Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

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Final health and environmental risk assessment of genetically modified LLcotton25

Scientific opinion on glufosinate-tolerant, genetically modified LLcotton25 from Bayer CropScience for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (Application EFSA/GMO/NL/2005/13)

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) 2016:10
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Final health and environment assessment of genetically modified LLcotton25 (EFSA/GMO/NL/2005/13)

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Assessed and approved

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

Acknowledgment

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

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

Genetically modified LLcotton25 from Bayer CropScience expresses the *bar* gene from *Streptomyces hygroscopicus* ATCC21705 encoding the phosphinothricin-acetyl–transferase (PAT) enzyme, which confers tolerance to the active herbicide glufosinate-ammonium.

Updated bioinformatics analyses of the inserted DNA and flanking sequences in LLCotton25 have not indicated potential production of putatively harmful toxins or allergens caused by the genetic modification. Genomic stability of the functional insert and consistent expression of the *bar* gene have been shown over several generations of LLCotton25.

Data from field trials indicate that with the exception of the newly introduced trait, LLCotton25 is compositionally, phenotypically and agronomically equivalent to its conventional counterpart Coker 312 and other cotton cultivars.

A 33-day nutritional assessment trial with broilers has not revealed adverse effects of cottonseed meal from LLCotton25. Toxicity testing of the PAT protein in a repeated-dose dietary exposure test with rats did not indicate adverse effects. The PAT protein produced in LLCotton25 does not show amino acid sequence resemblance to known toxins or IgE-dependent allergens, nor has it been reported to cause IgE-mediated allergic reactions. It is therefore unlikely that the PAT protein will cause toxic or IgE-mediated allergic reactions to food or feed containing LLCotton25 compared to conventional cotton cultivars.

Cotton is not cultivated in Norway, and there are no cross-compatible wild or weedy relatives of cotton in Europe.

Based on current knowledge and with the exception of the introduced traits, the VKM GMO Panel concludes that LLCotton25 is nutritionally, compositionally, phenotypically and agronomically equivalent to and as safe as its conventional counterpart and other cotton cultivars.

Considering the intended uses, which exclude cultivation, the VKM GMO Panel concludes that LLCotton25 does not represent an environmental risk in Norway.

Summary

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Scientific Committee for Food Safety (VKM) has been requested by the Norwegian Environment Agency 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 glufosinate-ammonium tolerant genetically modified LLCotton25 (Unique Identifier ACS-GHØØ1-3) from Bayer CropSciences is approved in EU under Regulation (EC) No 1829/2003 for food and feed uses, import and processing since 13th of April 2007 (Application EFSA/GMO/NL/2005/13, Commission Implementing Decision 2008/837/EC).

LLCotton25 has previously been assessed by the VKM GMO Panel commissioned by the NFSA related to the EFSAs public hearing of the application EFSA/GMO/NL/2005/13 in 2005 (VKM, 2005). LLCotton25 has been used as a component of the stacked GM event GHB614 x LLCotton25 (EFSA/GMO/NL/2010/77), which has been evaluated by EFSA (EFSA, 2014), but not by VKM.

The current food, feed and environmental risk assessment of the LLCotton25 is based on information provided in the application EFSA/GMO/NL/2005/13, relevant peer-reviewed scientific literature including scientific opinions and comments from EFSA (EFSA, 2006a), VKM (VKM, 2005) and statements provided by other member states made available on the EFSA GMO Extranet. Except for a synopsis of more recent literature, this draft opinion is to a large extent a summary of the above-mentioned VKM and EFSA opinions, which are provided in Appendix I and II respectively, and readers are referred to these for details.

The VKM GMO Panel has evaluated LLCotton25 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 also takes account of the appropriate principles described in the EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA, 2006b and 2011b), the environmental risk assessment of GM plants (EFSA, 2010a), selection of comparators for the risk assessment of GM plants (EFSA, 2011a) and for the post-market environmental monitoring of GM plants (EFSA, 2011c).

The scientific risk assessment of LLCotton25 includes molecular characterisation of the inserted DNA and expression of the novel protein, comparative assessment of agronomic and

phenotypic characteristics, nutritional assessments, toxicity and allergenicity, unintended effects on plant fitness, potential for gene transfer, interactions between the GM plant, target and non-target organisms, and effects on biogeochemical processes.

It is emphasised that the VKM mandate does not include assessments of contribution to sustainable development, societal utility or ethical considerations, included in 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 possible herbicide residues in food and feed from genetically modified plants specifically.

The event LLCotton25 was developed by *Agrobacterium tumefaciens* mediated transformation to express a modified *bar* gene from *Streptomyces hygroscopicus*. The gene encodes the enzyme phosphinothricin-acetyl-transferase (PAT) that acetylates L-glufosinate, which renders LLCotton25 tolerant to glufosinate ammonium based herbicides.

Molecular characterisation

The LLCotton25 genome has a complete, single integrated copy of the *bar*-expression cassette. Even though the PAT concentration is low, 0.21-0.35% of total crude protein in the leaves, it is highest in the plant parts exposed to herbicide treatment. This is consistent with the regulation by the inserted *35S* promoter, with highest activity in leaves and stems.

Out of 26 putative novel open reading frames (ORFs) identified in the GM cotton, only three short ORFs located in the 3' region of the insert were theoretically found to encode potential novel gene products. No relevant homologies were found between these theoretically predicted translation products and known toxins or allergens. Southern hybridisation, ELISA and segregation analyses show that the introduced gene elements were stably inherited and expressed over multiple generations in parallel with the observed phenotypic characteristics of the event.

Based on current knowledge and information provided by the applicant, the VKM GMO panel concludes that the intended changes in LLCotton25 have been sufficiently characterised, and that no unintended changes have been identified that requires particular attention in the further assessment.

Comparative assessments

Field trials have been conducted in the USA during 2000 and 2001 for compositional assessments of whole linted cottonseeds, cotton lint, and different processed cottonseed products. Field trials in USA, Brazil and Australia during numerous growing seasons were performed for agronomic and GM phenotype assessments. In all trials, LLCotton25 was compared to its conventional counterpart, parent line Coker 312. LLCotton25 was grown using conventional or glufosinate-based herbicide while cotton Coker 312 was grown using conventional herbicides.

With the exception of the changes caused by the introduced transgenic trait, data provided by the applicant revealed no biologically relevant differences between LLCotton25 and its conventional counterpart Coker 312. The few statistically significant differences observed were only present in material from some of the locations in some years and the values were within or close to the range of historical values observed in conventional cotton cultivars. The differences were therefore considered to reflect the natural variability of the analytes.

Based on current knowledge and excluding the new PAT-protein, the VKM GMO Panel concludes that LLCotton25 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart and other cotton cultivars.

Food and feed risk assessment

A 33-day nutritional assessment trial with broilers did not reveal biologically relevant adverse effects or differences in the performance of animals fed diets containing cottonseed meal from LLCotton25 compared to conventional counterpart Coker 312 or another cotton cultivar. Bioinformatics analysis of the amino acid sequence of the PAT protein did not show sequence resemblance to known toxins or IgE-dependent allergens, nor has the protein been reported to cause IgE-mediated allergic reactions. It is therefore unlikely that the PAT protein will cause toxic or IgE-mediated allergic reactions to food or feed containing LLCotton25 compared to conventional cotton cultivars.

Based on current knowledge, and considering the intended uses, the VKM GMO Panel concludes that LLCotton25 is nutritionally equivalent to and as safe as its conventional counterpart Coker 312 and other cotton cultivars.

Environmental assessment

Considering the intended uses of LLCotton25, which exclude cultivation, the environmental risk assessment is concerned with the accidental release into the environment of viable seeds during transport and/or processing, and with indirect exposure to microorganisms in the gastrointestinal tract and in soil or water, mainly via intestinal content and faeces from animals fed feeds containing LLCotton25.

With the exception of the introduced tolerance to the herbicide glufosinate-ammonium, LLCotton25 has no altered survival, multiplication or dissemination characteristics compared to conventional cotton cultivars, and there are no indications of an increased likelihood of spread and establishment of cotton plants in the case of accidental release of seeds from LLCotton25 into the environment. Cotton is not cultivated in Norway, and there are no cross-compatible wild or weedy relatives of cotton in Europe. Plant to plant gene flow is therefore not considered to be an issue. There are no indications that transfer of recombinant genes from LLCotton25 products to microorganisms in the gastrointestinal tract or in soil or water could occur at higher frequencies than from naturally occurring microbial sources.

Based on current knowledge and considering its intended uses, which exclude cultivation, the VKM GMO Panel concludes that LLCotton25 does not represent an environmental risk in Norway.

