Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

VKM Report 2016:09

Final health and environmental risk assessment of genetically modified cotton GHB614

Scientific opinion on glyphosate-tolerant, genetically modified cotton GHB614 from Bayer CropScience for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (Application EFSA/GMO/NL/2008/51)

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) 2016:09
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Final health and environment assessment of genetically modified GHB614 (EFSA/GMO/NL/2008/51)

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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 cotton GHB614 from Bayer CropScience expresses a modified *epsps* gene (*2mepsps*) gene from maize encoding the enzyme 5-enolpyruvylshikimate 3-phosphate synthase (2mEPSPS), which confers tolerance to the herbicide glyphosate.

Updated bioinformatics analyses of the inserted DNA and flanking sequences in GHB614 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 *2mepsps* gene has been shown over several generations of cotton GHB614.

Field trials indicate that with the exception of the introduced trait, cotton GHB614 is compositionally, phenotypically and agronomically equivalent to its conventional counterpart Coker 312 and other cotton cultivars.

A 42-day nutritional assessment trial with broilers did not reveal adverse effects of cottonseed meal from GHB614. The 2mEPSPS protein produced in GHB614 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 2mEPSPS protein will cause toxic or IgE-mediated allergic reactions to food or feed containing cotton GHB614 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 trait, the VKM GMO Panel concludes that cotton GHB614 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 GHB614 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 glyphosate-tolerant genetically modified cotton GHB614 (Unique Identifier BCS-GHØØ2-5) from Bayer CropSciences is approved in EU under Regulation (EC) No 1829/2003 for food and feed uses, import and processing since 17 of June 2011 (Application EFSA/GMO/NL/2008/51, Commission Implementing Decision 2011/354/EU).

Cotton GHB14 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/2008/51 in 2008 (VKM, 2009). Cotton GHB614 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 cotton GHB614 is based on information provided by the applicant in the application EFSA/GMO/NL/2008/51, relevant peer-reviewed scientific literature, including scientific opinions and comments from EFSA (EFSA, 2009a), VKM (VKM, 2009) and statements provided by 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 opinions, which are provided in Appendix I and II respectively, and readers are referred to these for details.

The VKM GMO Panel has evaluated cotton GHB14 with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Food Act, the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed. VKM has also decided to take account of the appropriate principles described in the EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA, 2006 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 cotton GHB14 includes molecular characterisation of the inserted DNA and expression of novel proteins, comparative assessment of agronomic and phenotypic characteristics, nutritional assessments, toxicity and allergenicity, unintended effects on plant fitness, potential for gene transfer, interactions between the GM plant, target and non-target organisms, and effects on biogeochemical processes.

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

The cotton event GHB14 was developed by *Agrobacterium tumefaciens* mediated transformation to express a modified *epsps* gene (*2mepsps*) from maize. The 2*mepsps* gene encodes a variant of the enzyme 5-enolpyruvylshikimate 3-phosphate synthase (2mEPSPS), which renders GHB614 tolerant to glyphosate-based herbicides.

Molecular characterisation

The GHB614 genome has a complete, single integrated copy of the modified *epsps* (*2mepsps*) expression cassette. Determination of 2mEPSPS protein levels in samples obtained from green house cultured plants, field trials, and processed cottonseed fractions, show that expression levels varied depending on growth stage and tissue type. Expression of the 2mEPSPS protein was generally higher in rapidly growing plant parts, in accordance with the activity of the promoter used to control expression of 2mEPSPS. Fourteen putative novel open reading frames (ORFs) have been identified spanning the 5-prime upstream and the 3-prime downstream junctions of the inserted DNA. No relevant homologies were found between their 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 GHB614 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 2005 and 2006 for compositional assessments of whole linted cottonseeds, cotton lint, and different processed cottonseed products. Field trials in 2004 and 2005 were performed for agronomic and GM phenotype assessments. In all trials, the GM cotton line GHB614 was compared to its conventional counterpart, parent line Coker 312. Cotton GHB614 was grown using conventional or glyphosate 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 cotton GHB614 and its conventional counterpart Coker 312. The 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 data reported for other conventional cotton cultivars. The differences were therefore considered to reflect the natural variability of the analytes.

Based on current knowledge and excluding the new protein 2mEPSPS, the VKM GMO Panel concludes that cotton GHB614 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart and other cotton cultivars.

Food and feed risk assessment

A 42-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 GHB614 compared to conventional counterpart Coker 312 or another cotton cultivar. Bioinformatics analysis of the amino acid sequence of the 2mEPSPS 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 2mEPSPS protein will cause toxic or IgE-mediated allergic reactions to food or feed containing cotton GHB614 compared to conventional cotton cultivars.

Based on current knowledge, and considering the intended uses, the VKM GMO Panel concludes that GHB614 is nutritionally equivalent to and as safe as its conventional counterpart Coker 312 and other cotton cultivars.

