



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

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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 EFSA's 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 *2mepsps* gene encodes a variant of the enzyme 5-enolpyruvylshikimate 3-phosphate synthase (2mEPSPS), which renders GHB14 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 (Kommissjonsbeslutning 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 bomullshybriden 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, urensset og rensset 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 <i>Agrobacterium tumefaciens</i> plasmid <i>pTi15955</i>
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	<i>Bacillus thuringiensis</i>
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
<i>In planta</i>	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 denatured 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 Pesteel 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 EFSA's 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 than 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 EFSA's 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.

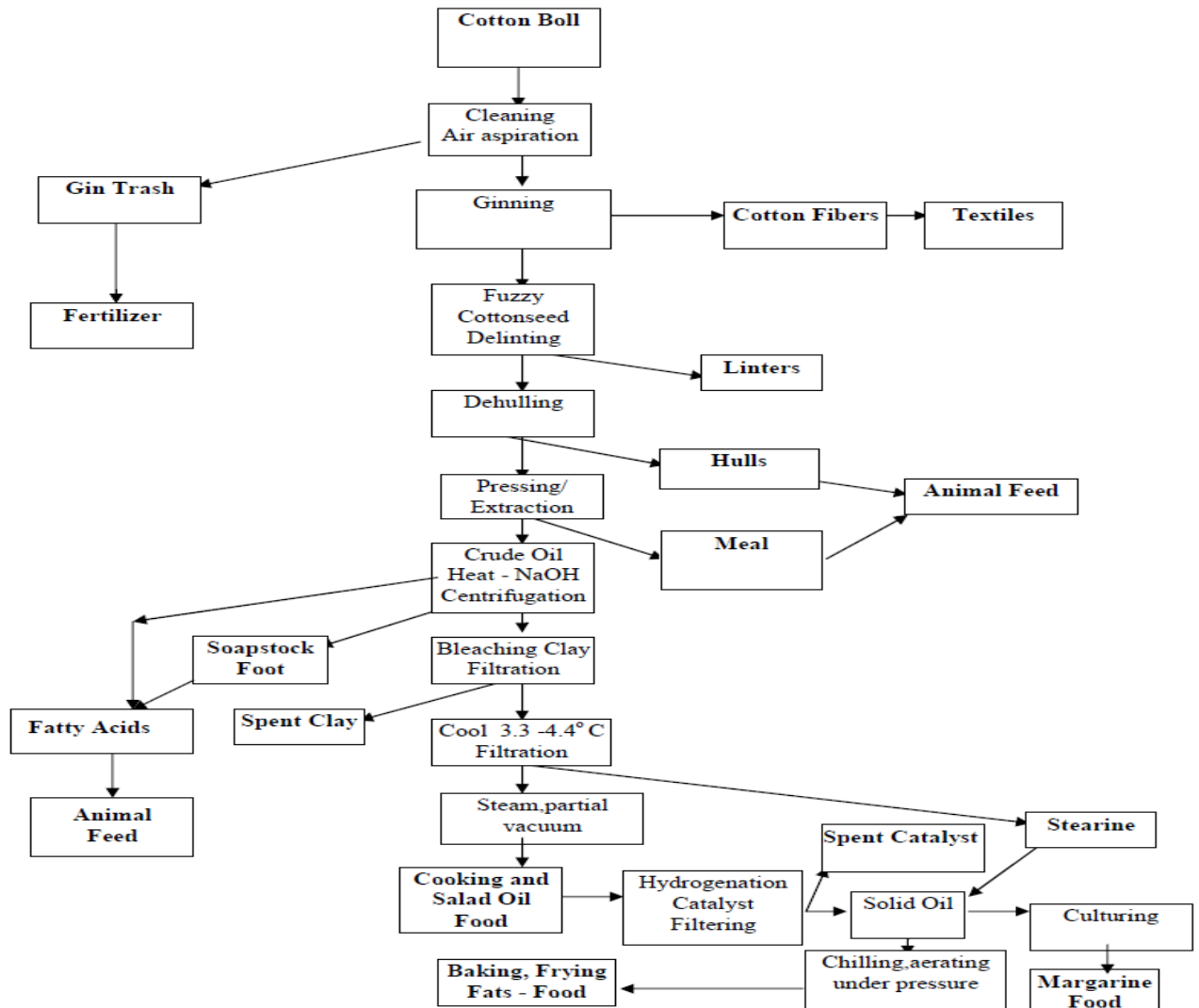


Figure 4.2-1: Processing of cotton boll, adapted from OECD (2004)

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

LD₅₀ 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-Guichenev 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 at 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 LD₅₀ 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 cyclopropanoid 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 allotetraploid species, four of these (*G. arboreum*, *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/statistikbanken).

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



**Uttalelse fra Faggruppe for genmodifiserte organismer i
Vitenskapskomiteen for mattrygghet**

16.02.09

**Helse- og miljørisikovurdering av genmodifisert bomull
GHB614 fra Bayer CropScience
(EFSA/GMO/NL/2008/51)**

BIDRAGSYTERE

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

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SAMMENDRAG

Helse- og miljørisikovurderingen av den herbicidtolerante bomullslinjen GHB614 fra Bayer CropScience (EFSA/GMO/NL/2008/51) er utført av Faggruppe for genmodifiserte organismer i Vitenskapskomiteen for mattrygghet. Mattilsynet og Direktoratet for naturforvaltning (DN) ber Vitenskapskomiteen for mattrygghet om å vurdere den genmodifiserte bomullslinjen GHB614 til import og prosessering, og til bruk i næringsmidler og fôrvarer. Søknaden gjelder ikke dyrking eller bruk av hele bomullsfrø som mat.

Risikovurderingen av den genmodifiserte bomullen er basert på dokumentasjon som er gjort tilgjengelig på EFSAAs nettside GMO EFSAAnet. I tillegg er det benyttet informasjon fra uavhengige vitenskapelige publikasjoner i vurderingen. GHB614 er risikovurdert i henhold til tiltenkt bruk og faggruppen har derfor ikke vurdert helse- og miljøeffekter knyttet til dyrking av bomullslinjen. Vurderingen er gjort i overensstemmelse med miljø- og helsekravene i genteknologiloven med forskrifter, først og fremst og fremst forskrift om konsekvensutredning etter genteknologiloven, samt kravene i EUs forordning 1829/2003/EF og utsettingsdirektiv 2001/18/EF med annekser. Det presiseres at de deler av den norske konsekvensutredningsforskriften som vedrører bærekraft, samfunnsnytte og etikk ikke er vurdert av VKM. Videre er EFSAAs retningslinjer for risikovurdering av genmodifiserte planter (EFSA2006a) og Organisation for Economic Co-operation and Development (OECD) konsensusdokument for bomull (OECD 2004) 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 og genoverføring vurdert.

Bomullslinjen GHB614 er fremkommet ved *Agrobacterium*-mediert transformasjon av planteceller fra den kommersielle bomullssorten 'Coker 312'. Bomullslinjen har fått satt inn en genkonstruksjon med et modifisert *epsps*-gen (*2mepsps*) fra mais. Genene *epsps* og *2mepsps* koder for enzymet 5-enolpyruvylsiki-3-fosfat-3-fosfat-syntetase (EPSPS- og 2mEPSPS-enzym), som omdanner fosfoenolpyruvat og sikimat-3-fosfat til 5-enolpyruvylsiki-3-fosfat, en viktig metabolitt i syntesen av aromatiske aminosyrer. N-fosfonometylglycin (glyfosat) hemmer generelt EPSPS-enzymet og blokkerer derved biosyntesen av aromatiske aminosyrer i planter. I motsetning til plantens eget EPSPS-enzym er det modifiserte mais-enzymet 2mEPSPS også aktivt ved nærvær av glyfosat. De transgene plantene vil derfor tolerere høyere doser av herbicider med virkestoff glyfosat sammenlignet med konkurrerende ugras.

GHB614 har fått satt inn et modifisert *epsps*-gen (*2mepsps*) fra mais. *2mepsps*-genet ble dannet ved å klonere villtypemais *epsps*-genet inn i et plasmid og deretter introdusere to mutasjoner med *in vitro*-mutasjonsteknikk. Genet ble så klonet inn i den binære vektoren pTEM2. *2mEpsps*- og *epsps*- genene koder for enzymet 5-enolpyruvylsiki-3-fosfat-syntetase, som omdanner fosfoenolpyruvat og sikimat-3-fosfat til 5-enolpyruvylsiki-3-fosfat, en viktig metabolitt i syntesen av aromatiske aminosyrer. Bomullslinjen GHB614 uttrykker 2mEPSPS-proteinet som i motsetning til plantens eget EPSPS enzym er aktivt ved nærvær av N-fosfonometylglycin (glyfosat). N-fosfonometylglycin (glyfosat) hemmer generelt EPSPS-enzymet og blokkerer derved biosyntesen av aromatiske aminosyrer i planter. GHB614 inneholder ingen funksjonelle markørgener for antibiotikaresistens.