Overall conclusion

Based on current knowledge and with the exception of the introduced trait, the VKM GMO Panel concludes that LLCotton25 is nutritionally, compositionally, phenotypically and agronomically equivalent to and as safe as its conventional counterpart and other cotton cultivars.

Considering the intended uses, which exclude cultivation, the VKM GMO Panel concludes that LLCotton25 does not represent an environmental risk in Norway.

Key words: VKM, risk assessment, Norwegian Scientific Committee for Food Safety, Norwegian Food Safety Authority/Norwegian Environment Agency. GMO, cotton (*Gossypium hirsutum* L.), LLCotton25, unique identifier ACS-GHØØ1-3, EFSA/GMO/NL/2005/13, glufosinate tolerance, PAT protein, *bar* gene, food/feed safety, human and animal health, import and processing, Regulation (EC) No 1829/2003.

Sammendrag

Som en del av forberedelsene til implementering av forordning 1829/2003 i norsk rett, er Vitenskapskomiteen for mattrygghet (VKM) bedt av Miljødirektoratet 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 glufosinattolerante bomullssorten LLCotton25 (unik kode ACS-GØØ1-3) fra Bayer CropScience er fremkommet ved genmodifisering av bomullshybriden Coker 312. Hensikten med LLCotton25 er motstandsdyktighet mot ugressmidler som inneholder glufosinat-ammonium, f.eks. Basta, Buster, Finale, Ignite, Liberty og Rely.

LLCotton25 ble godkjent til import, videreforedling og til bruk som mat og fôr under forordning 1829/2003 den 29. oktober 2008 (Kommisjonsbeslutning 2008/837/EC). Søknaden og godkjenningen omfatter ikke dyrking.

LLCotton25 ble første gang vurdert av VKMs faggruppe for GMO i 2005 (VKM, 2005) i forbindelse med den offentlige høring av søknad EFSA/GMO/NL/2005/13 i 2005. EFSAs endelig vurdering ble publisert i 2006 (EFSA, 2006a). LLCotton25 har også blitt brukt som en komponent i bomullhybriden GHB614 x LLCotton25 (søknad EFSA/GMO/NL/2010/77), som har blitt vurdert av EFSA (EFSA, 2014), men ikke av VKM.

Risikovurderingen av den genmodifiserte bomullen er basert på søkers dokumentasjon som er gjort tilgjengelig på EFSAs GMO Extranet, og uavhengige vitenskapelige publikasjoner, inklusiv vitenskapelige vurderinger fra EFSA (EFSA, 2006a) og VKM (VKM, 2005). Bortsett fra gjennomgang av nylig offentliggjort publikasjoner er resten av teksten i denne vurderingen en oppsummering av de tidligere VKM (VKM, 2005) og EFSA (EFSA, 2006a) vurderingene, som er vedlagt i hhv. Appendix I og II. For utfyllende detaljer henvises leserne til disse.

Vurderingen er gjort i henhold til tiltenkt bruk i EU/EØS-området, og i overensstemmelse med matloven, miljøkravene i genteknologiloven med forskrifter, først og fremst, forskrift om konsekvensutredning etter genteknologiloven. Videre er kravene i 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, 2006b, 2010a, 2011a, 2011b og 2011c) 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.

LLCotton25 er utviklet ved hjelp av *Agrobacterium*-mediert transformasjon til å uttrykke et modifisert *bar* -gen fra *Streptomyces hygroscopicus*. Genet koder for enzymet phosphinothricin-acetyl-transferase (PAT) som gir LLCotton25 en økt toleranse overfor glufosinat-ammonium baserte ugressmidler.

Molekylær karakterisering

Den molekylære karakteriseringen fra søker viser at det kun er inkorporert én intakt kopi av det transgene innskudds-DNAet (T-DNAet) bestående av en *35S* promoter og *bar*-genet som til sammen fører til uttrykk av proteinet PAT. Proteinmålinger utført på prøver av LLCotton25 viser at PAT proteinet er uttrykket og aktivt i blad, stengel, rot, pollen og bomullsfrø og hovedsakelig i blad. Det er identifisert 26 mulige nye åpne leserammer (ORFs), i og ved den innsatte T-DNA-sekvensen i plantens genom, hvorav kun tre er antatt å kunne føre til produksjon av korte genprodukt i LLCotton25. Databasesøk utført av søker viser derimot ingen likheter mellom de tre antatte genproduktene og kjente toksiner eller allergener. Southern analyser, ELISA, og nedarvingsmønstre over flere generasjoner bekrefter at de introduserte genetiske elementene er stabilt nedarvet og samsvarer med de observerte fenotypiske egenskapene til LLCotton25.

Ut i fra dagens kunnskap og informasjon fra søker, konkluderer VKMs faggruppe for GMO med at den molekylære karakteriseringen av de tilsiktede endringene i LLCotton25 er tilstrekkelig og at det ikke er identifisert utilsiktede endringer som krever spesifikk oppfølging i den videre vurderingen.

Komparative analyser

Søker har utført flere feltforsøk i USA i 2000 og 2001 med påfølgende analyse av næringsstoffer, antinæringsstoffer og andre relevante, biologisk aktive stoffer i bomullsfrø, bomullsfrømel, urenset og renset bomullsfrøolje og øvrig prosessert plantemateriale. Registrering av agronomiske og fenotypiske egenskaper har blitt utført under feltstudier i USA, Australia og Brazil over flere år. For alle feltstudiene ble data fra LLCotton25 sammenlignet med konvensjonell kontroll Coker 312.

Tilgjengelig data fra søker viser at med unntak av den ønskede endringen, var det ingen biologisk relevante forskjeller i enkeltparametere mellom den genmodifiserte LLCotton25 og konvensjonell kontroll Coker 312. De registrerte statistisk signifikante forskjellene varierte mellom lokalitet og/eller år, og nivåene lå innenfor eller svært nær spredningen i verdier rapportert for andre bomullssorter. Forskjellene skyldes sannsynligvis den naturlige variasjonen for de enkelte parameterne.

Ut i fra dagens kunnskap, og med unntak av det introduserte proteinet PAT, konkluderer VKMs faggruppe for GMO med at LLCotton25 er vesentlig lik konvensjonell kontroll og andre bomullssorter med hensyn til næringsstoffsammensetning og agronomiske og fenotypiske egenskaper.

Helserisiko

Et 33-dagers fôringsforsøk med broilere har blitt utført med bomullsfrømel fra LLCotton25, konvensjonell kontroll Coker 312 og en annen konvensjonell bomullssort. Studien viste ikke negative effekter eller andre relevante forskjeller hos broilere gitt fôr med frømel fra LLCotton25 sammenlignet med de konvensjonelle bomullssortene. Databasesøk viser ingen relevante sekvenslikheter mellom PAT-proteinet og kjente toksiner eller IgE-avhengige allergener, og er ikke rapportert å ha forårsaket IgE-medierte allergiske reaksjoner. Det foreligger derfor ikke data som tilsier at PAT proteinet vil føre til toksiske eller IgE-medierte allergiske reaksjoner fra mat og fôr som inneholder LLCotton25 sammenlignet med konvensjonelle bomullssorter.

Ut i fra dagens kunnskap og tiltenkt bruk, konkluderer VKMs faggruppe for GMO med at LLCotton25 er ernæringsmessig lik og like trygg som konvensjonell kontroll Coker 312 og andre bomullssorter.

Miljørisiko

Miljørisikovurderingen av LLCotton25 er avgrenset til mulige effekter av utilsiktet spredning av spiredyktige frø i forbindelse med transport og prosessering, samt indirekte eksponering gjennom gjødsel fra husdyr fôret med den genmodifisert bomullen. Faggruppen har ikke vurdert mulige miljøeffekter knyttet til dyrking av LLCotton25 i Norge.

Genmodifiseringen av LLCotton25 har ikke medført endringer i egenskaper knyttet til fitness, oppformering eller spredning sammenlignet med konvensjonell bomull, og det er ingen indikasjoner på økt sannsynlighet for spredning og etablering av viltvoksende bomullplanter fra utilsiktet frøspill av LLCotton25. Bomull 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. Det er ingen indikasjoner for at nyinnsatte gener fra LLCotton25 vil kunne overføres horisontalt til mikroorganismer i mage-tarm trakt eller i jord eller vann, ved høyere frekvenser enn fra de naturlig forekommende mikrobielle kildene til de innsatte genene.

Med bakgrunn i tiltenkt bruksområde, som ekskluderer dyrking, konkluderer VKMs faggruppe for GMO med at LLCotton25 ikke vil medføre miljørisiko i Norge.