Environmental assessment

Considering the intended uses of cotton line GHB614, 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 GHB614.

With the exception of the introduced tolerance to the herbicide glyphosate, GHB614 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 plants in the case of accidental release into the environment of seeds from GHB614. 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 GHB614 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 GHB614 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 GHB614 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 GHB614 does not represent an environmental risk in Norway.

Key words

VKM, (benefit and) risk assessment, Norwegian Scientific Committee for Food Safety, Norwegian Food Safety Authority/Norwegian Environment Agency. GMO, cotton (*Gossypium hirsutum* L.), EFSA/GMO/NL/2008/51, genetically modified cotton GHB14, unique identifier BCS-GHØØ2-5, herbicide glyphosate, glyphosate tolerant 2mEPSPS protein, *2mepsps* 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 glyfosattolerante bomullssorten GHB614 (unik kode: BCS-GHØØ2-5) fra Bayer CropScience er fremkommet ved genmodifisering av bomullshybriden Cocker312. Hensikten med bomull GHB614 er motstandsdyktighet mot ugressmidler som inneholder glyfosat, f.eks. RoundUp.

Bomullen GHB614 ble godkjent til import, videreforedling og til bruk som mat og fôr under forordning 1829/2003 den 17. juni 2011 (Kommisjonsbeslutning 2011/354/EC). Søknaden og godkjenningen omfatter ikke kultivering.

Bomullen GHB614 ble første gang vurdert av VKMs faggruppe for GMO i 2008 (VKM, 2009) i forbindelse med den offentlige høring av søknad EFSA/GMO/NL/2008/51. EFSAs endelig vurdering ble publisert i 2009 (EFSA, 2009a). Bomull GHB614 har også blitt brukt som en komponent i bomullhybriden GHB614 x LLCotton25 (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 nettside GMO Extranet, og uavhengige vitenskapelige publikasjoner, inklusiv vitenskapelige vurderinger fra EFSA (EFSA, 2009a) og VKM (VKM, 2009). Bortsett fra gjennomgang av nylig offentliggjort publikasjoner er resten av teksten i denne vurderingen en oppsummering av de tidligere VKM (VKM, 2009) og EFSA (EFSA, 2009a) 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, 2006, 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 ikke tilsiktede 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.

Bomullssorten GHB614 er utviklet ved hjelp av *Agrobacterium*-mediert transformasjon til å uttrykke et modifisert *epsps* -gen (*2mepsps*) fra mais. Genet koder for enzymet 5-enolpyruvylshikimat 3-fosfat syntase (2mEPSPS) som gir GHB614 en økt toleranse overfor glyfosat baserte ugressmidler.

Molekylær karakterisering

Den molekylære karakteriseringen fra søker viser at det kun er inkorporert én kopi av det transgene innskudds-DNAet (T-DNA), og at *2mepsps* genet er intakt. Proteinmålinger utført på prøver av GHB614 fra veksthusforsøk, feltforsøk og fraksjonene fra prosesserte bomullsfrø, viser at mengden 2mEPSPS-protein varierte i henhold til vekststadiene og type plantevev – generelt høyere i hurtigvoksende vev – og i henhold til fraksjonstypen fra prosesserte frø. Det er identifisert fjorten nye potensielle åpne leserammer (ORFs), i og ved det innsatte T-DNAet i plantens genom. Databasesøk viser derimot ingen relevante samsvar / homologier mellom de antatte genproduktene fra de tilførte åpne leserammene, 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 GHB614.

Ut i fra dagens kunnskap og informasjon fra søker, konkluderer VKMs faggruppe for GMO med at den molekylære karakteriseringen av de tilsiktede endringene i GHB614 er tilstrekkelig og at det ikke er identifisert utilsiktede endringer som krever spesifikk oppfølging i den videre vurderingen.

Komparative analyser

Søker har utført feltforsøk i USA i 2005 og 2006 med påfølgende analyse av næringsstoffer, antinæringsstoffer og andre relevante, biologisk aktive stoffer i hele bomullsfrø, bomullsfrømel, urenset og renset bomullsfrøolje og øvrig prosessert plantemateriale. Registrering av agronomiske og fenotypiske egenskaper ble også utført fra feltstudier i USA i 2004 og 2005. For alle feltstudiene ble data fra bomull GHB614 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 bomullen GHB614 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 2mEPSPS, konkluderer VKMs faggruppe for GMO med at GHB614 er vesentlig lik konvensjonell kontroll og andre bomullssorter med hensyn til næringsstoffsammensetning og agronomiske og fenotypiske egenskaper.

Helserisiko

Et 42-dagers fôringsforsøk med broilere har blitt utført med bomullsfrømel fra GHB614, 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 bomull GHB614 sammenlignet med de konvensjonelle bomullene. Databasesøk viser ingen relevante sekvenslikheter mellom 2mEPSPS 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 2mEPSPS proteinet vil føre til toksiske eller IgE-medierte allergiske reaksjoner fra mat og fôr som inneholder bomull GHB614 sammenlignet med konvensjonelle bomullssorter.