Bomullsfrø hvor bomullsfibrene er fjernet blir bearbeidet til fire hovedprodukter, olje (16 %), mel (45 %), frøskall (26 %) og "bomullshår/fiber"(lint) (9 %). Om lag 4 % går tapt ved prosessering av frøene (OECD 2004). Det er hovedsakelig olje fra bomullsfrø som brukes som menneskeføde, mens hele bomullsfrø og biprodukter som mel og kli fra oljeproduksjonen brukes som fôr. Etter det faggruppen kjenner til benyttes ikke bomullsfrøolje til produksjon av dyrefôr.

Analysen av ernæringsmessige viktige komponenter er utført i tråd med OECDs konsensusdokument for bomull (OECD 2004). Faggruppen anser analysene for å være tilstrekkelige for en vurdering av bomullslinjen GHB614 til bruk som fôr, samt for olje til bruk som mat. Det er påvist statistisk signifikante forskjeller for enkelte av komponentene som er analyserte, men forskjellene er ikke konsistente over forsøksfelt og verdiene ligger innenfor variasjonsområde for typiske verdier for andre bomullssorter som er rapportert i litteraturen.

Flere studier viser at 2mEPSPS-proteinet som blir uttrykt som følge av genmodifiseringen ikke er akutt toksisk eller allergent. Bayer CropScience har utført og henviser til akuttstudier på mus og fôringsforsøk på broilere med det aktuelle proteinet. Disse studiene viser at proteinet ikke fører til påvisbare helseeffekter på dyrene.

Faggruppen konkluderer med at det er lite sannsynlig at eksponering for 2mEPSPS-proteinet i seg selv, og i de mengder som tilføres via fôr fra den genmodifiserte bomull fører til allergi eller toksiske effekter.

Faggruppe for genmodifiserte organismer konkluderer med at bomullsfrøolje og fôrvarer fra GHB614 er vesentlig lik olje og fôrvarer fra umodifiserte bomullsfrø, og finner at bruk av olje og fôrvarer fra den transgene bomullslinjen ikke utgjør noen større helseisiko enn kommersiell olje og fôrvarer fra umodifiserte bomullsplanter.

Søknaden gjelder godkjenning av bomullslinjen GHB614 for import, prosessering, mat og fôr. Faggruppen har derfor ikke vurdert mulige miljøeffekter knyttet til dyrking av bomullslinjen. Det er ingen indikasjoner på økt sannsynlighet for spredning, etablering og invasjon av bomullslinjen i naturlige habitater eller andre arealer utenfor jordbruksområder som resultat av frøspill i forbindelse med transport og prosessering. Bomull dyrkes ikke i Norge, og det er ingen stedegne eller introduserte viltvoksende arter i den europeiske flora som bomull kan hybridisere med. Det er derfor ikke risiko for utkryssing med dyrkede sorter eller ville planter i Norge.

Samlet vurdering

Faggruppen finner det lite trolig at den omsøkte bruken av bomullslinjen GHB614 vil medføre endret risiko for helse og miljø i forhold til annen bomull.

NØKKELOD

Bomull, *Gossypium hirsutum* L., genmodifisert bomull, GHB614, herbicidtoleranse, 2mepsps-gen, 2mEPSPS-protein, helsemessig trygghet, helse, miljø, forordning 1829/2003/EF, direktiv 2001/18/EF

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BAKGRUNN

Faggruppe for genmodifiserte organismer i Vitenskapskomiteen for mattrygghet er blitt bedt av Mattilsynet og Direktoratet for naturforvaltning om å foreta en utredning av helse- og miljørisiko ved en eventuell godkjenning av den genmodifiserte bomullslinjen GHB614 fra Bayer CropScience (EFSA/GMO/NL/2008/51). Bomullslinjen er søkt omsatt i EU/EØS-området under Forordning (EF) No. 1829/2003 om genmodifiserte næringsmidler og fôrvarer (artiklene 3(1) og 15(1)). I henhold til Bayer CropScience omfatter søknaden bruksområdene import, prosessering, næringsmidler og fôrvarer, men omfatter ikke dyrking eller bruk av hele frø som mat.

Søknaden ble fremmet og anbefalt av nederlandske myndigheter i januar 2008. Dokumentasjonen knyttet til søknaden ble lagt ut på EFSA-nett 11. mars 2008, med frist på 90-dager for innspill fra EU- og EØS/EFTA-landene. Norge har ikke tidligere uttalt seg om bomullslinjen.

Utenfor EU/EØS-området er bomullslinjen GHB614 søkt godkjent for dyrking og omsetning i USA, og til bruk som mat, fôr og industriell bruk i Australia, New Zealand, Canada, Japan, Korea og Mexico (Bayer CropScience 2008).

OPPDRAG FRA DIREKTORATET FOR NATURFORVALTING OG MATTILSYNET

Mattilsynet og Direktoratet for naturforvaltning har i brev datert 12.5.2006 (ref. 2006/17817) og 23.4.2008 (ref. 2008/4367 ART-BI-BRH) gitt Vitenskapskomiteen for mattrygghet i oppdrag å foreta løpende risikovurderinger av genmodifiserte næringsmidler og fôrvarer som faller inn under EUs forordning 1829/2003/EF. VKM er bedt om å vurdere helse- og miljøaspekter ved slike produkter, og på bakgrunn av vurderingene gi innspill til EFSA-nett.

Søknad EFSA/GMO/NL/2008/51, genmodifisert bomull GHB614, ble lagt ut på EFSA-nett 11. mars 2008. Faggruppe for genmodifiserte organismer skal i tråd med oppdragsbrev utarbeide helse- og miljørisikovurdering av bomullslinjen til import og industriell prosessering, samt til bruk som næringsmiddel og fôrvarer. Søknaden omfatter ikke dyrking.

Vurderingen av GHB614 skal utføres i henhold til tiltenkt bruk og i overensstemmelse med prinsippene som er nedfelt i EFSAAs retningslinjer for vurdering av genmodifiserte planter ("Guidance document of the scientific panel on genetically modified organisms for the risk assessment of genetically modified plants and derived food and feed") (EFSA2006a).

I henhold til oppdragsbrev fra DN skal VKM primært fokusere på miljørisiko i EØS-området, og på miljørisiko som er spesifikke for Norge. Det skal også gis en samlet konklusjon om miljørisiko i tråd med kravene i forskrift om konsekvensutredning etter genteknologiloven, vedlegg 2 C.

Produktet som ønskes vurdert:

Genmodifisert bomull, EFSA/GMO/NL/2008/51 (GHB614).

Unik kode: BCS-GHØØ2-5.

Status i EU: Søknad under 1829/2003/EF. EFSAAs frist for innspill er 11.06.08.

Ønsket svarfrist til Mattilsynet/DN: 8. juni 2008.

RISIKOVURDERING

1. Innledning

Helse- og miljørisikovurderingen av den genmodifiserte bomullslinjen GHB614 er basert på informasjon som er gjort tilgjengelig på EFSAAs nettside GMO EFSA.net. I tillegg er det benyttet uavhengige vitenskapelige publikasjoner med referee i vurderingene. Vurderingen er gjort i henhold til tiltenkt bruk og i overensstemmelse med miljø- og helsekravene i genteknologiloven med forskrifter, først og fremst forskrift om konsekvensutredning etter genteknologiloven. Videre er kravene i EUs forordning 1829/2003/EF og utsettingsdirektiv 2001/18/EF med annekser lagt til grunn for vurderingen.

I tråd med VKMs mandat presiseres det at vurderinger av etikk, bærekraft og samfunnsnytte i henhold til kravene i genteknologiloven og dens konsekvensutredningsforskrift ikke skal utføres av Faggruppe for genmodifiserte organismer. Faggruppen har derfor ikke vurdert mulige helse- og miljøeffekter ved dyrking og prosessering utenfor EØS-området.

Faggruppen har vedtatt å benytte EFSAAs retningslinjer som gruppens retningslinjer for vurdering av genmodifiserte planter. Prinsippene som er lagt til grunn for vurderingen, er derfor hentet fra EFSAAs dokument "Guidance document of the scientific panel on genetically organisms for the risk assessment of genetically modified plants and derived food and feed" (EFSA2006a). Ved vurdering av vesentlig likhet har faggruppen lagt vekt på OECDs konsensusdokument for bomull (OECD 2004), som gir anbefalinger over hvilke parametere som bør undersøkes.

Det er kun medlemmene i Faggruppen som har vurdert den genmodifiserte bomullen.

1.1. Beskrivelse av egenskaper(er) og virkningsmekanismer

Bomullslinjen GHB614 uttrykker et 2mEPSPS-enzym, som er resultat av introduksjon av et modifisert *epsps*-gen (*2mepsps*) fra mais. Genene *epsps* og *2mepsps* koder for enzymet 5-enolpyruvylsukinat-3-fosfatsyntetasen (EPSPS- og 2mEPSPS-enzym), som omdanner fosfoenolpyruvat og sukimat-3-fosfat til 5-enolpyruvylsukinat-3-fosfat, en viktig metabolitt i syntesen av aromatiske aminosyrer. N-fosfonometylglisic (glyfosat) hemmer generelt EPSPS-enzymet og blokkerer derved biosyntesen av aromatiske aminosyrer i planter. I motsetning til plantens eget EPSPS-enzym er det modifiserte mais-enzymet 2mEPSPS også aktivt ved nærvær av glyfosat. De transgene plantene vil derfor tolerere høyere doser av herbicider med virkestoff glyfosat sammenlignet med konkurrerende ugras.