Samlet vurdering

Ut i fra dagens kunnskap, og med unntak av den introdusert egenskapen, konkluderer VKMs faggruppe for GMO med at LLCotton25 har lik næringsstoffsammensetning, og er ernæringsmessig, fenotypisk og agronomisk lik og like trygg som konvensjonell kontroll og andre bomullssorter.

Med bakgrunn i tiltenkt bruksområde, som ekskluderer dyrking, konkluderer VKMs faggruppe for GMO med at LLCotton25 ikke vil medføre miljørisiko i Norge.

Abbreviations and/or glossary

4ocsΔMas2	'Mannopine synthase promoter from *Agrobacterium tumefasiens* plasmid *pTi15955*
Abiotic	Of or characterised by the absence of life or living organisms
Annuals	A plant that complete its life cycle within one year, then dies
ARMG	Antibiotic resistance marker gene
Bt	*Bacillus thuringiensis*
bw	Body weight
Crude fiber	Fibrous food residue that is left over after treatment with dilute acid and alkali
Cultivar	A race or variety of a plant that has been intentionally created or selected and maintained through cultivation
Delinted	Pertains to cottonseed from which any leftover lint (see below) has been removed
DNA	Deoxyribonucleic acid
Dw	Dry weight
Dwt	Dry weight tissue
EC	European Commission
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
ERA	Environmental risk assessment
EU	European Union
FAO	Food and Agriculture Organisation
Fitness	Describes an individual's ability to reproduce successfully relative to that of other members of its population.
Glandless cotton	Genotypes of cotton that are devoid of the gossypol-containing glands distributed in various tissues of the cotton plant
GM	Genetically modified
GMO	Genetically modified organism
GMP	Genetically modified plant
Hemizygous	The transformation process produces hemizygous plants, i.e. the transgene is inserted without an allelic counterpart (i.e. Cry1A/-; CryF/-;PAT/-) that are inbred to generate selected homozygotes for the transgene in the final GMOs
IgE	Immunoglobulin E
ILSI	International Life Sciences Institute
In planta	Within the living plant
Lint	Leftover fibres attached to the cottonseed following deseeding of the cotton boll
Linted	Cottonseed with leftover fibres (lint) attached
mRNA	Messenger RNA
MT/NFSA	Norwegian Food Safety Authority (Mattilsynet)

NDF	Neutral detergent fibre, measure of fibre used for animal feed analysis. NDF measures most of the structural components in plant cells (i.e. lignin, hemicellulose and cellulose), but not pectin.
Northern blot	A technique used to study gene expression by detection of RNA or cDNA separated in a gel according to size.
Novel gene(s)	Newly introduced gene(s) as a result of genetic modification
NTO	Non-target organism
OECD	Organisation for Economic Co-operation and Development
ORF	Open Reading Frame; a molecular reading frame that can code for amino acids between two successive stop codons.
PAT	Phosphinothricin-acetyl-transferase
PCR	Polymerase chain reaction, a technique to amplify DNA by copying
Perennial	Plant that lives for more than two years
Selfing	Self-pollination. Pollen grains from the anther are transferred to the stigma of the same flower
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis. Technique to separate proteins according to their approximate size
Southern blot	Method used for transfer of electrophoresis-separated DNA fragments to a filter membrane and possible subsequent fragment detection by probe hybridisation
Transgene copy number	Defined as the number of exogenous DNA insert(s) in the genome. If the exogenous DNA fragment inserts only once at a single locus of the genome, it is a single copy transgenic event.
Western blot	Technique used to transfer proteins separated by gel electrophoresis by 3-D structure or denaturated proteins by the length of the polypeptide to a membrane, where they might be identified by antibody labelling.

Background

On 7 March 2005, the European Food Safety Authority (EFSA) received from the Dutch Competent Authority an application (Reference EFSA/GMO/NL/2005/13) for authorisation of the glufosinate-tolerant genetically modified LLCotton25 (Unique Identifier ACS-GHØØ1-3), 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/2005/13 and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed the EU- and EFTA Member States (MS) and the European Commission and made the summary of the dossier publicly available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of regulation (EC) No 1829/2003. Following receipt of additional information from the applicant, EFSA declared on 5 August 2005 that the application was valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the EC and consulted nominated risk assessment bodies of the MS, including the Competent Authorities within the meaning of Directive 2001/18/EC (EC 2001), following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Within three months following the date of validity, all MS could submit via the EFSA GMO Extranet to

EFSA comments or questions on the valid application under assessment. The VKM GMO Panel assessed the application in connection with the EFSA official hearing, and submitted a preliminary opinion in December 2005 (VKM, 2005). EFSA published its scientific opinion 6 December 2006 (EFSA, 2006a), and LLcotton25 was approved for food and feed uses, import and processing 29 October 2008 (Commission Implementing Decision 2008/837/EC).

LLCotton25 has been used as a component of the stacked GM event GHB614 x LLCotton25 (EFSA/GMO/NL/2010/77), which has been evaluated by EFSA (EFSA, 2014), but not by VKM.

Terms of reference

The Norwegian Environment Agency 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, 2006b, 2010a, 2011b and 2011c), 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

The current food, feed and environmental risk assessment of the genetically modified LLcotton25 is assessed with reference to the intended use. The risk assessment is based on information provided by the applicant in the application EFSA/GMO/NL/2005/13, relevant peer-reviewed scientific literature, and scientific opinion and comments from VKM (VKM, 2005), EFSA (EFSA, 2006a) and other member states made available on the EFSA website GMO Extranet. Except for a synopsis of more recent literature, this draft opinion is to a large extent a summary of the above-mentioned VKM and EFSA reports, which are provided in Appendix I and II, respectively, and readers are referred to these for details.

LLCotton25 has been used as a component of the stacked GM event GHB614 x LLCotton25 (EFSA/GMO/NL/2010/77), which has been evaluated by EFSA (EFSA, 2014), but not by VKM.

Genetically modified LLcotton25 (Unique Identifier ACS-GHØØ1-3) is derived from the cotton variety Coker 312 which was transformed by *Agrobacterium*-mediated gene transfer (Zambryski, 1992). LLCotton25 was genetically modified to express the *bar* gene. The *bar* gene encodes the enzyme phosphinothricin-acetyl-transferase (PAT), which confers tolerance to the herbicide glufosinate ammonium (commercial names Liberty®, Basta®).

Molecular analysis shows that LLCotton25 contains a single insert and does not retain backbone sequences from the vector. The purpose of the modification is to allow for effective weed control during the cultivation of LLCotton25. The genetic modification in LLCotton25 is intended to improve agronomic performance only and is not intended to influence the nutritional properties, the processing characteristics or the overall use of cotton as a crop.

The source of the *bar* gene is the bacterium *Streptomyces hygroscopius* strain ATCC21705 (Murkami et al., 1986).

Streptomyces hygroscopius belongs to the *Streptomyceta*, and is generally soil-borne, although it may be isolated from water. *Streptomyces* are not typically pathogenic to animals or humans, and few species have been shown to be phytopathogenic (Bradbury, 1986; Kutzner, 1981). The bacteria *S. hygroscopius*, also naturally produces the toxin bialaphos, which is an effective broad-spectrum herbicide. The PAT enzyme prevents autotoxicity in the bacterial organism and generates complete resistance towards high doses of PPT by acetylating the free amino group of PPT, bialaphos or the synthetically produced glufosinate-ammonium.

Glufosinate ammonium (also referred to as phosphinothricin; PPT) is a non-selective, contact herbicide that is phytotoxic to many broadleaf and grassy weeds. Glufosinate-ammonium inhibits glutamine synthetase, leading to glutamine deficiency, ammonia accumulation and eventually to plant death. The PAT protein in LLCotton25 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.

LLCotton25 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, 2006b and 2011b), the environmental risk assessment of GM plants (EFSA, 2010a), the selection of comparators for the risk assessment of GM plants (EFSA, 2011a), and for the post-market environmental monitoring of GM plants (EFSA, 2011c).