Ut i fra dagens kunnskap og tiltenkt bruk, konkluderer VKMs faggruppe for GMO med at GHB614 er ernæringsmessig lik og like trygg som konvensjonell kontroll Coker 312 og andre bomullssorter.

Miljørisiko

Miljørisikovurderingen av bomull GHB614 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 GHB614 i Norge.

Genmodifiseringen av bomull GHB614 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 bomull GHB614. 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 GHB614 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 bomull GHB614 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 bomull GHB614 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 bomull GHB614 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
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
ERA	Environmental risk assessment
EU	European Union
FAO	Food and Agriculture Organisation
Fitness	Describes an individual's ability to reproduce successfully relative to that of other members of its population.
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.
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 25 January 2008, the European Food Safety Authority (EFSA) received from the Dutch Competent Authority an application (Reference EFSA/GMO/NL/2008/51) for authorisation of the glyphosate-tolerant genetically modified cotton GHB614 (Unique Identifier BCS-GHØØ2-5), 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/2008/51 and in accordance with Articles 5(2)(b) and 17(2)(b) of Regulation (EC) No 1829/2003, EFSA informed the EU- and EFTA Member States (MS) and the European Commission and made the summary of the dossier publicly available on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of regulation (EC) No 1829/2003. Following receipt of additional information from the applicant, EFSA declared on 11 March 2008 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 included Norway 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 February 2009 (VKM, 2009). EFSA published its scientific opinion 05 March 2009 (EFSA, 2009a), and cotton GHB614 was approved for food and feed uses, import and processing on 17 June 2011 (Commission Implementing Decision 2011/354/EC).

Cotton GHB614 has been used as a component of the stacked GM events GHB614 x LLCotton25 (EFSA/GMO/NL/2010/77), which have 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, 2006, 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 cotton GHB614 is assessed with reference to the intended use. The risk assessment is based on information provided by the applicant in the application EFSA/GMO/UK/2008/51, relevant peer-reviewed scientific literature, and scientific opinions and comments from VKM (VKM, 2009), EFSA (EFSA, 2009a) 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.

Cotton GHB614 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 cotton GHB614 (Unique Identifier BCS-GHØØ2-5) is developed to provide tolerance to glyphosate-based herbicides. The genetic modification in cotton line GHB614 consists of a single glyphosate tolerance trait introduced by the transfer of a gene encoding a modified form of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) from maize. Two simple mutations were introduced into the wild type *epsps* gene, using site directed mutagenesis. The mutations introduced into the 2mEPSPS enzyme significantly reduce its sensitivity to glyphosate, allowing continued function in the presence of the glyphosate. Plants expressing 2mEPSPS are therefore able to tolerate treatment with glyphosate-containing herbicides.

The purpose of the modification is to allow for effective weed control during the cultivation of GHB614. The genetic modification in cotton GHB614 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.

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

Cotton GHB614 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, 2006 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 2009, Appendix I; EFSA, 2009a, Appendix II) have previously assessed the molecular characterisation of the cotton event GHB614 (*2mepsps* – gene insert) with regards to the following:

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

Both Panels concluded that the applicant had provided sufficient analyses for the molecular characterisation.

Cotton tissue from *Gossypium hirsutum* variety Coker 312 was transformed by *Agrobacterium tumefaciens* mediated gene transfer with the binary transformation vector pTEM2. The vector contained the T-DNA region, with the left and right borders (LB and RB) delimiting a single gene cassette for expression of a modified *epsps* gene of maize origin. The modified *2mepsps* gene was generated with two single nucleotide mutations introduced by site direct mutagenesis The mutated maize *2mepsps* gene produces a 47 kDa version of the 5-enolpyruvylshikimate-3-phosphate enzyme (2mEPSPS protein). The two amino acid changes in the 2mEPSPS protein significantly lower its affinity for glyphosate, allowing the enzyme to continue to function in the presence of glyphosate based herbicides. This property makes plant tissue expressing the 2mEPSPS protein tolerant to glyphosate-based herbicides such as RoundUp Ready.

The inserted T-DNA region in cotton GHB614 comprises the following elements: the *Arabidopsis thaliana* promoter *Ph4a748At*, the intron *1 h3At+TPotp C*, the modified *2mepsps* gene from maize coding for glyphosate tolerance, and the *3'histonAt* terminator sequence from *A. thaliana.* Extensive molecular analyses were performed for the molecular characterisation; Southern hybridisation after digesting DNA with many different enzymes, Northern hybridisation, PCR, BLAST searches, and ELISA, to determine the number of insertions, copy number, integrity of the insert, evaluation of the presence or absence of plasmid backbone sequences, expression levels of *2mepsps,* and levels of 2mEPSPS protein. The wild type cotton variety Coker 312 was used as the negative control for these analyses.