2. Molekylær karakterisering

2.1. Transformasjonssystem og vektorkonstruksjon

Bomullslinjen GHB614 er fremkommet ved *Agrobacterium*-mediert transformering. Det modifiserte *epsps*-genet (*2mepsps*) ble dannet ved at et *epsps*-gen fra mais fikk introdusert to mutasjoner med *in vitro*- mutasjonsteknikk. *2mepsps*-ekspresjonskassetten ble klonet inn i den binære vektoren pTEM2. pTEM2 inneholder foruten andre gener også antibiotikaresistensgenene *aadA*, som koder for streptomycin/spectinomycinresistens og *nptII* som koder for neomycinresistens. Den binære vektoren pTEM2 inneholder venstre og høyre grense T-DNA-sekvenser. Antibiotikaresistensgenene ligger utenfor T-DNA-sekvensene, og blir derfor ikke overført til bomullscellene. T-DNA-området inneholder *2mepsps*-ekspresjonskassetten som ble overført til bomullens genom av *Agrobacterium tumefaciens* under *in vitro* transformasjonsprosess. Det rekombinante DNA-fragmentet er på 3978 basepar. Foruten *2mepsps*-genet inneholder DNA-fragmentet en *Arabidopsis thaliana* histon H4

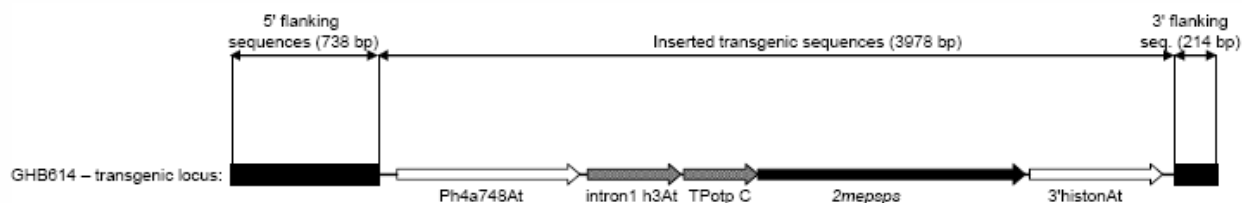
promoter (Ph4a748At) og *Arabidopsis thaliana* histon H3-intron (intron1 h3At), et optimalisert kloroplast overføringspeptid (TPotpC) og en *Arabidopsis thaliana* 3'-ende terminatorsekvens (3' histon At). Det rekombinante DNA-fragmentet inneholder ikke antibiotikaresistensen. Transformanter ble selektert ved at de overlevde og vokste i nærvær av glyfosat. Kutting av plante-DNA med restriksjonsenzymet *KpnI* og Southern blot analyse av kuttet DNA, viser at det rekombinante DNA-fragmentet er en del av et 14 kb store *KpnI* restriksjonsenzymfragmentet.

2.2. Karakterisering av geninnsettingen/genkonstruksjonen

Southern blot, PCR analyse og sekvensanalyse av PCR-fragmentene er benyttet til karakterisering av det rekombinante DNA-fragment i planten. Både innskutt DNA og flankerende genomisk DNA er blitt sekvensert. Molekylærbiolegisk karakterisering viser at det bare er satt inn en kopi av DNA-fragmentet i bomullens genom. DNA-fragmentene sitter på et 14 kilobasepar(kb) stort *KpnI*-restriksjonsenzymfragment (GHB614 rekombinant DNA fragment) (se figur 1). Genelementer i bomullsplantens rekombinante DNA-fragment er vist i tabell 1.

Tabell 1. Genelementer i 2mepsps- ekspresjonskassetten (Bayer CropScience 2008)

Symbol	Definition	Source	Size (bp)	Reference	Function
LB	Left border repeat	<i>Agrobacterium tumefaciens</i>	24	Zambryski, 1988	Cis-acting element for T-DNA transfer
Ph4a748At	Promoter	<i>Arabidopsis thaliana</i>	1010	Chaboute <i>et al.</i> , 1987	High level constitutive expression, especially in the rapidly growing plant tissues
intron1 h3At	Intron	<i>Arabidopsis thaliana</i>	516	Chaubet <i>et al.</i> , 1992	
TPotpC	Transit peptide	<i>Zea mays</i> , <i>Helianthus annuus</i>	372	Lebrun <i>et al.</i> , 1997b	Targeting of the protein to the plastids
2mepsps	Glyphosate tolerance 2mepsps gene	<i>Zea mays</i>	1337	Lebrun <i>et al.</i> , 2003	Herbicide tolerance and selectable marker
3'histon At	Terminating signal of 2mepsps gene	<i>Arabidopsis thaliana</i>	742	Chaboute <i>et al.</i> , 1987	Stop signal
RB	Right border repeat	<i>Agrobacterium tumefaciens</i>	24	Zambryski, 1988	Cis-acting element for T-DNA transfer



Figur 1. Rekombinant DNA- fragment i GHB614 bomullens genom. DNA-fragmentet er på 3978 bp.

Den genmodifiserte bomullslinjen GHB614 uttrykker glyfosattoleranse ved at GHB614 har fått satt inn et modifisert *epsps*-gen fra mais (*2mepsps*). *2mepsps*-genet koder for enzymet 5-enolpyruvylsikumat-3-fosfat syntetase (2mEPSPS-enzym) I 2mEPSPS-proteinet er treonin 102 byttet ut med isoleusin og prolin 106 byttet ut med serin (Lebrun *et al.* 2003). EPSPS- og 2mEPSPS-enzymene omdanner fosfoenolpyruvat og sikimat-3-fosfat til 5-enolpyruvylsikumat-3-fosfat, som er en viktig metabolitt i syntesen av aromatiske aminosyrer. I motsetning til hos dyr, inneholder alle planter og mikroorganismer dette enzymet. Dyr må dermed få de aromatiske aminosyrene fra føden. Planters EPSPS-enzym er imidlertid sensitiv for glyfosat, mens endringene i proteinets aminosyresekvens medfører toleranse mot fosfonometylglycinherbicer, som glyfosat, sulfosat og fosametin.

Molekylærbiologiske analyser

For å få tilstrekkelig mengde 2mEPSPS protein til diverse analyser ble *2mepsps*-genet klonet inn i *E. coli*.

Molekylærbiologiske analyser viser at det rekombinante fragmentet på ca. 3978 bp i planten inneholder det samme genet og genelementer som er på det tilsvarende fragmentet i vektoren pTEM2. 2mEPSPS-proteinet som uttrykkes i bomullsblad er undersøkt med Western-blot analyse og densitometri, SDS-PAGE-analyse og densitometri, trypsindegradering og HPLC/elektrospray massespektrometri, N-ende sekvensanalyse, samt glykosyleringsanalyse og 2mEPSPS enzymaktivitets analyse. Enzymaktivitetsanalysen viser at 2mEPSPS-proteinet er funksjonelt lik det *E. coli*-produserte proteinet. Det ble ikke påvist glykoliseringssteder på proteinene.

Sammenlignende analyser av 2mEPSPS-protein fra henholdsvis plante og bakterie viser 427 av 445 aminosyrer i *E. coli*-produsert 2mEPSPS-protein, og 407 av 445 aminosyrer i planteprodusert 2mEPSPS. Analysene ble foretatt ved hjelp av trypsindegradering og HPLC/elektronspraymetode. Søkers forklaring på at det er funnet 20 færre aminosyrer i plante-2mEPSPS enn i bakterie-2mEPSPS er Edman-degradering, delvis trypsindegradering, kjemiske endringer under proteinrensing eller posttranslasjon-modifisering av proteinet. SDS-PAGE-analyse og Westernblot viser omtrentlig lik størrelse til de to proteinene.

Det er foretatt sekvenseringsanalyser av flankesekvensene til det rekombinante DNA fragmentet, 738 bp oppstrøms fra 5'-flanke-enden og 214 bp nedstrøms fra 3'-flanke-enden til DNA fragmentet. Sekvensanalysene av transgen homozygot bomull (BC₂F₅) og villtype (varietet FM966) viser at et fragment på 17 bp i villtypen er kuttet bort i den transgene linjen. Disse 17 bp-ene ble kuttet ut under integreringen av T-DNAet.