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

2 Molecular characterisation

2.1 Previous molecular assessment

The VKM and EFSA GMO Panels (VKM, 2005, Appendix I; EFSA, 2006a, Appendix II) have previously assessed the molecular characterisation of the cotton event LLCotten25 (inserted *bar*-gene) with regards to the following:

1. The transformation system and vector construct
2. Characterisation of transgene insertion and construct
3. Information on the expression of the insert
4. Analyses of new open reading frame(s) (ORFs)
5. Inheritance and the stability of the inserted DNA

Both Panels concluded that the applicant had provided sufficient analyses for the molecular characterisation. Initially, cotton tissue from *Gossypium hirsutum*, variety Coker 312, was transformed by *Agrobacterium tumefaciens* mediated gene transfer with the binary plasmid vector pGSV71. The vector contained the T-DNA region, with the left and right borders (LB and RB) delimiting a gene construct for expression of a modified *bar* gene derived from *Streptomyces hygroscopicus*. The *bar* gene encodes the enzyme phosphinothricin acetyltransferase (PAT) that acetylates L-glufosinate-ammonium and provides tolerance to glufosinate-based herbicides. This makes event LLCotten25 tolerant to herbicides based upon glufosinate ammonium such as Basta®, Buster®, Finale®, Ignite®, Liberty® and Rely®. In addition to the *bar*-gene the inserted T-DNA sequence in LLCotten25 contains the 35S promoter from Cauliflower Mosaic Virus, and the 3'nos terminator sequence including the 3' untranslated region of the nopaline synthase gene from the T-DNA of pTiT37 (nopaline Ti plasmid).

A number of molecular analyses, Southern and Northern hybridisations, PCR, BLAST searches and ELISA, have been performed by the applicant to determine the number of insertion sites, copy number, integrity of the insert, evaluation of the presence or absence of plasmid backbone sequences, expression levels of *bar,* and levels of PAT protein. The cotton variety Coker 312 was used as the negative control for these analyses.

The results show the presence of a single insertion site consisting of one copy of the *bar* gene construct in LLCotten25 of 2319 bp, equal to the original transgene cassette in vector pGSV71. No vector backbone sequences were detected in LLCotten25. The inserted *bar* gene contains two intended sequence alterations compared to the native *bar* gene derived from *Streptomyces hygroscopicus*. The GTG translation initiation codon has been changed to the ATG translation codon, and the second codon of the *bar* gene (AGC encoding serine) has been modified to GAC (encoding aspartic acid) to ensure correct translation initiation in plants.

PAT protein expression levels were measured by ELISA with samples from plants grown under greenhouse conditions. Stems, roots, seeds, leaves and pollen, from both glufosinate-treated and untreated LLCotton25 plants, were examined. The PAT protein was detected in all tissues tested. The level of PAT protein accumulation was measured as PAT protein content relative to total extractable protein. Leaves and stems contained more PAT than roots, and considerably more than seeds and pollen. The average level of PAT protein in four growth stages of the life cycle of the plant (2-4 leaf-stage, 4-6 leaf-stage, early and full flowering stages) ranged from approximately 58 to 98 µg PAT/g fresh weight (fw) in both, glufosinate-treated and untreated leaf samples. PAT protein content declined in the later growth stage in leaves of both treated and untreated LLCotton25. PAT protein comprised an average of 0.21-0.35% of the total crude protein in the leaves of LLCotton25.

Insertion of the gene cassette introduced 26 novel ORFs for putative peptides spanning the 5-prime upstream and 3-prime downstream junctions of the inserted DNA. According to the applicant, only three were found to potentially give rise to short putative peptides. Further bioinformatics analysis of these three ORFs revealed no relevant sequence homologies between the theoretically predicted translation products with known toxins or allergens.

The stability of the insert in LLCotton25 plants was analysed by Southern hybridisation of leaf tissues over multiple generations. The expected integration pattern was present in all samples analysed. Phenotypic stability was demonstrated by Mendelian inheritance of the glufosinate-ammonium tolerance trait over multiple generations and field locations.

2.2 Conclusion

Based on current knowledge and information provided by the applicant, the VKM GMO panel concludes that the intended changes in LLCotton25 have been sufficiently characterised, and that no unintended changes have been identified that requires particular attention in the further assessment.

3 Comparative assessments

Compositional and agronomic data provided by the applicant from various field trials with LLCotton25 has previously been assessed as food and feed by the VKM GMO Panel (VKM, 2005; Appendix I) commissioned by the Norwegian Food Safety Authority and the Norwegian Environment Agency related to the EFSAs public hearing of the application EFSA/GMO/NL/2005/13 in 2005 and in EFSA's final opinion (EFSA, 2006a; Appendix II). A brief summary from these reports are provided below.

3.1 Production of material for comparative assessment

For compositional studies, LLCotton25 was compared to its parent non-GM variety Coker 312, which is a commercial cotton variety grown in the Southern US since 1990. The comparison also included data from the scientific literature regarding the natural ranges of key compounds in various conventional cotton cultivars. Field trials were performed in year 2000 and 2001 in Arkansas, Georgia, Mississippi, Missouri, North Carolina and Texas, all belonging to the cotton growing regions of Southern United States. Each year trials were performed at 15 locations, three treatments at each location and three replications per treatment (except in one site where the sample plot was harvested three times per treatment). One site was excluded for the analysis of fatty acids as different methods had been used for the different treatment samples within the site. The three treatments consisted of: (a) non-GM cotton Coker 312 grown using conventional herbicide weed control, (b) GM LLcotton25 grown using conventional herbicide weed control, and (c) GM LLcotton25 grown with glufosinate-ammonium (Liberty®) herbicide weed control. Isolation distances of 12 m were maintained in order to avoid cross-pollination and herbicide treatment drift.

Compositional analysis was performed on whole, linted (fuzzy) cottonseed, delinted cottonseed, untoasted cottonseed meal, toasted cottonseed meal, cottonseed oil, both crude and refined, deodorised (proximates, fatty acids and tocopherol); linters (proximates only) and cotton seed hulls (proximates only) obtained from LLCotton25 and the parent line Coker 312 from the field trials conducted in the USA in 2000 and 2001. For the whole, linted cottonseeds, all material from all 15 sites in both 2000 and 2001 were analysed. For the other cottonseed products, cottonseeds from two sites for each year were processed to provide samples. Unless otherwise specified (see above), the samples were analysed for the components of importance for cotton and its various products used as food/feed as defined by the OECD consensus document for cotton (OECD, 2004), including proximates, amino acids, fatty acids, vitamin E, minerals, and the anti-nutrients gossypol, cyclopropenoid fatty acids and phytic acid, a total of 52 components. The statistical analysis of the data was carried out using a commercially available statistical package (SAS version 6.12).

The applicant also provided information on agronomic performance and phenotypic characteristics derived from several field trials in the USA, Australia and Brazil during multiple seasons. The characteristics that were analysed in these studies included parameters related

to plant morphology, seeds and plant development, reproductive traits, disease and pest susceptibility, weediness, weed control, volunteers, yields, cotton seed and fibre quality.

3.2 Compositional analysis

For data on whole, linted cottonseeds pooled from the 15 sites analysed over two years, statistically significant differences were observed for calcium and the cyclopropenoid fatty acid dihydrosterculic acid content between conventional counterpart Coker 312 and LLCotton25 treated with conventional herbicides. No differences were found between Coker 312 treated with conventional herbicides and LLCotton25 treated with Liberty herbicide. However, the differences were small and the concentrations of both analytes were within the range of values reported for other conventional cotton cultivars. Statistical comparison between the composition of LLCotton25 treated with conventional herbicides and LLCotton25 treated with Liberty were not provided by the applicant, but differences in data provided for these two groups were consistently small. Values for nearly all analytes provided for Coker 312 and conventionally and Liberty-treated LLCotton25 were within or close to the range of values reported for other conventional cotton cultivars, with the notable exception of free gossypol, which were higher in all three tested groups compared to the reported range of values. However, no statistically significant differences in total or free gossypol levels were observed in LLcotton25, treated either with conventional herbicides or Liberty, compared to the conventional counterpart Coker 312. Furthermore, the applicant pointed out that gossypol levels in the three tested groups were within the range reported by the International Life Science Institute's (ILSI) crop composition database (www.cropcomposition.org).

For the other cotton products analysed, statistically significant differences between Coker 312 and LLCotton25 were identified for a number of analytes for each product, but most of these were not considered biologically relevant as they were generally within or close to the range of values available for other conventional cotton cultivars. Possible exceptions were identified for vitamin E and zinc: observed vitamin E levels in delinted cottonseeds were variable and highest in Coker 312, and zinc levels in delinted cottonseeds and untoasted and toasted cottonseed meals from material harvested in 2000 were higher than literature values, especially for LLCotton25 treated with Liberty. The high zinc levels were attributed by the applicant to environmental conditions and/or contamination.

3.3 Agronomic traits and GM phenotype

The data from the field trials conducted in USA, Australia and Brazil showed that LLCotton25 did not differ significantly in terms of plant morphology, growth, agronomic performance, and susceptibility to diseases and pests from the non-transformed parent Coker 312. LLCotton25 did not exhibit any increased tendency towards weediness, compared to the unmodified parental line.