Analyses of the insert in cotton GHB614 show the presence of a single intact T-DNA region of 3978 bp. The inserted region is equal to the original T-DNA region in vector pTEM2. No vector backbone sequences were detected in cotton GHB614. The 5' (738 bp) and 3' (214 bp) flanking regions of the insertion site were also sequenced. Analyses of the sequencing

results demonstrated that a 17 bp fragment was removed as a result of the integration and that the T-DNA region was inserted near a gene of a protein with unknown function. Results from comparative agronomic performance and compositional analyses, suggest that the proximity of the insert to this gene has not caused any noticeable unintended effects.

The expression levels of the 2mEPSPS protein was measured by ELISA in cotton tissues from green house samples, field trials, and in cotton products. Greenhouse grown cotton samples were measured at the 2-3 and 4-6 leaf stages of growth, pre-flowering and at flowering. Protein levels varied depending on growth stage and type of plant tissue, and were found to be higher in rapidly growing plant parts. Expressed as a percentage of total extractable protein, the 2mEPSPS protein showed a maximum of 0.39% in leaves, 0.34% in apices, 0.18% in roots and squares, 0.06% in stems and 0.001% in pollen in greenhouse-cultivated plants.

Levels of 2mEPSPS protein in seeds and processed seed fractions from Roundup treated and untreated plants were tested during field trials in the US in 2004/2005. The average 2mEPSPS protein content per test site in the field trial ranged from 15.8 to 25.5 µg/g fresh weight (fw) in untreated fuzzy seed (overall average value of 19.2 ± 3.1 µg/g fw, or 21.2 µg/g dry matter [dm]), and from 16.2 to 30.5 µg/g (fw) in treated fuzzy seed (overall average value of 21.2 ± 4.0 µg/g fw, or 23.3 µg/g dm).

Of nine processed individual fractions of cottonseed tested, 2mEPSPS protein was only found in detectable amounts in three fractions; delinted cottonseed: 102 ± 2 µg/g fw; hulls: 6.93 ± 0.40 µg/g fw; and defatted meal: 0.26 ± 0.10 µg/g fw. The other fractions contained 6.63 µg/g fw combined.

Upon request from the EFSA GMO Panel the applicant has performed additional sequence analyses for newly created ORFs following the original submission, (De Pestel 2008). The analyses revealed 12 novel ORFs for putative peptides spanning the 5-prime upstream and the 3-prime downstream junctions of the inserted DNA, in addition to the two ORFs previously reported. According to the applicant, further bioinformatics analyses revealed no relevant homologies between the theoretically predicted translation products of these ORFs and known toxins or allergens.

The stability of the insert in GHB614 cotton was analysed by Southern hybridisation of leaf tissues over multiple generations. The expected integration patterns were present in all samples analysed. Phenotypic stability was demonstrated by Mendelian inheritance of the glyphosate tolerance trait over multiple generations and field locations, as well as throughout the development of commercial lines based upon cotton event GHB614.

2.2 Conclusions

Based on current knowledge and information provided by the applicant, the VKM GMO panel concludes that the intended changes in cotton GHB614 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 cotton GHB614 has previously been assessed as food and feed by the VKM GMO Panel (VKM, 2009; 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/2008/51 in 2008 and in EFSA's final opinion (EFSA, 2009a; Appendix II). A brief summary from these reports are provided below.

3.1 Production of material for comparative assessment

For compositional studies, GHB614 was compared to its parent variety Coker 312, which is a commercial non-GM 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 2005 and 2006 in Arkansas, Florida, Georgia, Mississippi and Texas, all belonging to the cotton growing regions of Southern United States. In 2005, trials were performed at 9 locations, three treatments at each location and three replications per treatment. In the year 2006, 8 trials were conducted at the same locations used the year before. The three treatments consisted of: (a) non-GM cotton Coker 312 grown under conventional herbicide weed control, (b) GM cotton GHB614 grown under conventional herbicide weed control, and (c) GM cotton GHB614 grown with glyphosate-based 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 cottonseed, cottonseed linters, hulls, delinted seeds meal, toasted meal, crude oil and refined, deodorised oil obtained from cotton GHB614 and the parent line Cocker 312 from the field trials. For the whole, linted cottonseed, all material from all 17 sites in 2005 and 2006 were analysed. For the other cottonseed products, cottonseeds from one field trial were processed to provide samples. The samples were analysed for the components of importance for cotton as defined by the OECD consensus document for cotton (OECD, 2004), a total of 81 components, including proximates, amino acids, fatty acids, vitamin E, minerals, the antinutrient cyclopropenoid fatty acids and the toxicant gossypol.

The statistical analysis of the data was carried out with t-tests and analysis of variance (ANOVA) using a commercially available statistical package (SAS version 8.2) with data from three replicates per location for each year, as well as on the combine data from all sites for both years.