2.3. Informasjon vedr. uttrykk av introduserte gener og åpne leserammer (ORF)

Ekspressjonen av *2mepsps*-mRNA og 2mEPSPS-protein ble analysert ved hjelp av henholdsvis Northern blot og ELISA. Konsentrasjon av 2mEPSPS-protein ble målt i prøver fra blad, stilk, rot, apikalt meristem (toppskudd/vekstpunkt), blomsterknopper og pollen. GHB614-plantene ble dyrket i veksthus, og det ble tatt ut prøver på fire ulike vekststadier. Som forventet ut fra at de regulatoriske elementene er aktive i meristematisk vev, ble det høyeste nivået av 2mEPSPS-protein funnet i raskt voksende plantedeler som blad og toppskudd, og lavest innhold i pollen. Konsentrasjonen av proteinet ble målt til $7,94 \pm 2,87$ µg/g råvekt i blad på et tidlig vekststadium, mens innholdet av 2mEPSPS ble målt til henholdsvis $5,47 \pm 0,22$ og $5,35 \pm 0,25$ µg/g råvekt i apikalt meristem og blomsterknopp ved blomstring. I frø ble nivået av 2mEPSPS-protein målt til $36,3 \pm 7,2$ (variasjonsbredde (VB)= 28,7 til 47,1) og $40,2 \pm 9,0$ (VB= 28,6 til 55,8) µg/g råvekt for henholdsvis usprøytet og sprøytet bomullsplante. I mel og frøskall ble det påvist henholdsvis $0,26 \pm 0,10$ (VB= 0,16 til 0,36) og $6,93 \pm 0,40$ (VB= 6,48 til 7,41) µg/g råvekt.

Ved sekvensering av hele DNA-fragmentet ble det påvist to åpne leserammer i 5'-flankerende sekvens, mens det ikke ble påvist åpne leserammer i 3'-flankerende sekvens. De to leserammene i 5' flankerende sekvens bestod av bomullssekvenser som ikke hadde elementer som trengs for

transkripsjon av DNA. I 5'- og 3'enden ble det ikke påvist kjente bomullsgener, mRNA, cDNA eller EST. Leserammer ble testet *in silico* for homologer til kjente toksiner og allergener, ingen slike homologer ble påvist. Northern blot analyse viste fravær av kryptisk ekspresjon.

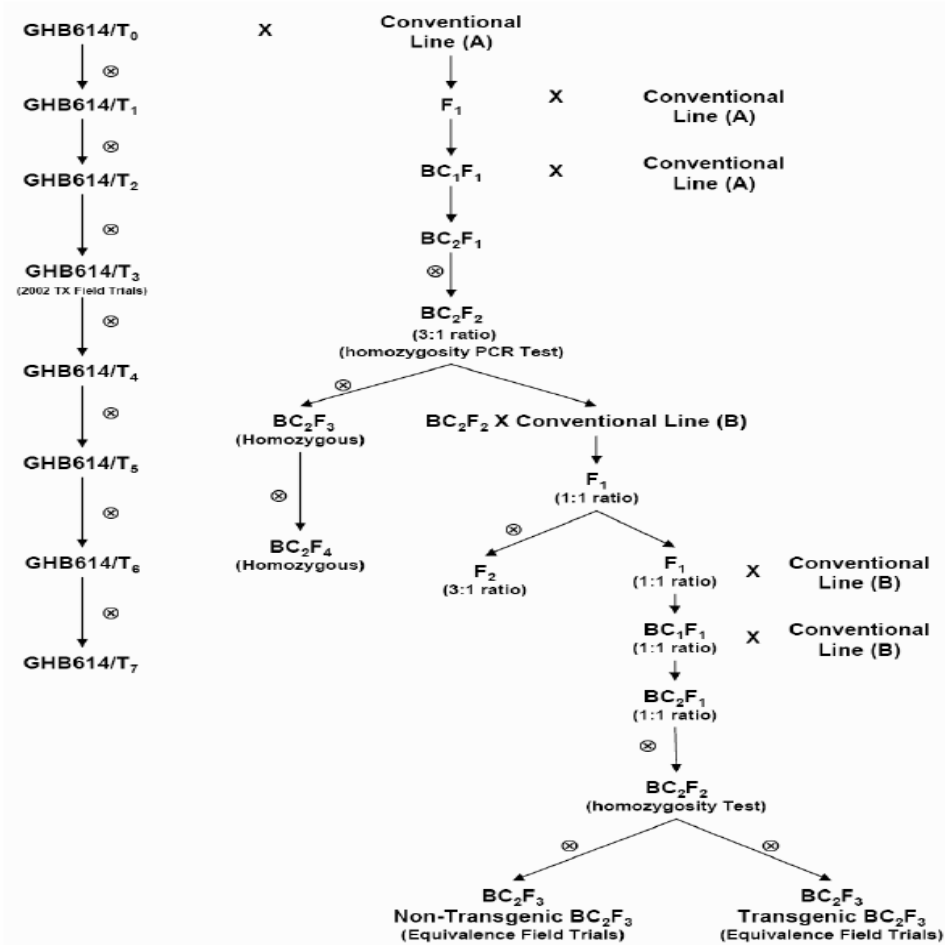
2.4. Nedarving og stabilitet av innsatt DNA

I henhold til dokumentasjonen fra Bayer CropScience er genotypisk og fenotypisk stabilitet vist ved Southern blot, samt analyser av proteinekspressjon og fenotypisk/agronomiske karakterer. Genetisk stabilitet ble evaluert i generasjonene T₃, T₄, T₅, T₆ og BC₂F₂ (se figur 2), der GHB614 var krysset inn i ulike genetisk bakgrunner (Coker 612, FiberMax966). Det ble også foretatt analyser av genomisk DNA fra planter dyrket på 6 ulike lokaliteter. Resultatene av Southern blot-analysene viser at det rekombinante DNA-innsluddet er stabilt integrert i genomet og nedarves stabilt over generasjoner under varierende dyrkingsbetingelser. Analyse av spaltingsdata fra fem ulike generasjoner viser forventet segregeringsmønster på henholdsvis 1:1, og 3:1 for det rekombinante DNA-fragmentet. Søker konkluderer med at nedarvingen av DNA-fragmentet følger mønsteret for mendelsk nedarving av et enkelt, dominant lokus. Analyser av stabiliteten av det innsatte fragmentet synes å være tilfredsstillende.

2.5. Delkonklusjon

Faggruppen har vurdert karakteriseringen av det rekombinante innskuddet i GHB614, de fysiske, kjemiske og funksjonelle karakteriseringene av proteinet til å være tilfredsstillende.

Figure 11 Breeding tree for the development of GlyTol cotton



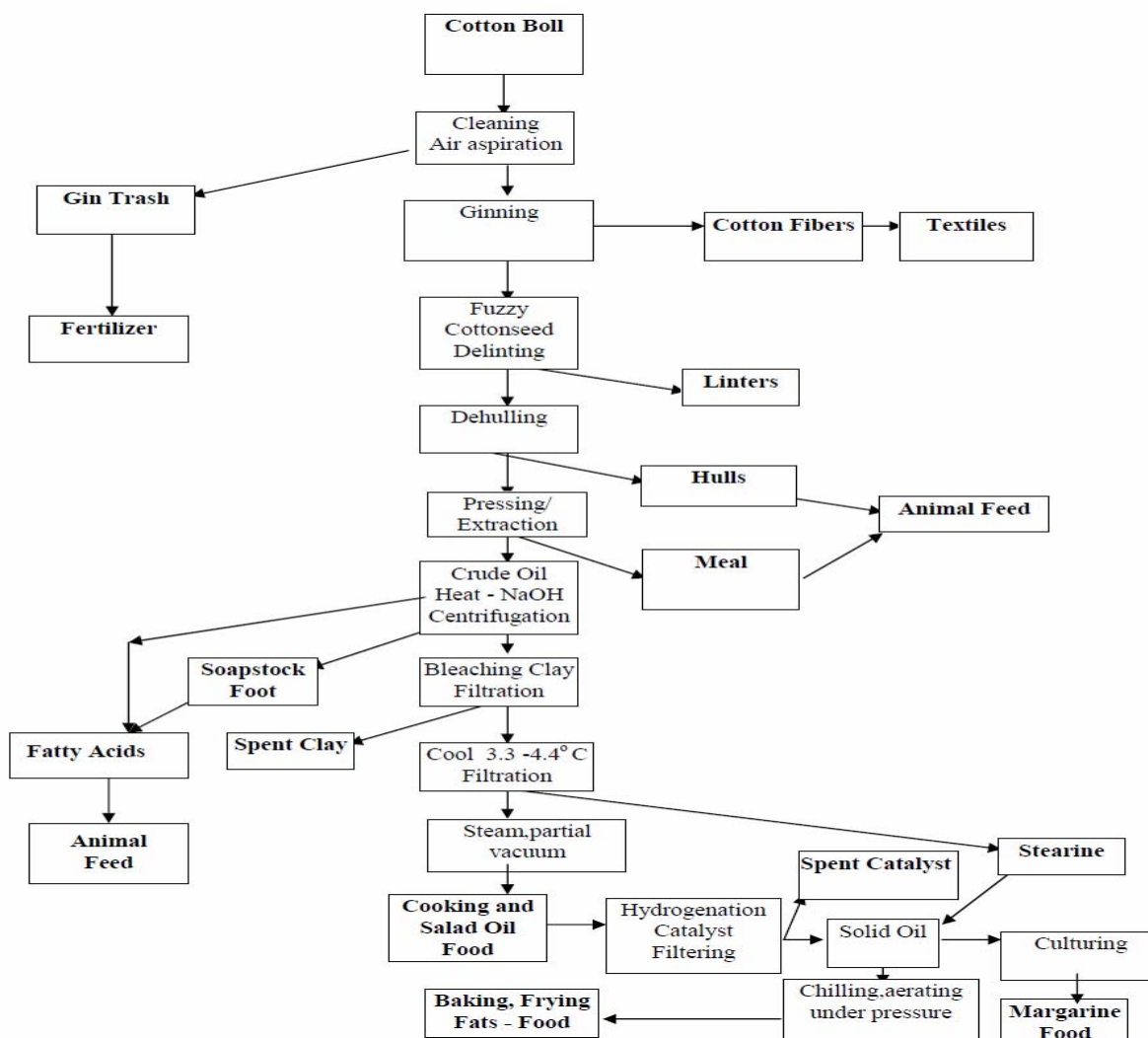
Notes for Figure 11.