3.4 Conclusion

The VKM GMO Panel has considered the data supplied by the applicant on compositional, agronomic and phenotypic characteristics and confirms that with the exception of the new protein, no biologically relevant differences were observed between LLCotton25, the conventional counterpart Coker 312, and other cotton cultivars. The few statistically significant differences observed were only present in material from some of the locations in some years, and the values were within or close to the range of historical values observed in conventional cotton cultivars. The differences were therefore considered to reflect the natural variability of the analytes.

Based on current knowledge and excluding the new PAT-protein, the VKM GMO Panel concludes that LLCotton25 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart and other cotton cultivars.

4 Food and feed safety assessment

Spain and Greece are the only two EU member states that grow cotton, and Greece is the largest cotton growing country in Europe. Greece's MY (Marketing Year) 2013/14 cotton production was 200,000 MT (Metric Tons) (Gain Report, 2014a), and Spain's MY 2013/2014 cotton production was 145,000 MT (Gain Report, 2014b). No GM cotton is planted in these two countries.

Bulgaria produces cotton on less than 1 000 ha. Cotton production has ceased in Italy in 1991 and in Portugal in 1996.

LLCotton25, Trade Name: Fibermax™ Liberty Link™ was first cultivated in the USA in 2003 (food, feed, cultivation).

4.1 Previous evaluations by the VKM and EFSA GMO panels

LLCotton25 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 hearings of the application EFSA/GMO/NL/2005/13 in 2005 (VKM, 2005; Appendix I). EFSA has also published a final opinion on LLcotton25 (EFSA, 2006a; Appendix II). The VKM GMO Panel and EFSA concluded that LLCotton25 was nutritionally equivalent to conventional cotton cultivars and it was unlikely that the inserted protein would cause toxic or allergic reaction to food or feed containing LLCotton25 compared to conventional cotton.

4.2 Product description and intended uses

According to the applicant, the genetic modification in LLCotton25 will not impact the existing post-harvest production processes used for cotton. Cotton is mainly grown for its commodity product the cotton boll. The fibres on the cotton boll are separated from the seeds by a cotton gin machine. The fibres, which consist mainly of cellulose, are primarily used for textiles, but also have some application for food or feed (see Figure 4.2-1). Especially the fibres that are too short to be spun into textiles can be used as food additives. Cellulose and methylcellulose can be used as thickeners, stabilisers, emulsifiers, or fillers. The protein- and oil-rich whole cottonseeds (WCS) are used for oil extraction and the oil is used in food and feed. Following oil extraction, the cottonseed can be processed into various other side-products, such as cottonseed meal, various protein preparations, and cottonseed milk, all used in food and feed. Protein-rich cottonseed meal is mostly used as an animal feed ingredient. Another major processed product derived from cottonseed is the fibre-rich hulls, which may also be used in animal feeds (Figure 4.2-1). For more information see Appendix III.

Cottonseed and its derived products have a history of safe use in foods and feeds as long as dietary intake of the naturally occurring toxicants gossypol and cyclopropenoid fatty acids is restricted to acceptable levels. This is accomplished either by processing to reduce or eliminate these toxicants or by limiting the inclusion level of cottonseed products in foods and feeds. Current EU regulations (Annex I of Council Directive 2002/32/EC; as assessed in EFSA, 2008) specifies maximum levels of free gossypol in various feed commodities and animal feeds. For more information see Appendix III.

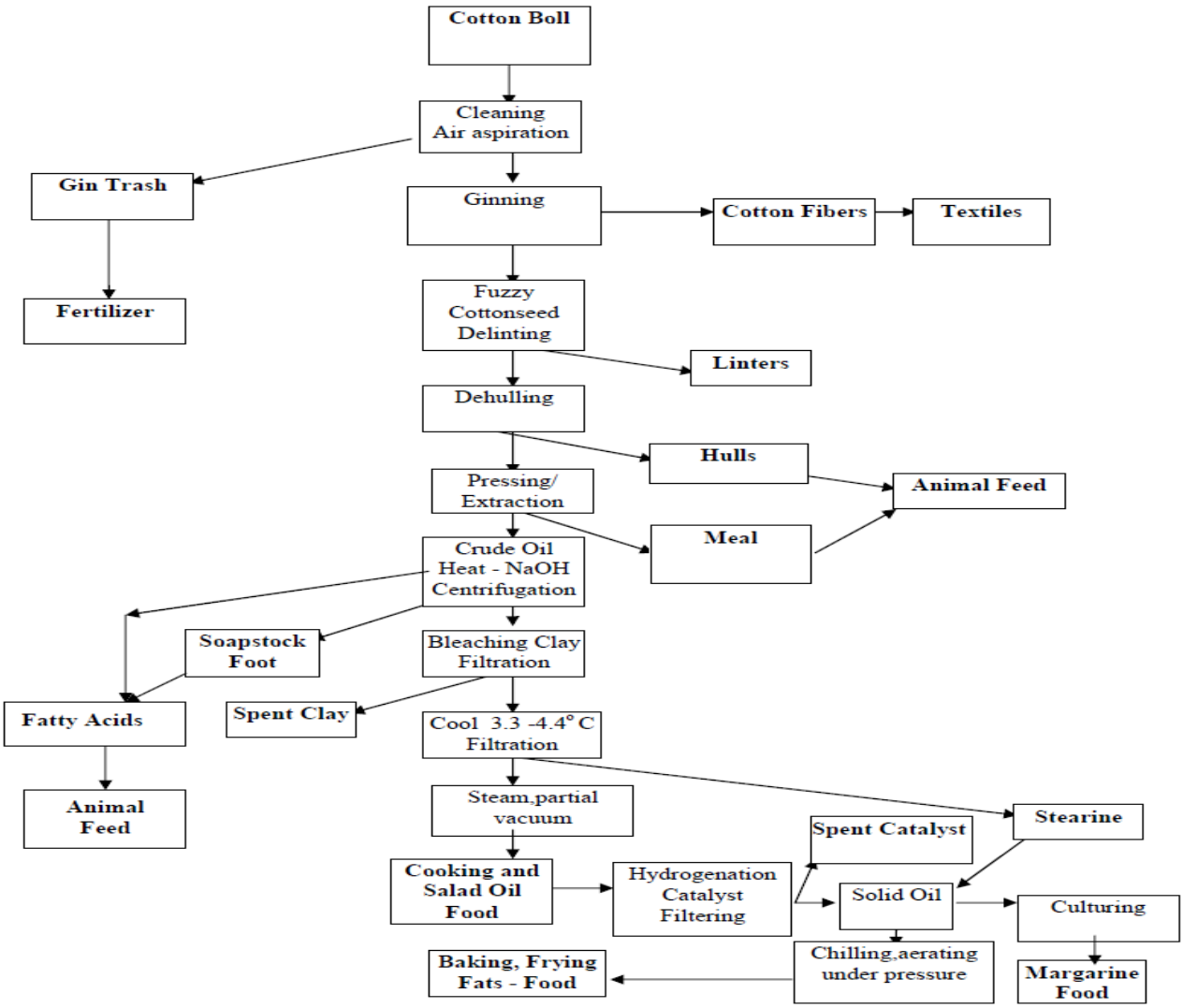

Figure 4.2-1: Processing of cotton boll, adapted from OECD (2004)

4.3 Effects of processing

According to the applicant, the commercial experiences have confirmed that the production and processing of LLCotton25 do not differ from the production and processing of the equivalent foods and feeds originating from conventional cotton cultivars.

4.3.1 Effects of processing on whole cotton products

The processing steps that are used to produce the various cotton products are shown in figure 4.2-1. The processing of whole cottonseed (WCS) may include delinting, dehulling, crushing, flaking, extruding, extracting, roasting, bleaching and deodorizing. WCS are first cracked and de-hulled, then heated to approximately 60°C, ground to flakes with rollers, and are then treated with solvent to remove the oil. The flakes are toasted (steamed), cooled and ground. Roasting (baking; dry heat), extruding, and cracking whole cottonseed has improved digestibility in some trials but also has increased the availability of free gossypol in several circumstances. By-products of processing can be included in human diet, such as linters and oil, or in animal diet such as hulls and cottonseed meal. For more information see Appendix III.

Cottonseed from LLCotton25 contains comparable levels of the naturally occurring toxicants gossypol and cyclopropenoid fatty acids relative to its conventional cotton counterpart and other conventional cultivars (see section 3.2). Therefore, processing to reduce or remove these toxicants, or practices used to limit their levels in foods and feeds are not expected to change.

4.3.2 Effect of processing on PAT proteins

The processing steps used to produce various cotton products are shown in Figure 4.2-1. According to information provided by the applicant, the processing conditions used for cottonseed and oil will reduce the PAT protein to very low or non-detectable levels in hulls and cottonseed meal, and were not detectable in refined oil.