The applicant also provided information on agronomic performance and phenotypic characteristics derived from several field trials in the US performed in 2004 and 2005 with the same control and test groups described for the compositional studies, as well as an additional comparator FiberMax9740. The characteristics that were analysed in these studies

included parameters related to plant morphology, seed and plant development, reproductive traits, disease and pest susceptibility, weediness, weed control, volunteers, yield, and cottonseed and fibre quality.

3.2 Compositional analysis

For the linted cottonseeds, analysis of the combined mean values of the proximates, amino acids, fatty acids, minerals, vitamin E and gossypol from all 17 sites for both years indicate that statistically significant differences were observed for the minority (0-6) of analytes in the conventional counterpart Coker 312, cotton GHB614 treated with conventional herbicides and cotton GHB614 treated with glyphosate. The exceptions were for the fatty acids C16:1, C18:0, C18:1, C18:2 and C18:3 and the cyclopropenoid fatty acids (antinutrients) malvalic, sterculic and dihydrosterculic acids, which were significantly different in the majority (>50%) of site samples analysed. The mean levels of the cyclopropenoid fatty acids were all lower in the cotton GHB614 groups that the conventional counterpart Coker 312. In all cases, any differences observed were small, were not consistent between sites and years, and the mean values for all analytes were within the range of values reported for conventional cotton cultivars. Thus any statistically significant differences detected between linted cottonseeds from conventional counterpart Coker 312 and GHB614, either glyphosate treated or not, were not considered biologically relevant.

For the other, processed cottonseed products analysed from one field trial, few differences in analyte levels were consistently observed across the products from conventional counterpart Coker 312 compared to those from cotton GHB614. Those differences in analytes reported by the applicant either corresponded to those observed for the linted cottonseeds, or were inconsistent between the products and therefore considered to be due to factors other than the genetic modification, e.g. processing conditions or contamination during processing. Most values fell within or were close to the range of values reported for the corresponding products from conventional cotton cultivars. Thus the statistically significant differences detected between specific processed products from conventional counterpart Coker 312 and those from GHB614 were not considered biologically relevant.

3.3 Agronomic traits and GM phenotype

The data supplied by the applicant from the field trials conducted in 2004 and 2005 indicated differences between cotton GHB614 and its conventional counterpart Coker 312 in some instances with regard to several characteristics related to yield, lint percentage, and reproduction. However, the differences did not occur consistently between the various locations and years, and were therefore not considered to be related to the genetic modification, but rather an indication of natural variability.

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 identified between cotton GHB614, the conventional counterpart Coker 312 and other conventional cotton cultivars. The 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 2mEPSPS-protein, the VKM GMO Panel concludes that cotton GHB614 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.

4.1 Previous evaluations by the VKM and EFSA GMO panels

Cotton GHB614 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 this application EFSA/GMO/NL/2008/51 in 2008 (VKM, 2009; Appendix I). EFSA published their final opinion in 2009 (EFSA, 2009a; Appendix II). EFSA and the VKM GMO Panel concluded that cotton GHB614 was nutritionally equivalent to conventional cotton cultivars and it was unlikely that the inserted protein would cause toxic or allergic reactions to food or feed containing cotton GHB614 compared to conventional cotton.

4.2 Product description and intended uses

According to the applicant, the genetic modification in GHB614 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.