- At each generation, plants were sprayed with glyphosate to eliminate those not expressing the *2mepsps* gene
- ⊗ = self-cross
- Homozygous T₃ plants were identified by planting 25 seed, spraying with glyphosate to identify segregating seed lots. Homozygosity PCR based Invader analysis was also performed as a secondary means of identifying homozygous plants.
- Selfed T₃ homozygous seed (no segregation for resistance) was used to produce homozygous T₄ seed and was the source of the lines that were used in early event agronomic and stability studies.
- Generation BC₂F₄ (homozygous) was used for detailed insert characterization and protein/RNA expression levels.
- Generations T₃, T₄, T₅, T₆ and BC₂F₂ were used for molecular stability analyses.
- Generation T₅ was used for seed composition analysis.
- Generations T₅ and BC₂F₃ were used for replicated agronomic field tests.
- Generation T₇ was used for analyses on absence/presence of vector backbone sequences.

Figur 2. Kryssingsskjema for genmodifisert bomullslinje GHB614 (Bayer CropScience 2008).

3. Komparative analyser

Bomullsfør hvor bomullsfibrene er fjernet blir bearbeidet til fire hovedprodukter, olje (16 %), mel (45 %), frøskall (26 %) og ”bomullshår (lint)” (9 %), ca. 4 % går tapt ved prosessering av frøene (OECD 2004). Det er hovedsakelig olje fra bomullsfør som brukes som menneskeføde, mens hele bomullsfør og biprodukter som mel og kli fra oljeproduksjonen brukes som fôr (se figur 3).



Figur 3. Bearbeiding av bomullsfør til bomullsfiber, fôr og olje. Diagrammet er fra OECDs konsensusdokument (OECD 2004).

3.1. Valg av komparator og forsøksdesign

I følge dokumentasjon fra Bayer CropScience er det er foretatt analyser av ernæringsmessige viktige komponenter og registreringer av agronomiske karakterer i en serie feltforsøk i sentrale dyrkingsområder for bomull i USA.

Undersøkelser av ernæringsmessige komponenter ble foretatt på 17 lokaliteter i 5 stater i vekstsesongene 2005 og 2006 (se tabell 2). Forsøksfeltene bestod av et fullstendig randomisert

blokkdesign med 3 gjentak, og inkluderte testlinjen GHB614 og en konvensjonell bomullssort ('cv. Coker 312') som tradisjonell kontroll. Det inngikk ikke kommersielle referansesorter i forsøkene. Forsøksruter med testlinjen ble behandlet med henholdsvis glyfosat eller annet konvensjonelt herbicid, mens det ble benyttet konvensjonelle sprøyteregimer på den umodifiserte kontrollen. Sprøytingene med glyfosat ble foretatt på henholdsvis 3, 12 og 16 bladstadiet.

Tabell 2. Oversikt over forsøkssteder for analyser av ernæringsmessige komponenter.

	Year	Site	County / State	EPA Region	Comparator	Treatment	Design	Replicates
Nutritional composition	2005	201	Tift, GA	II	Coker 312	A / B / C	RCBD	3
		302	Escambia, FL	III	Coker 312	A / B / C	RCBD	3
		403	Jackson, AR	IV	Coker 312	A / B / C	RCBD	3
		404	Crittenden, AR	IV	Coker 312	A / B / C	RCBD	3
		405	Drew, AR	IV	Coker 312	A / B / C	RCBD	3
		406	Tate, MS	IV	Coker 312	A / B / C	RCBD	3
		407	Tate, MS	IV	Coker 312	A / B / C	RCBD	3
		608	Wharton, TX	VI	Coker 312	A / B / C	RCBD	3
		809	Hockley, TX	VIII	Coker 312	A / B / C	RCBD	3
	2006	201	Tift, GA	II	Coker 312	A / B / C	RCBD	3
		302	Escambia, FL	III	Coker 312	A / B / C	RCBD	3
		403	Jackson, AR	IV	Coker 312	A / B / C	RCBD	3
		404	Crittenden, AR	IV	Coker 312	A / B / C	RCBD	3
		405	Drew, AR	IV	Coker 312	A / B / C	RCBD	3
		406	Tate, MS	IV	Coker 312	A / B / C	RCBD	3
		612	Wharton, TX	VI	Coker 312	A / B / C	RCBD	3
814	Hockley, TX	VIII	Coker 312	A / B / C	RCBD	3		

Trial design: complete randomized block design with three replications and three treatments:

- A) conventional cotton grown using conventional herbicide weed control,
- B) GHB614 cotton grown using conventional herbicide weed control,
- C) GHB614 cotton grown with glyphosate herbicide weed control (three applications, at the 3-leaf stage, the 12-leaf stage and 16-leaf stage, of 840 g a.i. glyphosate acid equivalent per hectare per application).

Kilde: Søknad EFSA/GMO/UK/2007/42

Analyser av variasjon i morfologiske og agronomiske karakterer er basert feltforsøk med bomullslinjen GHB614 i USA i 2004 og 2005. I tillegg ble karakterer knyttet til frøkvile og spireegenskaper testet ved hjelp av standardiserte spireanalyser. I vekstsesongene 2004 og 2005 ble det gjennomført fenotypiske registreringer på henholdsvis 8 og 12 lokaliteter i representative områder for bomullsdyrking i 5 ulike stater. De ikke-transgene linjene 'Coker312' og 'FM9740' ble benyttet som kontrollsorter i forsøkene. Forsøksfeltene i begge feltsesongene bestod både av et split-plot blokkdesign og fullstendig randomisert blokkdesign med 3 gjentak (se tabell 3).

Tabell 3. Oversikt over feltforsøk for registrering av agronomiske karakterer.

	Year	Region	County / State	Comparator	Design	Replicates	
Agronomic performance	2004	Southeast	Dillon, SC	Coker 312	split-plot	3	
			Jefferson, GA	Coker 312	split-plot	3	
			Halifax, NC	Coker 312	split-plot	3	
		Southwest	Lubbock, TX	Coker 312	split-plot	3	
			Swisher, TX	Coker 312	split-plot	3	
		Midsouth	Washington (2 sites), MS	Coker 312	split-plot	3	
			Cohoma, MS	Coker 312	split-plot	3	
		2005	Southeast	Bulloch, GA	Coker 312	split-plot	3
				Halifax, NC	Coker 312	split-plot	3
	Dillon, SC			Coker 312	split-plot	3	
	Dillon, SC			Coker 312, FM9740, BC ₂ F ₃ in FM9740 genetic background	RCBD	3	
	Southwest		Lubbock, TX (3 sites)	Coker 312	split-plot	3	
			Lubbock, TX	Coker 312, FM9740, BC ₂ F ₃ in FM9740 genetic background	RCBD	3	
	Midsouth		Washington (2 sites) MS	Coker 312	split-plot	3	
			Cohoma, MS	Coker 312	split-plot	3	
			Washington, MS	Coker 312, FM9740, BC ₂ F ₃ in FM9740 genetic background	RCBD	3	

RCBD – Randomized Complete Block Design

Statistiske analyser

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

3.2. Analyser av ernæringsmessige komponenter

Hovedkomponenter i hele bomullsfrø, bomullsmel, skall, lint og olje

Valget av analyseparametre er gjort i henhold til OECDs konsensusdokument for bomull (OECD 2004). Det er foretatt ulike analyser av hovedkomponenter for de forskjellige produktene fra bomullsfrø.

Skall og lint

Analyser av skall og lint er utført i tråd med OECDs konsensusdokument, og omfatter aske, fett, protein, vann, ADF (acid detergent fibre), NDF (neutral detergent fibre) (se tabell 4). En sammenligning mot referanseverdier for lint er ikke mulig fordi, i henhold til Bayer, slike verdier ikke er tilgjengelige. Det er kun påvist signifikante forskjeller mellom GHB614 og kontroll for innhold av fett i skall og lint.

Tabell 4. Analyser av komponenter som det i henhold til OECDs konsensusdokument bør analyseres for i skall og lint.

