presence of antibodies against SE. For both CBA mice fed diet with soy protein concentrate derived from transgenic soybean line A5547-127 and mice fed protein concentrate from conventional soybean, the antibody appeared on Day 14 post-immunization at the titer of 1:16. According to the Russian investigators the synthesis rate of the antibodies raised against SE were similar in C57Bl/6 and CBA mice lines fed diet with soy protein concentrate derived from transgenic soybean and in mice of the same lines fed on conventional soy protein concentrate.

Hypersensitivity reaction to sheep erythrocytes
The possible immunomodulating effect of transgenic soybean was assessed with delayed hypersensitivity reaction to sheep erythrocytes (SE) (Tutelyan, 2013). C57Bl/6 and CBA mice were used in this test. Each strain was divided in three groups, one group was fed soybean protein concentrate from A5547-127, one was fed conventional soybean protein concentrate

and one was fed without soybean (control group). The soybean protein concentrate was added to the diet for 21 days; thereafter, sheep erythrocytes (SE) was injected subcutaneously (1 million cells per mouse). On post-injection Day 5, SE (0.02 mL, 109 cells) were injected into the finger-pad of the right hindleg of control and test mice. The left hindleg was injected with 0.02 mL physiological saline solution. Local inflammatory reaction was assessed 18 h after the injections by comparison of the weights of both injected paws.

The studies demonstrated the absence of any significant differences in the parameters of local inflammatory reaction in mice of either examined lines fed diet with soy protein concentrate derived either from transgenic soybean line A5547-127 or from conventional soybean. However, test C57Bl/6 mice fed diet with soy protein concentrate derived from transgenic soybean line A5547-127 demonstrated a small but statistically significant ($p <$ 0.05) elevation of this parameter in comparison with the control group fed diet with conventional soy protein concentrate, but no significant differences were observed when compared with the control mice maintained on the standard vivarium diet.

These results showed that soybean A5547-127 did not contain elevated sensitising components compared to the control soybean.

Potential Effect on Cellular Component of Immune System

Effect of soybean A5547-127 on susceptibility to Salmonella typhimurium
Effect of soybean A5547-127 on susceptibility to Salmonella typhimurium was investigated in mice (Tutelyan, 2013). Mice fed diets supplemented with protein concentrate derived from conventional or transgenic soybean for three weeks were subsequently injected intraperitoneally with various doses of Salmonella typhimurium strain 415. The injected doses ranged from 100 to 105 microbial cells per mouse and varied on a 10-fold basis. The post-injection observation period was 18 days, and all mice were dead prior to day 18. The lifetime of the mice in the control group was somewhat longer than that of the test mice: the test mice lived 11.3 and the control lived 14.7 days. The lower doses did not reveal any difference in the lifetime of mice in both groups. These data showed that Salmonella typhimurium produced typical infection both in control mice fed diet with conventional soybean protein concentrate and in the test mice fed diet with transgenic protein concentrate. According to the difference in the time to death, the control group survived longer than the test mice, although the differences in LD50 values (LD50 control and test was 72 and 69, respectively) remained within the experimental error. Thus, introduction of protein concentrate derived from transgenic soybean line A5547-127 into mouse diet produced no effect on the humoral and cellular components of the immune system, did not sensitize the mouse organism, and did not disturb the natural resistance against typical infection such as murine typhus. Taken together, these data support the conclusion that transgenic soybean line A5547-127 has no immunomodulating properties.

Assessment of systemic anaphylaxis

The potential impact of soybean A5547-127 on systemic anaphylaxis was investigated in rats (Tutelyan, 2013). The model employed was according to standard protocols as described in the Russian Methodical Guidelines (MUK 2.3.2.970-00, 2000). The study was performed on male Wistar rats (n = 47) weighing 140 ± 10 g. After a 7-day adaptation period to standard vivarium diet, the rats were fed a diet supplemented with protein concentrate (3.1 g protein concentrate/ 100 g diet) derived from conventional soybean (control group) or from soybean line A5547-127 for 28 days. During the entire experiment the rats of both groups grew normally.

On experimental days 1, 3 and 5, the rats were sensitized intraperitoneally with 100 µg ovalbumin from hens' eggs (OVA). On Day 21, another portion of 10 µg OVA was administered under the same conditions to induce the secondary immune response. After termination of feeding animals with the diets on experimental Day 29, blood (0.2 mL) was drawn from the tail vein in order to assess the antibody response. Then a booster dose of OVA (30 mg/kg in 0.5 mL isotonic apyrogenic 0.15 M NaCl saline) was injected intravenously. During the following 24 h, the development of any symptoms of active anaphylactic shock were evaluated by observation. Severity of anaphylactic shock was scored as follows: +(1), shiver, chill, dyspnea; ++(2), asthenia, ataxia, peripheral cyanosis; +++(3), convulsions, paralysis; ++++(4) fatal outcome. The anaphylactic index (AI) was calculated according to the Russian Methodical Guidelines (MUK 2.3.2.970-00, 2000) as the mean of anaphylactic severity scores in a group at 24 h after injection of the booster dose. Intensity of humoral immune response was assessed according to concentration of circulating specific immunoglobulin antibodies (the sum of IgG_1 and IgG_4 fractions) by the method of indirect solid-phase enzyme-linked immunosorbent assay (standard ELISA) on polystyrene. Results showed that rats fed diets with protein concentrate derived from line A5547-127 displayed a humoral immune response that was significantly lower in intensity compared to control rats. There was only an insignificant increase of anaphylactic reaction and mortality in the A5547-127 group. The degree of sensitization by ovalbumin in these rats did not increase compared with the rats fed diet with protein concentrate derived from conventional soybean. It was concluded from the study that the protein concentrate prepared from transgenic soybean line A5547-127 did not elevate allergic reactivity and sensitization towards the model allergen in test rats in comparison with the control rats fed conventional soybean.