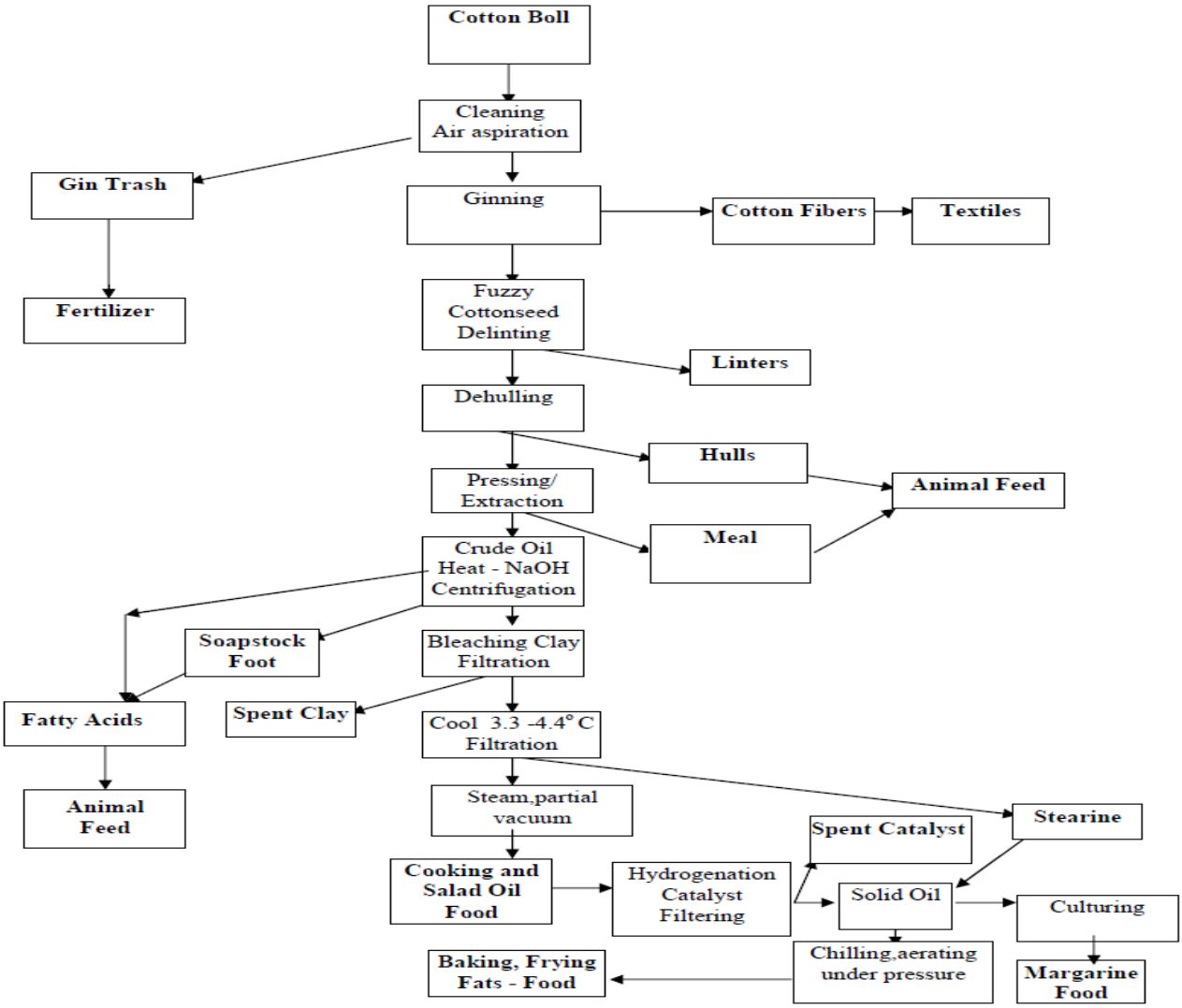

Figure 4.2-1: Processing of cotton boll, adapted from OECD (2004)

4.3 Effects of processing

According to the applicant, the commercial experiences have confirmed that the production and processing of cotton GHB614 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 cotton GHB614 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 2mEPSPS protein

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 2mEPSPS protein to very low or non-detectable levels in hulls and toasted cottonseed meal, and was not detectable in refined oil. At 60°C, the 2mEPSPS protein was inactivated after 10 minutes and at 75°C the enzyme had lost total activity.

4.4 Toxicological assessment of cotton GHB614

4.4.1 Toxicological assessment of the expressed novel protein

The 2mEPSPS protein expressed in cotton GHB614 is also expressed in other genetically modified plants that have been assessed and considered safe by both VKM and EFSA.

The applicant's Technical Dossier provides the following data regarding the toxicological assessment of the expressed novel proteins in cotton GHB614:

- Acute oral toxicity testing of 2mEPSPS 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, 2009a)

Due to the low levels of 2mEPSPS in cotton and the difficult task of isolating a sufficient quantity of purified protein from the cotton, the acute toxicity testing studies described and referred to in the Applicant Dossier were conducted with 2mEPSPS protein produced in *Escherichia coli*. The applicant has performed analysis of structural similarity, physicochemical and functional equivalence of the microbially-produced 2mEPSPS protein and the proteins produced by the cotton. These indicate that plant-produced and bacterially-produced 2mEPSPS protein is biologically, biochemically, and immunologically equivalent.

4.4.1.1 *Acute toxicity testing of novel protein*

In an acute oral toxicity study with mice, the purified (>99 % pure) 2mEPSPS protein produced in *E. coli* was used. The study was performed in accordance with the principles of Good Laboratory Practices, U.S. E.P.A. Health Effects Test Guidelines OPPTS 870.1100 and on the OECD Test Guideline 425, adopted in 2001 (OECD, 2001).

Groups of 5 female OF1 mice were administered either the 2mEPSPS protein or bovine serum albumin (a negative control) by oral gavage at a single limit dose of 2000 mg protein/kg body weight. The animals were in a weight range from 21.69 to 23.98 g on the day of treatment. Each animal was identified by a stainless steel ear tag bearing a unique animal number. All animals were observed for clinical signs daily for fifteen days while their body weights were measured weekly. At termination, animals were subjected to necropsy including macroscopic examination, i.e. abdominal and thoracic cavities, major organs and tissues.