Component (on a dry matter basis)	Crop/herbicide regime				
	Linters		Hulls		
	Coker 312/ Conventional Herbicide ^a	GHB614/ Glyphosate Herbicide ^a	Coker 312/ Conventional Herbicide ^a	GHB614/ Glyphosate Herbicide ^a	Reference Range from Literature
Moisture % FW	7.08	6.11	11.86	10.38	8.5 - 12.3
Protein %	6.31	6.54	15.74	14.49	4.0 - 13.1
Fat %	3.38	1.56	2.26	4.40	1.0 - 3.3
Ash %	3.75	2.76	4.77	4.80	2.39 - 3.97
Total carbohydrates % ^a	85.40	89.19	79.09	78.73	85.8 - 92.6
Crude Fiber %	74.80	79.14	42.21	38.61	41.5 - 56.2
Acid detergent fibre %	87.00	85.40	66.90	61.40	57.9 - 71.6
Neutral detergent fibre %	88.80	90.60	82.10	76.30	76.3 - 92.6

^a Total Carbohydrates calculated as 100% - (crude protein %DM + crude fat %DM + ash %DM)

Frø og olje

Analysene av frø ble foretatt i henhold til OECDs konsensusdokument, og inkluderte parametrene protein, fett, aske, vann, karbohydrater, ADF, NDF, total fiber (TDF), aminosyrer, fettsyrer (C8-C22), fosfor, kalsium, vitaminene E, tokoferoler, anti-næringsstoffet gossypol (fritt og totalt), syklopropenoide fettsyrer (malvalin-, sterkulin-, og dihydrosterkulin syre). I tillegg ble det analysert for innhold av jern, kalium, magnesium og sink. Av de 81 analyserte parametrene ble det påvist statistisk signifikante forskjeller for flere komponenter, men forskjellene er ikke konsistente over forsøksfelt.

Hovedkomponenter og fiber

Analysene av hovedkomponenter og fiber viste statistisk signifikante forskjeller mellom den transgene linjen og kontrollsorter innen enkeltlokaliteter, men ikke over alle (se tabell 5).

Tabell 5. Resultater fra variansanalyse (ANOVA) av hovedkomponenter og fiber.

Summary t-tests procedures [‡]	A vs B		A vs C	
	Significant	not significant	Significant	not significant
Moisture	4	13	2	15
Protein	1	16	1	16
Crude fat	2	15	1	16
Ash	1	16	1	16
Carbohydrates	-	17	-	17
Acid detergent fibre	-	17	2	15
Neutral detergent fibre	2	15	-	17

[‡] Number of sites with significant (p < 0.05) and not significant (p > 0.05) treatment differences

A = counterpart, control samples
B = GHB614 samples, not sprayed with glyphosate
C = GHB614 samples, sprayed with glyphosate

Mineraler og vitaminer

I henhold til dokumentasjonen fra søker er det foretatt analyser av følgende mineraler: fosfor, jern, kalium, kalsium, magnesium og sink. I OECDs konsensusdokument for bomull er det kun anbefalt analyser av kalsium og fosfor i bomull. For samtlige mineraler og vitamin E viste de statistiske analysene for de enkelte komponentene signifikante forskjeller for enkelte lokaliteter, men forskjellene er ikke konsistente over forsøksfelt (tabell 6).

I henhold til OECDs konsensusdokument er vitamin E det eneste vitaminet som anbefales analysert i bomullsolje og -frø. Bayer CropScience har målt totalinnhold av vitamin E i hele -, litted - og delitted frø, samt i uraffinert og raffinert olje. I tillegg er det også analysert for innhold av alfa- og gamma-tokoferol i uraffinert og raffinert olje. Det er ikke funnet store statistisk signifikante forskjeller for de fleste komponentene (tabell 6).

Tabell 6: Resultater fra variansanalyser (ANOVA) for mineraler, vitamin E, alfa- og gamma tokoferol i bomullsfør.

Summary t- test procedures *	A vs B		A vs C	
	significant	not significant	significant	not significant
Calcium	3	14	4	13
Phosphorus	2	15	-	17
Potassium	3	14	2	15
Magnesium	2	15	3	14
Iron	-	17	2	15
Zinc	1	16	-	17
Alpha Tocopherol	1	16	1	16
Gamma Tocopherol	-	17	1	16
Vitamin E (Tocopherols)	1	16	1	16

* Number of sites with significant ($p < 0.05$) and not significant ($p > 0.05$) treatment differences
A = counterpart, control samples
B = GHB614 samples, not sprayed with glyphosate
C = GHB614 samples, sprayed with glyphosate

Fettsyresammensetning i bomullsfør og olje

Fettsyresammensetningen i hele frø, samt uraffinert og raffinert olje fra GHB614 og umodifisert kontrollsort er målt i henhold til OECDs konsensusdokument for bomull. Det ble analysert for innhold av 13 ulike fettsyrer. Det er funnet statistisk signifikante forskjeller for flere fettsyrer (se tabell 7). I henhold til søker er det funnet flertydige resultater for palmitolsyre, og signifikante forskjeller for stearin-, olje- og linolsyre for begge sammenligningene. Søker hevder at imidlertid at variansanalysen (ANOVA) over lokaliteter ikke er gyldig fordi det ble påvist signifikante effekter av lokalitet og herbicidbehandling. Med unntak for linolensyre, der forskjellen var ca. 25 %, er de gjennomsnittlige forskjellene over alle lokalitetene for de øvrige fettsyrene på mindre enn ± 10 %. For råolje, rensert og deodorisert olje er det ikke funnet statistisk signifikante forskjeller. Det er imidlertid ikke påvist myristinsyre i rensert og deodorisert olje. Søker forklarer dette med at det ble gjort feil ved evaluering av kromatogrammet.

Tabell 7. Resultater fra variansanalyse (ANOVA) for fettsyrer i bomullsfør

Summary t-test procedures *	A vs B		A vs C	
	significant	not significant	significant	not significant
C14:0 Tetradecanoic (myristic)	-	17	3	14
C16:0 Hexadecanoic (palmitic)	5	12	4	13
C16:1 Hexadecanoic (palmitoleic)	8^a	9^a	8^a	9^a
C18:0 Octadecanoic (stearic)	11^a	6^a	12^a	5^a
C18:1 Octadecanoic (oleic)	13^a	4^a	13^a	4^a
C18:2 Octadecadienoic (linoleic)	12^a	5^a	13^a	4^a
C18:3 Octadecatrienoic (linolenic)	17^a	-	17^a	-
C20:0 Eicosanoic (arachidic)	6	10	5	11
C22:0 Docosanoic (behenic)	5	11	5	11
C22:5 Docosapentaenoic	-	8	2	6
C22:5 Docosapentaenoic ^b	-	17	2	15
C24:0 Tetracosanoic (lignoceric)	-	9	1	8
C24:0 Tetracosanoic (lignoceric) ^b	-	15	1	14

* Number of sites with significant (p < 0.05) and not significant (p > 0.05) treatment differences
A = counterpart, control samples
B = GHB614 samples, not sprayed with glyphosate
C = GHB614 samples, sprayed with glyphosate
^a figures in bold indicate that at the majority of sites were found significant differences
^b 'not significant' was also assumed if all samples of a site were equal or below the limit of quantification for the two respective treatments

Aminosyrer i bomullsfør

Det er analysert for innhold av både essensielle og ikke-essensielle aminosyrer i hele frø, samt i ubehandlet og varmebehandlet mel. Analysene er foretatt i henhold til OECDs retningslinjer. Det ble ikke funnet statistisk signifikante forskjeller over lokalitetene (se tabell 8). Verdiene avviker ikke utover ± 10 %, og for samtlige aminosyrer ligger verdiene innenfor typiske verdier som er rapportert i litteraturen.

Tabell 8. Resultater fra variansanalyse (ANOVA) for aminosyrer i frø.

Summary t-tests procedures *)	A vs B		A vs C	
	significant	not significant	significant	not significant
Alanine	1	16	1	16
Arginine	3	14	-	17
Aspartic Acid	2	15	1	16
Cystine	1	16	-	17
Glutamic acid	2	15	1	16
Glycine	1	16	1	16
Histidine	1	16	1	16
Isoleucine	1	16	1	16
Leucine	1	16	1	16
Lysine	1	16	-	17
Methionine	1	16	-	17
Phenylalanine	1	16	-	17
Proline	-	17	1	16
Serine	2	15	-	17
Threonine	2	15	1	16
Tryptophan	3	14	-	17
Tyrosine	3	14	-	17
Valine	1	16	1	16

* Number of sites with significant (p < 0.05) and not significant (p > 0.05) treatment differences
A = counterpart, control samples
B = GHB614 samples, not sprayed with glyphosate
C = GHB614 samples, sprayed with glyphosate

Antinæringsstoffer

Det ble påvist statistisk signifikante forskjeller for antinæringsstoffene som er analysert i 'lited frø'. Resultatene viser relativt store signifikante forskjeller for variablene malvin-, sterkul- og dihydrosterkulsyre (se tabell 9). Statistiske analyser over forsøkssteder viser forskjeller på henholdsvis ca. 30 %, 25 % og 50 % for disse parametrene sammenlignet med den umodifiserte kontrollen 'Coker 312'. Søker hevder imidlertid at de statistiske undersøkelsen for malvalin- og sterkulsyre ikke er gyldige fordi ANOVA-analysen viser signifikante effekter av herbicidbehandling, lokalitet og år. Når det gjelder uraffinert olje ble det funnet signifikante forskjeller over 20 % mellom GHB614 og 'Coker 312' for innhold av total gossypol og dihydrosterkulsyre. Det ble ikke funnet signifikante forskjeller for raffinert olje.