4.4 Toxicological assessment of LLCotton25

4.4.1 Toxicological assessment of the expressed novel protein

The PAT protein expressed in LLCotton25 is also expressed in numerous other genetically modified plants that have been assessed and considered safe by both VKM and EFSA, and has also been reviewed by others (OECD, 1999; Herouet et al., 2005). The toxicological evaluation of PAT protein produced by *E. coli* was conducted by Pfister et al. (1999), which has since then formed the basis for the safety assessment of other transgenic crops expressing the *pat* gene (see below).

The applicant's Technical Dossier provides the following data regarding the toxicological assessment of the expressed novel proteins in LLCotton25:

- Acute toxicity testing of PAT protein with mice
- Degradation in simulated digestive fluids
- Thermolability (see section 4.3.2)
- Amino acid sequence comparisons with known toxins and allergens (see also sections 2.1 and 4.4.3; EFSA, 2006a)

Otherwise the applicant refers to previously generated data from repeated dose toxicity trials conducted by others (see below).

Due to the low levels of PAT in cotton and the difficult task of isolating a sufficient quantity of purified protein from the cottons, the acute toxicity testing studies described and referred to in the Applicant Dossier were conducted with PAT protein produced in *Escherichia coli*. The applicant has performed analysis of structural similarity, physicochemical and functional equivalence of the microbially-produced PAT protein and the proteins produced by the cotton. These indicate that plant-produced and bacterially-produced PAT proteins are biologically, biochemically, and immunologically equivalent. PAT protein has been shown to be rapidly degraded in simulated gastric fluid.

4.4.1.1 *Acute toxicity testing of novel protein PAT*

The applicant provided data on an acute toxicity study in mice with a PAT protein encoded by the *bar* gene generated in *E. coli*. Because of the expected efficient proteolytic degradation in digestive environments, the potential toxicity of the protein was studied after intravenous injection at the dose levels of 1 and 10 mg/kg body weight. At 10 mg/kg body weight, no signs of systemic toxicity were observed.

The VKM GMO panel agrees with the EFSA's guideline (EFSA, 2011b) that acute toxicity testing of newly expressed proteins is discouraged since this is of little additional or applicable value to the risk assessment for human and animal consumption of food and feed derived from GM plants. The VKM GMO panel recognizes that the applicant submitted the application prior to the last guidance document from EFSA.

4.4.1.2 *Repeated dose toxicity testing*

The applicant has not provided data from repeated dose toxicity trials with the novel protein PAT expressed in LLCotton25. However, a trial has been conducted with this protein and used in the assessment of numerous other transgenic crops with the same inserted *pat* genes. This is summarised below.

A repeated dose feeding study of reduced duration (14-day) relative to prevailing guidelines (OECD guideline 407; OECD, 1995) was conducted in rats with the PAT protein encoded by the *pat*-gene generated in *E. coli* (Pfister et al., 1999). Groups of five male and female

Wistar rats (HanIbm: WIST) received diets containing the PAT protein (lyophilized powder) at levels of 0, 5 and 50 g/kg diet. The high level corresponded to a dose of 7.6 and 7.9 g/kg body weight/day for males and females, respectively. A reference group received standard rat diet. No remarkable findings were observed apart from statistically significant increases in blood cholesterol levels in males of groups fed the 5 and 50 g PAT-supplemented diets and blood phospholipid levels in females fed the 50 g and males fed the 5 and 50 g PAT-supplemented diets. The applicant concluded that since these effects were also observed in the control, unsupplemented group (0 PAT protein), they were not regarded as toxicologically relevant.

According to the OECD guideline 407, the duration of exposure should normally be 28 days. Although a 14-day study may be appropriate in certain circumstances, justification for use of a 14-day exposure period should be provided. No justification for using 14-days was found in the report.

4.4.2 Toxicological assessment of the whole GM food/feed

4.4.2.1 *90-day sub-chronic toxicity study of whole GM food/feed*

No 90-day sub-chronic oral toxicity study with LLCotton25 has been performed by the applicant. Since the compositional studies indicated that LLCotton25 was compositionally equivalent to its conventional counterpart Coker 312 and other cotton cultivars, and the molecular and compositional analyses did not indicate any unintended effects of the genetic modification, EFSA concluded that further toxicity studies with laboratory animals were not needed (EFSA, 2006a).

4.4.3 Allergenicity

The strategies used when assessing the potential allergenic risk focus on the characterisation of the source of the recombinant protein, the potential of the newly expressed protein to induce sensitisation or to elicit IgE-dependent allergic reactions in already sensitised persons and whether the transformation may have altered the allergenic properties of the modified food. A weight-of-evidence approach is recommended, taking into account all of the information obtained with various test methods, since no single experimental method yields decisive evidence for allergenicity (Codex Alimentarius, 2003; EFSA, 2006b and 2010b).

4.4.3.1 *Assessment of allergenicity of the newly expressed protein*

In order to assess the potential for introduced IgE-dependent allergens in LLCotton25 sequence evaluation schemes were used to assess the similarity of the PAT protein to known protein allergen sequences contained in several widely accepted databases. An immunologically significant sequence identity requires a match of at least eight contiguous identical amino acids. In studies conducted on the PAT protein, no immunologically significant sequence identity was detected, indicating no homology to known IgE-dependent allergens, based on amino acid sequences in PAT.

In vitro simulated gastric fluid (SGF) digestibility studies were also conducted on the protein. According to the applicant PAT was rapidly digested and no longer detectable by SDS-PAGE or western blot analysis within one minute of exposure to SGF. Thermolability results for PAT protein also indicated that the protein was not biologically active following exposure to elevated temperature (>75°C).

The results of these studies indicate that the PAT protein does not exhibit characteristics commonly attributed to an IgE-dependent allergenic protein.

4.4.3.2 *Assessment of allergenicity of the whole GM plant*

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

This issue does not appear relevant since cotton is not considered to be a common allergenic food, and only rare cases of occupational allergy have been reported.

4.4.3.3 *Assessment of allergenicity of proteins derived from the GM plant*

Food products from cottonseed are limited to highly processed products due to the presence of the natural toxicants gossypol and cyclopropenoid fatty acids in the seed. These substances are removed or reduced by processing (OECD, 2004).

The main cottonseed product in human food, cottonseed oil, is highly purified. Edible oils that are refined, bleached and deodorised do not appear to pose a risk to allergic individuals, as they contain virtually no proteins. Linters are also highly processed (alkaline pH, high temperature) to remove non-cellulose components. Linters are composed of greater than 99 % cellulose, and are a major source of cellulose for chemical and food use.

Exposure to proteins through consumption of oil and linters derived from LLCotton25 would be very low to negligible.

4.4.4 Assessment of adjuvanticity

According to the EFSA Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed from GM plants (EFSA, 2010b) and the VKM risk assessment of the adjuvant properties of Cry-protein (VKM, 2012), adjuvants are substances that, when co-administered with an antigen increases the immune response to that antigen and therefore might increase the allergic response. Adjuvanticity has not been routinely considered in the assessment of allergenicity of GMOs.

LLCotton25 contains the PAT protein. Interaction between the newly expressed PAT protein impacting on allergenicity and/or adjuvanticity is not expected given the lack of indications of allergenicity and adjuvanticity of the protein. Also, there is no information available on the structure or function of the newly expressed PAT protein that would suggest an adjuvant effect resulting in or increasing an eventual IgE response to a bystander protein. In cases when known functional aspects of the newly expressed protein or structural similarity to known strong adjuvants may indicate possible adjuvant activity, the possible role of this protein 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.

4.5 Nutritional assessment of GM food and feed

Cottonseed oil and processed cotton linters are the primary cotton products used for human food. Both products undergo extensive processing procedures before use for human consumption. The processed linter pulp product is composed of almost pure cellulose, and is used in food mainly in the production of casings for bologna, sausages, and frankfurters. However, the total amount of linters used is very small. Cotton fibre is used in ice cream and salad dressings to increase viscosity (OECD, 2004).

Cottonseed meal is an important ingredient in animal feed. Depending on the oil extraction process, cottonseed meal is used in feed for cattle, monogastrics, and laying hens. Cottonseed meal is not used for human consumption in the EU, however, it has been approved for use in human food in the USA and other countries, when derived from gossypol-free varieties of cotton or after processing to remove the gossypol. Human consumption of cottonseed meal is reported mainly in Central American countries and India where it is used as a low cost, high quality protein ingredient.