The applicant reported that no clinical signs, mortalities, treatment related effects on body weight or other macroscopic signs of systemic toxicity during necropsy in female OF1 mice at 2000 mg/kg body weight were observed during the study. Based on this test the acute oral

LD_{50} was estimated to be greater than 2000 mg/kg body weight, and that 2mEPSPS protein is not acutely toxic.

More recently, a report of a study conducted by Bayer CropScience (Herouet-Guicheney et al., 2009) has appeared in the peer-reviewed scientific literature. The study was performed in accordance with the OECD Test Guideline 425, adopted in 2001 (OECD, 2001). Groups of 5 female OF1 mice were intravenously injected with 2mEPSPS protein, aprotinin (negative controls at both doses) or melittin (negative control at dose 1 mg/kg and positive control at dose 10 mg/kg body weight) in physiological saline solution a dose levels of 1 and 10 mg/kg body weight at a constant volume of 10 ml/kg body weight. All animals were observed for clinical signs daily for 15 days, with particular attention given to the first four hours following injection. Their body weights were measured weekly. At termination, animals were subjected to necropsy including macroscopic examination, i.e. abdominal and thoracic cavities, major organs and tissues.

The scientists reported that negative control female mice treated with 1 mg/kg melittin or 1 and 10 mg/kg aprotinin showed no signs of systemic toxicity, while melittin at 10 mg/kg caused 100% mortality within 10 minutes of application (positive control). In the test groups, female mice treated with 1 and 10 mg/kg 2mEPSPS protein reportedly showed no mortalities or toxic effects. Based on this test the acute intravenous LD_{50} of 2mEPSPS protein was estimated to be greater than 10 mg/kg body weight, and that 2mEPSPS protein is not acutely toxic.

The VKM GMO panel agrees with 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.

4.4.1.2 *Repeated dose toxicity testing*

The applicant has not provided data from repeated dose toxicity trials. No reports of such studies have been found in the peer-reviewed scientific literature.

4.4.2 Toxicological assessment of the whole GM food/feed

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

No 90-day sub-chronic feeding study with cotton GHB614 has been performed by the applicant. Since the compositional studies indicated that cotton GHB614 was compositionally similar 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, 2009a).

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, 2006 and 2010b).

4.4.3.1 *Assessment of allergenicity of the newly expressed proteins*

In order to assess the potential for introduced IgE-dependent allergens in GHB614, sequence evaluation schemes were used to assess the similarity of the 2mEPSPS 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 2mEPSPS protein, no immunologically significant sequence identity was detected, indicating that no homology to known IgE-dependent allergens, based on amino acid sequences in 2mEPSPS.

In vitro simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) digestibility studies were also conducted on the protein. Within 30 s of exposure to SGF and SIF 2mEPSPS was rapidly digested and no longer detectable by SDS-PAGE or western blot analysis. Thermolability results for the 2mEPSPS 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 2mEPSPS 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 GHB614 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.

GHB614 contains the 2mEPSPS protein. Interaction between the newly expressed protein 2mEPSPS 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 2mEPSPS 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 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.

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 finds uses 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 cotton seed 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 is the main fatty acid in cottonseed oil, and it 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 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 cotton seeds, 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 (OGTR, 2008).

Analysis of cotton products derived from GHB614 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

Applicant's data for nutritional assessment

A 42-day broiler feeding study (Ross #708) was performed (Stafford, 2007). The data and report were produced and compiled in accordance with all pertinent U.S. EPA Good Laboratory Practice regulations (40 CFR, Part 160,1989), OECD Principles of Good Laboratory Practice (OECD, 1998) and Japan MAFF (12 Nousan, Notification No. 8623, Agricultural Product Bureau) with the following exceptions: routine water contaminant screening analyses for pesticides, polychlorinated biphenyls (PCB) and toxic metals were conducted with standard U.S. EPA procedures. None of these compounds were detected at concentrations that are considered toxic in any of the samples analysed. Herbicide residue levels in the feeds were below detection limits. Levels of the anti-nutrient gossypol in the toasted cottonseed meal and the test diets are reported in Appendix III.

The Analysis of Variance function in SYSTAT, for Windows, Version 9 (SPSS, Inc., Chicago, IL 60611, USA, SPSS, 2000) was used to conduct the statistical analyses. Two-factor analysis of variance (ANOVA) was used to test for significant main effects (treatment group and gender) on the dependent variables. The ANOVA model included an interaction statement to detect significant "group x gender" interactions.

Three groups of 140 animals consisting of 14 pens (7 pens/sex) with 10 animals in each were fed diets containing toasted meal obtained from seeds of cotton GHB614 sprayed with glyphosate based herbicide. The non-GM counterpart Coker 312 or another, unspecified conventional non-GM cultivar, both treated with conventional herbicides. The inclusion level of cottonseed meal in the starter, grower and finisher diets was 10%. Broilers were randomised to treatment groups and received one of the three test diets immediately at cage assignment and throughout the 42 days of the study. Water and feed were provided *ad libitum* throughout the study. All birds were monitored at least once a day for health status, overt signs of toxicity, and mortality. Body weights were recorded initially and at days 7, 21, 35 and 42. Feed consumption was measured for each pen on a weekly basis and used to calculate feed conversion ratios. Carcass and tissue weights were recorded for 126 of the 420 broilers in this study (21 birds/sex/treatment group).