Tabell 9. Resultater fra variasjonsanalyser (ANOVA) for antinæringsstoffer i frø

Summary t- test procedures *	A vs B		A vs C	
	significant	not significant	significant	not significant
Free Gossypol	2	15	1	16
(+) Gossypol	4	13	4	13
(-) Gossypol	4	13	2	15
Total Gossypol	1	16	4	13
Malvalic acid	10^a	4^a	9^a	5^a
Sterculic acid	9^a	7^a	10^a	6^a
Dihydrosterculic acid	3^a	-	3^a	-

* Number of sites with significant ($p < 0.05$) and not significant ($p > 0.05$) treatment differences

A = counterpart, control samples

B = GHB614 samples, not sprayed with glyphosate

C = GHB614 samples, sprayed with glyphosate

^a figures in bold indicate that at the majority of sites significant differences were found

Toksiner og allergener

I henhold til søkers dokumentasjon ble innholdet av aflatoksiner målt i røstet mel. Nivået aflatoksiner i melet, som ble benyttet i føringsforsøk med kylling, var under 5 ppb. Den norske grenseverdien for totalinnhold av aflatoksiner i korn og kornprodukter er på 4 ppb.

Søker har videre undersøkt aminosyresekvenshomologi for 2mEPSPS-proteinet til kjente toksiner og allergener i offentlig tilgjengelige databaser. Kriterier som ble benyttet var 35 % homologi og et vindu på 80 aminosyrer. Det ble ikke funnet homologe sekvenser med kjente toksiner eller allergener.

Analyse av protein og DNA i raffinert bomullsolje.

Bayer CropScience har analysert raffinert bomullsolje for protein og DNA. Verken 2mEPSPS-protein eller DNA ble påvist over deteksjonsgrensen i raffinert olje. Deteksjonsgrense for DNA i olje er 0,1 µg/ml olje.

3.3. Agronomiske egenskaper*Forsøk 2004 (split-plot design)*

Total 23 ulike fenotypiske karakterer ble evaluert i løpet av vekstsesongen 2004. Bayer opplyser at det er foretatt registreringer av egenskaper knyttet til reproduksjon, spredning, vekst og utvikling, morfologi, kvalitet (frø, fiber), sjukdoms- og insektsresistens, samt toleranse mot ulike abiotiske stressfaktorer. Det er foretatt statistiske analyser innen steder og kombinerte analyser over steder for hver karakter. De kombinerte analysene viser signifikante forskjeller ($p \leq 0,05$) mellom GHB614 og kontrollinjen for karakterene frøavling og fiberkvalitet. I følge søker er imidlertid gjennomsnittsverdiene for disse parametrene innenfor variasjonsområdene for referansessortene og 99 % toleranseintervall. For de øvrige karakterene ble det ikke funnet signifikante forskjeller.

Forsøk 2005 (split-plot design)

Totalt 23 ulike fenotypiske karakterer ble evaluert i løpet av vekstsesongen 2005, de samme variabler som i 2004. Statistiske analyser over steder viser signifikant ($p \leq 0,05$) lavere frøavling og fiberkvalitet hos testlinjen sammenlignet med kontrollen. Men igjen pekes det på at gjennomsnittsverdiene for denne karakteren ligger innenfor variasjonsområdene for referansessortene.

Forsøk 2005, (fullstendig randomisert blokkdesign)

På 3 ulike lokaliteter er forsøksoppsettet utført som fullstendig randomisert blokkdesign. Totalt er det undersøkt for 24 ulike fenotypiske karakterer. Det ble ikke benyttet glyfosat i disse forsøkene. De kombinerte analysene viser noen få signifikante forskjeller mellom GHB614 og kontrollinjen. I følge søker er imidlertid disse forskjellene regionale forskjeller og ikke konsistente over alle forsøkssteder.

3.4. Delkonklusjon

Analysene av ernæringsmessige komponenter er utført i tråd med OECDs konsensusdokument for bomull (OECD 2004). Analysene viser statistisk signifikante forskjeller for enkeltparametere, men forskjellene er ikke konsistente over forsøksfelt og ligger innenfor typiske verdier som er rapportert i litteraturen. Med unntak for dehydrosterulsyre, er forskjellene mellom testlinje og kontroll for samtlige komponenter mindre enn ± 20 %. Faggruppen anser at de forskjellene som er påvist ikke har helsemessig betydning.

Resultatene fra undersøkelsene av agronomiske og morfologiske karakterer viser at, med unntak av herbicidresistens, er det små eller ingen forskjeller mellom GHB614 og kontrollsorter.

4. Dokumentasjon av toksisitet og allergisitet

4.1. Toksisitet

Akuttforsøk på mus

Det er utført akuttstudie på mus med bakterielt produsert 2mEPSP-protein fra *E. coli*. Studiene er utført i henhold til U.S. E.P.A. Health Effects Test Guidelines OPPTS 870.1100 (godkjent i 2002) og O.E.C.D.s Test Guideline 425 (godkjent i 2001), for oral akuttoksisitetsstudier (US EPA 2002, OECD 2001). Akuttstudien på mus ble utført i henhold til "Good Laboratory Practice" vedtekter fra US EPA 1989, OECD 1998, EU direktiv 2004/10/EC, Japan MAFF 2000 og fransk dekret N°98-1312. I hver av studiene inngikk 5 hunn-mus. Det ble benyttet 2mEPSPS-doser på 2000 mg/kg kroppsvekt. Som kontroll ble det benyttet bovint serumalbumin (2000 mg/kg kroppsvekt). Alle dyr ble observert daglig for kliniske tegn på forgiftning, mens kroppsvekten ble målt ukentlig. Etter 15 dager ble det ikke registrert tegn på toksisk påvirkning i noen av forsøkene. Dyrene ble avlivet og det ble ikke påvist organskader ved grov patologisk undersøkelse. Faggruppen finner disse studiene tilfredsstillende. Generelt, med unntak for allergene proteiner, er proteiner ikke akuttoksiske.

Føringsforsøk på broiler

Søknaden inneholder dokumentasjon fra 42-dagers føringsforsøk på broilere. Undersøkelsen inkluderte 420 dyr, fordelt på 3 grupper à 140 dyr. Dyrene ble fordelt på 14 bur med 10 broiler per bur (7 bur med hanner og 7 bur med hunner). Studien er utført i henhold til "Good Laboratory Practice" vedtekter fra US EPA, OECD og Japan MAFF. Dyrene ble føret med ca. 10 % røstet bomullsmel innblandet i standardfôr. Bomullsmålet var fra en kommersiell bomullssort, umodifisert kontroll 'Coker 312' og den transgene linjen GHB614. Statistisk analyse indikerer signifikante forskjeller mellom gruppene for flere testparametre. Majoriteten av forskjellene ble funnet mellom kommersiell bomull og umodifisert tradisjonelt motstykke. Ved testdag 42 ble det vist signifikante forskjeller mellom kommersiell, umodifisert bomull og umodifisert kontrollinje for flere av de undersøkte

parametrene. Det ble ikke påvist signifikante forskjeller ved føring med transgen bomullsmel og mel fra 'Coker 312'. Det var heller ingen signifikante forskjeller ved føring med mel fra transgen bomull og kommersiell umodifisert bomull. Søker konkluderer med at forskjellene som er påvist ikke kan relateres til genmodifiseringen.

Subkronisk føringsforsøk på rotter

Bayar CropScience har ikke foretatt 13 ukers føringsforsøk med rotter.

4.2. Allergenitet

Aminosyresekvensen til de fleste viktige allergener, deriblant matallergener, er kjent. De viktige IgE-bindingsepitopene, dvs. aminosyresekvenser på 5-7 aminosyrer der IgE binder seg, er kartlagt for mange allergener. Det er utført *in silico* søk for aminosyresekvenshomologi for 2mEPPSPS-proteinet til aminosyresekvenser i databasene Uniprot_Swissprot, Uniprot_TrEMBL, PIR, NRL-3D, DAD og GenPept. Disse basene inneholder aminosyresekvenser til kjente allergener og toksiner. Analysene av 2mEPPSPS proteinet er gjort i henhold til FAO/WHO sine retningslinjer (FAO/WHO 2001). Kriterier som er benyttet er oppdeling i overlappende blokker på 8 aminosyrer. Det ble ikke funnet sekvenshomologi til epitoper til kjente allergener. Det er også foretatt undersøkelser for potensielle O- og N-glykosyleringsseter siden disse ofte finnes i allergener. Det ble ikke funnet potensielle glykosyleringsseter i 2mEPPSPS-proteinet.