Fat in cottonseed is mostly in the form of oil, and unsaturated fatty acids are the predominant fatty acids. The polyunsaturated fatty acid linoleic acid represents up to 50% of the total fat. Smaller quantities of oleic and palmitic acids are found in cottonseed oil.

The oil of conventional cottonseeds, particularly those of *Gossypium hirsutum*, generally contain about 0.5-1% of cyclopropenoid fatty acids (CPFA) such as malvalic, sterculic and dihydrosterculic acids. These fatty acids have been found to have deleterious effects on animal performance and various harmful effects on health (reproductive disorders, growth retardation and altered fat metabolism) in rainbow trout, rodents and poultry (OGTR, 2008). Rainbow trout fed glandless cottonseeds showed reduced weight gain and an increased prevalence of liver carcinomas (Hendricks et al., 1980). Glandless cottonseeds do not produce gossypol so the resulting effects have been attributed to CPFA (OTGR, 2008).

Analysis of cotton products derived from LLCotton25 confirmed that there is no detectable level of protein in either cottonseed oil or processed cotton linters.

4.5.1 Intake information/exposure assessment

According to FAO statistics (www.faostat3.fao.org), the total human consumption of cottonseed oil in the European Union was 17 500 metric tonnes in 2011. Consumption data of cottonseed products are not available for Norway. In the last five years, no registered import of cottonseed for use as food or feed in Norway was found in Statistics Norway's External Trade in Goods database (www.ssb.no). Thus, the intake of cottonseed products by humans and animals in Norway is considered to be negligible.

4.5.2 Nutritional assessment of feed derived from the GM-plant

Data from a 33-day broiler chicken nutritional assessment study with LLCotton25 was submitted by the applicant (Stafford, 2004). A total of 560 Ross #508 1 day-old chicks distributed in 56 pens with 10 birds/pen, 5 males and 5 females per pen, 14 replicate pens for each diet group, were given the same feed supplemented with 10% cottonseed meal from four different cotton plant sources: FiberMax (a current commercial non-transgenic variety), Coker 312 (non-transgenic isogenic control), as well as Liberty (glufosinate)-unsprayed and sprayed transgenic LLCotton25.

The nutritional assessment study was not conducted according to the latest EFSA guidelines (EFSA, 2011b), but the VKM GMO Panel recognizes that the applicant submitted the application prior to the last guidance document. The formulated experimental diets were analysed for proximates, amino acids, fibres, minerals, tocopherols, pesticides, PCBs and toxic metals, but not for the antinutrients free or total gossypol, cyclopropenoid fatty acids or phytic acid. The duration of the feeding trial was 33 days, which is shorter than the usually practiced study period of 42 days (ILSI, 2003 and 2007). According to the applicant, the feeding trial was shortened due to a shortage of feed. The VKM GMO Panel recognises that 33 days is within the range of regularly practiced production periods for broilers and includes the especially sensitive first 21-days of life when an approximate 15-fold increase in body weight is observed. The data reported from the 33-day broiler study can therefore be considered valid for the nutritional and toxic assessment of LLCotton25.

The data and report were generally produced in compliance with US EPA Good laboratory practice regulations (40 CFR, Part 160), OECD Principle of Good Laboratory Practice (ENV/MC/CHEM (98) 17) and Japan MAFF (59 NouSan, Notification No. 3850, Agriculture Bureau), with the exception of the routine water analyses and feed contaminant screening for pesticides, PCBs and toxic metals, which were conducted by external laboratories using standard U.S. EPA procedures, but who could not claim compliance to GLP procedures (e.g. no distinct protocol).

No statistically significant differences in total feed consumption, total weight gain, feed conversion to body weight (Table 4.6.2-1), survival, or mean chilled carcass weight among the cottonseed meal types tested were observed.

Table 4.5.2-1 Feed consumption, weight gain and feed conversion for broilers fed diets containing cottonseed meal from different sources

Cottonseed meal source	Mean feed consumption (g)						Mean total weight gain[1](g)	Feed conver-sion[2]
	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Total		
FiberMax™	101.5 ± 5.0	282.8 ± 15.8	552.6 ± 75.9	666.5 ± 49.6	356.5 ± 27.6	1963.6 ± 144.0	1114.0 ± 45.3	1.8
Coker 312	90.6 ± 6.8	280.0 ± 10.1	550.4 ± 77.6	688.0 ± 50.1	397.1 ± 50.0	2006.1 ± 119.0	1087.2 ± 93.3	1.9
LLCotton25 - unsprayed	104.6 ± 105.0	295.2 ± 295.0	587.0 ± 73.6	649.1 ± 48.2	364.9 ± 17.4	2000.7 ± 94.0	1071.5 ± 64.3	1.9
LLCotton25 - sprayed	99.8 ± 4.7	281.7 ± 12.1	533.8 ± 47.9	644.0 ± 33.4	360.4 ± 24.3	1919.8 ± 79.0	1097.7 ± 50.4	1.8

[1] Mean total weight gain = live weight on day 33 – live weight on day 0

[2] Feed conversion calculated as (total feed consumption)/(total weight gain)

However, statistically significant differences between treatment groups were observed for some carcass characteristics. For broilers fed the unsprayed LLCotton25, the mean breast weight was on average 8.9% lower than that of those fed the commercial variety FiberMax™, and thigh-weight was on average 7.3% lower than those fed either the commercial or isogenic control varieties. For broilers fed the Liberty-treated LLCotton25, however, no significant differences were observed in these variables compared to broilers fed the other three diets.

These results indicate that the cottonseed meal derived from LLCotton25 is nutritionally comparable with its near isogenic non-GM counterpart Coker 312 and the other conventional cultivars included in the study.

Feeding studies by independent investigators were not found by search in available databases.

4.6 Conclusions

A 33-day nutritional assessment trial with broilers did not reveal biologically relevant adverse effects or differences in the performance of animals fed diets containing cottonseed meal from LLCotton25 compared to conventional counterpart Coker 312 or another cotton cultivar. Bioinformatics analysis of the amino acid sequence of the PAT protein did not show sequence resemblance to known toxins or IgE-dependent allergens, nor has the protein been reported to cause IgE-mediated allergic reactions. It is therefore unlikely that the PAT protein will cause toxic or IgE-mediated allergic reactions to food or feed containing LLCotton25 compared to conventional cotton cultivars.

Based on current knowledge, and considering the intended uses, the VKM GMO Panel concludes that LLCotton25 is nutritionally equivalent to and as safe as its conventional counterpart Coker 312 and other cotton cultivars.

5 Environmental risk assessment

5.1 Introduction

Considering the scope of the application for the LLCotton25, which excludes cultivation, the environmental risk assessment is concerned with the accidental release into the environment of viable cotton seeds during transport and/or processing, and with indirect exposure to microorganisms in the gastrointestinal tract and in soil or water. The LLCotton25 line has tolerance to the glufosinate-ammonium herbicides. Use of glufosinate-ammonium is forbidden in Norway.

Genus *Gossypium* (Malvaceae) contains about 50 diploid or allotetrapleois species, four of these (*G. arboretum, G. barbadense, G. herbaceum* and *G. hirsutum)* are domesticated and cultivated (Brubaker et al., 1999). *G. herbaceum* and *G. hirsutum* have been cultivated in Southern Europe since the 19th century (Davis, 1967). Globally *G. hirsutum* is the most cultivated species today, and China, India, USA and Pakistan are the main producers of cotton (FAOSTAT, 2015). In Europe cotton is mainly grown in Greece and Spain, but five other countries have minor production (FAOSTAT, 2015).

G. hirsutum is originally a perennial plant, but the cultivars used today are grown as annuals. Cotton is adapted to tropical and subtropical conditions. *G. hirsutum* is tetraploid and mainly self-pollinated. Pollen grains are heavy and sticky, but pollen can be carried by bumble bees and bees. The degree of out-crossing varies between the cultivars, but generally it is very low (0-25%) (Xanthopoulos and Kechagia, 2000; Turley and Kloth, 2002). There are no native plant species in Europe which could hybridize with *G. hirsutum*. However, single plants of *G. herbaceum* and *G. hirsutum* have been found outside cultivated areas (Davis, 1967).

Being a tropical-subtropical plant, cotton is sensitive to low temperature. The optimum temperature for seed germination is 25-30°C and germination is inhibited at temperatures below 12-18°C, root growth is strongly reduced at temperatures below 20°C. Temperatures below 18°C result in chilling injuries (Stewart et al., 2010). Most of the commercial cultivars of cotton do not have any seed dormancy. For production of ripe seed, cotton needs a growth period of 120-200 days.

According to the national statistics, no food or feed grade cottonseed products have been imported into Norway in 2011-2015 (www.ssb.no/statistikkbanken).