According to the data provided by the applicant, no treatment-related differences were observed for clinical signs or mortality among the diet groups. Twenty-nine birds across the three treatment groups displayed clinical signs of disease, and of these, mortality was recorded for 14 birds, equivalent to 3% in this study, which was considered to be relatively low for the species and study conditions. Some statistically significant differences were noted, however, no biologically relevant differences in total feed consumption, body weight gain, or feed conversion ratio were observed. The group fed diets containing cottonseed meal from the non-transgenic commercial cotton consistently gained more weight and converted feed more efficiently than the other two groups. The values for weight gain and feed efficiency of the test group fed cotton GHB614 was consistently intermediate between the two conventional control groups. No biologically relevant differences in weights of chilled

carcass, abdominal fat pad, leg, thigh, wing and breast in animals fed cottonseed meal derived from cotton GHB614 compared with animals fed meal from the non-GM conventional cotton cultivars.

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

4.6 Conclusions

A 42-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 GHB614 compared to conventional counterpart Coker 312 or another cotton cultivar. Bioinformatics analysis of the amino acid sequence of the 2mEPSPS 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 2mEPSPS protein will cause toxic or IgE-mediated allergic reactions to food or feed containing GHB614 compared to conventional cotton cultivars.

Based on current knowledge, and considering the intended uses, the VKM GMO Panel concludes that GHB614 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 cotton line GHB614, 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 GHB614 line has tolerance to glyphosate-based herbicides.

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 biggest 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 GHB614 is similar to those of conventional, non-transformed cotton. Thus, under Norwegian conditions, it is highly unlikely that the seeds of the GM lines 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 lines will have selective advantages only if exposed to specific herbicide glyphosate. Consequently, the establishment of feral populations of GHB614 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 2009b).

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 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 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 cotton line GHB614, 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 cotton line GHB614, 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 GHB614.

With the exception of the introduced tolerance to the herbicide glyphosate, GHB614 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 plants in the case of accidental release into the environment of seeds from GHB614. 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 GHB614 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 GHB614 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 GHB614 genome has a complete, single integrated copy of the modified *epsps* (*2mepsps*) expression cassette. Determination of 2mEPSPS protein levels in samples obtained from green house cultured plants, field trials, and processed cottonseed fractions, show that expression levels varied depending on growth stage and tissue type. Expression of the 2mEPSPS protein was generally higher in rapidly growing plant parts, in accordance with the activity of the promoter used to control expression of 2mEPSPS. Fourteen putative novel open reading frames (ORFs) have been identified spanning the 5-prime upstream and the 3-prime downstream junctions of the inserted DNA. No relevant homologies were found between their 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 GHB614 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 2005 and 2006 for compositional assessments of whole linted cottonseeds, cotton lint, and different processed cottonseed products. Field trials in 2004 and 2005 were performed for agronomic and GM phenotype assessments. In all trials, the GM cotton line GHB614 was compared to its conventional counterpart, parent line Coker 312. Cotton GHB614 was grown using conventional or glyphosate 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 cotton GHB614 and its conventional counterpart Coker 312. The 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 data reported for other conventional cotton cultivars. The differences were therefore considered to reflect the natural variability of the analytes.

Based on current knowledge and excluding the new protein 2mEPSPS, the VKM GMO Panel concludes that cotton GHB614 is compositionally, agronomically and phenotypically equivalent to its conventional counterpart and other cotton cultivars.

Food and feed risk assessment

A 42-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 GHB614 compared to conventional counterpart Coker 312 or another cotton cultivar. Bioinformatics analysis of the amino acid sequence of the 2mEPSPS 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 2mEPSPS protein will cause toxic or IgE-mediated allergic reactions to food or feed containing cotton GHB614 compared to conventional cotton cultivars.

Based on current knowledge, and considering the intended uses, the VKM GMO Panel concludes that GHB614 is nutritionally equivalent to and as safe as its conventional counterpart Coker 312 and other cotton cultivars.

Environmental assessment

Considering the intended uses of cotton line GHB614, 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 GHB614.

With the exception of the introduced tolerance to the herbicide glyphosate, GHB614 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 plants in the case of accidental release into the environment of seeds from GHB614. 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 GHB614 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 GHB614 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 GHB614 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 GHB614 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 glyphosate as an in-crop selective herbicide to control a wide range of broadleaf and grass weeds without sustaining crop injury. This weed management strategy enables post-emergence spraying of established weeds and gives growers more flexibility to choose spraying times in comparison with the pre-emergence treatments of conventional crops.

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

VKM 2009

Appendix II

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Appendix III