5.2 Unintended effects on plant fitness due to the genetic modifications

Cotton is not a weed in Europe. Generally in Europe, spreading of cotton outside the cultivated areas is limited by the lack of seed dormancy and lack of tolerance to low temperatures. The genetic modifications of the lines in this assessment do not have any effects on seed dormancy or on temperature requirement for germination and growth. The fitness properties of the transgenic line LLCotton25 is similar to those of conventional, non-transformed cotton. Thus, under Norwegian conditions, it is highly unlikely that the seeds of the GM line of cotton will germinate, the growing season is too cold and short for production of ripe seed, and the plants or seeds cannot survive the winter. Further, feral populations of the modified cotton will have selective advantages only if exposed to glufosinate-ammonium. Consequently, the establishment of feral population of LLCotton25 in Norway is highly unlikely.

5.3 Potential for gene transfer

A prerequisite for gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer of DNA, or vertical gene flow via pollen or seed dispersal. Concerning the transgenic lines of cotton, gene transfer to microorganisms could take place in the digestive tract in humans and animals when cottonseed is used as food or feed, or in soil from faeces from animals fed with cottonseed. Under the Norwegian climatic conditions, gene flow via pollen or seed dispersal is not an issue. Use of extracted cottonseed oil as food or feed does not cause environmental concerns in Norway.

5.3.1 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 and Wackernagel, 2002; Bensasson et al., 2004; reviewed in EFSA, 2004 and 2009).

DNA is effectively degraded during digestion. The stability and uptake of DNA from the intestinal tract has been studied 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 feces 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. Nordgård

et al. (2012) concluded that, even after extensive ingestion of DNA, natural transformation of microorganisms in the gastrointestinal tract of rats was not detectable.

Considering the low level of exposure to recombinant DNA in connection with feeding cottonseed meal, horizontal gene transfer in the gastrointestinal system is highly unlikely.

5.3.2 Plant to plant gene flow

Cotton is not grown in Norway, establishment of feral populations from spilled seeds is highly unlikely, and there are no close relatives of cotton in the flora of Norway. Thus, gene flow from plant-to-plant is not an issue in Norway.

5.4 Interaction between the GM plant and the target organisms

Interaction between the transgenic lines of cotton and any target organisms is not an issue in Norway.

5.5 Interaction between the GM plant and the non-target organisms

Interaction between the transgenic lines of cotton and any non-target organisms is not an issue in Norway.

5.6 Potential interactions with the abiotic environment and biogeochemical cycles

Considering the intended uses of the LLCotton25, 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 are not considered an issue by the VKM GMO Panel.

5.7 Conclusion

Considering the intended uses of LLCotton25, which exclude cultivation, the environmental risk assessment is concerned with the accidental release into the environment of viable seeds during transport and/or processing, and with indirect exposure to microorganisms in the gastrointestinal tract and in soil or water, mainly via intestinal content and faeces from animals fed feeds containing LLCotton25.

With the exception of the introduced tolerance to the herbicide glufosinate-ammonium, LLCotton25 has no altered survival, multiplication or dissemination characteristics compared to conventional cotton cultivars, and there are no indications of an increased likelihood of spread and establishment of cotton plants in the case of accidental release of seeds from LLCotton25 into the environment. Cotton is not cultivated in Norway, and there are no cross-compatible wild or weedy relatives of cotton in Europe. Plant to plant gene flow is therefore not considered to be an issue. There are no indications that transfer of recombinant genes from LLCotton25 products to microorganisms in the gastrointestinal tract or in soil or water could occur at higher frequencies than from naturally occurring microbial sources.

Based on current knowledge and considering its intended uses, which exclude cultivation, the VKM GMO Panel concludes that LLCotton25 does not represent an environmental risk in Norway.

6 Post-market environmental monitoring

The objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC are to confirm that any assumptions regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment are correct and to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment which were not anticipated in the environmental risk assessment.

The environmental risk assessment did not identify any potential adverse environmental effects of the transgene lines of cotton. Thus, the general surveillance plan is sufficient and there is no need for a specific surveillance plan.

7 Conclusions

Molecular characterisation

The LLCotton25 genome has a complete, single integrated copy of the *bar*-expression cassette. Even though the PAT concentration is low, 0.21-0.35% of total crude protein in the leaves, it is highest in the plant parts exposed to herbicide treatment. This is consistent with the regulation by the inserted *35S* promoter, with highest activity in leaves and stems.

Out of 26 putative novel open reading frames (ORFs) identified in the GM cotton, only three short ORFs located in the 3' region of the insert were theoretically found to encode potential novel gene products. No relevant homologies were found between these theoretically predicted translation products and known toxins or allergens. Southern hybridisation, ELISA and segregation analyses show that the introduced gene elements were stably inherited and expressed over multiple generations in parallel with the observed phenotypic characteristics of the event.

Based on current knowledge and information provided by the applicant, the VKM GMO panel concludes that the intended changes in LLCotton25 have been sufficiently characterised, and that no unintended changes have been identified that requires particular attention in the further assessment.

Comparative assessments

Field trials have been conducted in the USA during 2000 and 2001 for compositional assessments of whole linted cottonseeds, cotton lint, and different processed cottonseed products. Field trials in USA, Brazil and Australia during numerous growing seasons were performed for agronomic and GM phenotype assessments. In all trials, LLCotton25 was compared to its conventional counterpart, parent line Coker 312. LLCotton25 was grown using conventional or glufosinate-based herbicide while cotton Coker 312 was grown using conventional herbicides.

With the exception of the changes caused by the introduced transgenic trait, data provided by the applicant revealed no biologically relevant differences between LLCotton25 and its conventional counterpart Coker 312. The few statistically significant differences observed were only present in material from some of the locations in some years and the values were within or close to the range of historical values observed in conventional cotton cultivars. The differences were therefore considered to reflect the natural variability of the analytes.

Based on current knowledge and excluding the new PAT-protein, the VKM GMO Panel concludes that LLCotton25 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart and other cotton cultivars.

Food and feed risk assessment

A 33-day nutritional assessment trial with broilers did not reveal biologically relevant adverse effects or differences in the performance of animals fed diets containing cottonseed meal from LLCotton25 compared to conventional counterpart Coker 312 or another cotton cultivar. Bioinformatics analysis of the amino acid sequence of the PAT protein did not show sequence resemblance to known toxins or IgE-dependent allergens, nor has the protein been reported to cause IgE-mediated allergic reactions. It is therefore unlikely that the PAT protein will cause toxic or IgE-mediated allergic reactions to food or feed containing LLCotton25 compared to conventional cotton cultivars.

Based on current knowledge, and considering the intended uses, the VKM GMO Panel concludes that LLCotton25 is nutritionally equivalent to and as safe as its conventional counterpart Coker 312 and other cotton cultivars.

Environmental assessment

Considering the intended uses of LLCotton25, which exclude cultivation, the environmental risk assessment is concerned with the accidental release into the environment of viable seeds during transport and/or processing, and with indirect exposure to microorganisms in the gastrointestinal tract and in soil or water, mainly via intestinal content and faeces from animals fed feeds containing LLCotton25.

With the exception of the introduced tolerance to the herbicide glufosinate-ammonium, LLCotton25 has no altered survival, multiplication or dissemination characteristics compared to conventional cotton cultivars, and there are no indications of an increased likelihood of spread and establishment of cotton plants in the case of accidental release of seeds from LLCotton25 into the environment. Cotton is not cultivated in Norway, and there are no cross-compatible wild or weedy relatives of cotton in Europe. Plant to plant gene flow is therefore not considered to be an issue. There are no indications that transfer of recombinant genes from LLCotton25 products to microorganisms in the gastrointestinal tract or in soil or water could occur at higher frequencies than from naturally occurring microbial sources.

Based on current knowledge and considering its intended uses, which exclude cultivation, the VKM GMO Panel concludes that LLCotton25 does not represent an environmental risk in Norway.

Overall conclusion

Based on current knowledge and with the exception of the introduced trait, the VKM GMO Panel concludes that LLCotton25 is nutritionally, compositionally, phenotypically and agronomically equivalent to and as safe as its conventional counterpart and other cotton cultivars.

Considering the intended uses, which exclude cultivation, the VKM GMO Panel concludes that LLCotton25 does not represent an environmental risk in Norway.

8 Data gaps

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

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

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

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

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 VKM GMO Panel.

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Appendix I

UTTALELSE OM BAYER CROPSCIENCE GENMODIFISERTE BOMULL LLCOTTON25 (EFSA/GMO/NL/2005/13)

Appendix II

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Appendix III