4.3. Delkonklusjon

Faggruppen konkluderer med at det på bakgrunn av disse forsøkene ikke er grunn til å anta at den ernæringsmessige kvaliteten til den genmodifiserte bomullen er forskjellig fra umodifisert bomull.

5. Miljørisikovurdering

Bayers søknad om godkjenning av den transgene bomullslinjen GHB614 under forordning 1829/2003/EF omfatter bruksområdene næringsmidler, fôrvarer, import og prosessering. Søknaden gjelder ikke dyrking. Miljørisikovurderingen av GHB614 er derfor avgrenset til mulige effekter av utilsiktet frøspredning i forbindelse med transport og prosessering til mat, fôr og industrielle formål. I tillegg vil indirekte eksponering gjennom gjødsel fra husdyr føret med biprodukter fra transgene bomullsfrø representere en mulig kilde til uønsket genspredning.

5.1. Potensiale for ikke tilsiktede effekter på fitness relatert til genmodifiseringen

Slekten *Gossypium* (*Malvaceae*) består av om lag 50 diploide og allotetraploide arter, av disse er *G. arboretum*, *G. barbadense*, *G. herbaceum* og *G. hirsutum* domestiserte og benyttet som landbruksplanter (Brubaker *et al.* 1999). *G. herbaceum* L. og *G. hirsutum* L. har vært dyrket i Sør-Europa siden 1800-tallet (EFSA 2006b). I dag er *G. hirsutum* L den arten som har størst dyrkingsomfang på verdensbasis, med India, Kina, USA og Pakistan som de største produsentlandene (FAOSTAT 2006). I Europa dyrkes det bomull i Hellas, Spania og noe i Bulgaria.

G. hirsutum L ('upland cotton') er opprinnelig en flerårig busk, men dagens kommersielle sorter dyrkes som ettårige kulturer. Bomullsplanten er tilpasset et subtropisk og tropisk klima og overvintring betinger månedlige gjennomsnittstemperaturer over 18 °C. *G. hirsutum* L er en tetraploid og overveiende selvbefruktende art. Pollenkornene er relativt store, tunge og klebrige, og eventuell pollenspredningen skjer primært med humler og bier som vektorer. Graden av utkryssing varierer mellom sorter og tilstedeværelse av pollinatorer, og skjer normalt ved lave frekvenser (0-25 %) (Xanthopoulos & Kechagia 2000; Turley & Kloth 2002). Det er ingen stedege eller introduserte viltvoksende arter i den europeiske flora som *G. hirsutum* L kan hybridisere med. Spredte forekomster

av forvillede planter fra *G. herbaceum* L. og *G. hirsutum* L. kan imidlertid forekomme (ref. EFSA 2006b).

Frø av dyrkede former av bomull har normalt ingen form for frøkvile (dormancy). Det er imidlertid kjent at ytre miljøbetingelser som lave jordtemperaturer og/eller fuktighet kan indusere sekundær (eksogen) frøkvile (OGTR 2002). Enkelte dyrkede sorter av bomull har endogen frøkvile, noe som skyldes forekomsten av 'harde frø'. Frøene må imidlertid ha mye sol og spirer bare under snevre klimatiske betingelser (optimal spiretemperatur 25 – 30 °C). Bomullsplanten krever en lang vekstsesong for frømodning (120-200 døgn), og under norske vekstforhold vil derfor eventuelle planter spirt fra spillfrø ikke kunne reproducere.

Spredning av bomull til andre habitater i Europa er i hovedsak begrenset av manglende frøkvile og liten toleranse for lave temperaturer. Det er ikke påvist forskjeller mellom den transgene bomullslinjen GHB614 og konvensjonelle sorter med tilsvarende genetisk bakgrunn for disse karakterene, og det er ikke grunn til å anta at den introduserte egenskapen vil medføre økt fitness utenfor dyrkingsmiljø i forhold til ikke-transgene sorter av bomull.

5.2. Potensiale for genoverføring

En forutsetning for genspredning er tilgjengelige veier for overføring av genetisk materiale, enten via horisontal genoverføring av DNA, eller vertikal genflyt i form av frøspredning og krysspollinering. Eksponering av mikroorganismer for rekombinant DNA skjer under nedbryting av plantemateriale på dyrket mark og/eller pollen i åkrer og omkringliggende arealer. Rekombinant DNA er også en komponent i en rekke mat- og fôrprodukter som er avledet av plantemateriale fra den transgene sorten. Dette medfører at mikroorganismer i fordøyelseskanalen hos mennesker og dyr kan eksponeres for rekombinant DNA.

5.2.1. Horisontal genoverføring

Data fra tilgjengelige eksperimentelle studier viser at genoverføring fra transgene planter til bakterier etter all sannsynlighet inntreffer svært sjelden under naturlige forhold, og at denne overføringen forutsetter sekvenshomologi mellom overført DNA og bakterien (EFSA 2004; VKM 2005).

Ut fra dagens vitenskapelig innsikt med hensyn til barrierer for genoverføring mellom ubeslektede arter og flere års forskning for om mulig å framprovosere tilfeldig overføring av genetisk materiale fra planter til mikroorganismer er det lite som tyder på at transgenene i GHB614 skal kunne overføres til andre enn naturens kryssingspartnere ved detekterbare frekvenser i laboratoriestudier. Det er gjort forsøk som ser på stabilitet og opptak av DNA fra tarmkanalen hvor mus er oralt tilført M13 DNA. Det tilførte DNAet var sporbart i avføring opp til syv timer etter fôring. Svært små mengder av M13 DNA (< 0,1 %) kunne spores i blodbanene i en periode på maksimum 24 timer, mens M13 DNA ble funnet i opptil 24 timer i lever og milt (Schubbert *et al.* 1994). Ved oralt inntak av genmodifisert soya er det vist at DNA er mer stabilt i tarmen hos personer med utlagt tarm sammenlignet med kontrollgruppen (Netherwood *et al.* 2004). I kontrollgruppen ble det ikke påvist GM DNA i feces. Nielsen *et al.* (2000) og De Vries & Wackernagel (2002) har undersøkt persistens av DNA og opptak av GM DNA i jord. I disse laboratorieforsøkene ble det påvist svært små mengder DNA som var overført fra planter til bakterier. Forutsetningen for at dette kunne skje var sekvenshomologi mellom plantetransgenet og mottagerbakterien. I hvilken grad det forekommer tilfeldig sekvenshomologi mellom plantetransgener og naturlig forekommende bakterier er usikkert, men siden de fleste transgenene inneholder rekombinerte DNA sekvenser fra jordbakterier kan dette ikke utelukkes (Bensasson *et al.* 2004)

Med bakgrunn i opprinnelse og karakter/egenskaper av det innsatte genet og mangel på seleksjonspress i fordøyelseskanal og/eller miljøet, er sannsynligheten for at horisontal genoverføring vil gi selektive fordeler eller økt fitness på mikroorganismer svært liten (Nielsen 2003). Det er derfor usannsynlig at gener fra GHB614 vil etableres stabilt i genomet til mikroorganismer i miljøet eller i fordøyelseskanalen hos mennesker eller dyr. Ut fra tilgjengelig kunnskap er det ikke grunn til å forvente at det vil skje horisontal genoverføring av DNA-materiale fra GHB614.

5.2.2. Vertikal genoverføring

Bomull dyrkes ikke i Norge, og arten har ikke viltvoksende populasjoner eller nærstående arter utenfor dyrking i Europa. Utsiktet frøspredning i forbindelse med transport, håndtering og prosessering vil derfor ikke medføre risiko for spredning av transgener til økologiske eller konvensjonelt dyrkede sorter, eller til ville populasjoner og arter utenfor jordbruksområder.

5.3. Delkonklusjon

Søknaden gjelder godkjenning av bomullslinjen GHB614 for import, prosessering og til bruk i næringsmidler og fôrvarer. Faggruppen har derfor ikke vurdert mulige miljøeffekter knyttet til dyrking av bomullslinjen. Det er ingen indikasjoner på økt sannsynlighet for spredning, etablering og invasjon av bomullslinjen i naturlige habitater eller andre arealer utenfor jordbruksområder som resultat av frøspill i forbindelse med transport og prosessering. 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.

5.4. Vurdering av søkers dokumentasjon/kunnskapshull

Ingen innspill fra FG3 til EFSA-nett.

KONKLUSJON

Analysen av ernæringsmessige viktige komponenter er utført i tråd med OECDs konsensusdokument for bomull (OECD 2004). Faggruppen anser analysene for å være tilstrekkelige for en vurdering av bomullslinjen GHB614 til bruk som fôr, samt for olje til bruk som mat.

Det er påvist statistisk signifikante forskjeller for enkelte komponenter, men forskjellene er ikke konsistente over forsøksfelt og verdiene ligger innenfor typiske verdier for andre bomullssorter som er rapportert i litteraturen.

Flere studier viser at 2mEPSPS-proteinet som blir uttrykt som følge av genmodifiseringen ikke er akutt toksisk eller allergent. Bayer CropScience har utført og henviser til akuttstudier på mus og fôringsforsøk på broilere med det aktuelle proteinet. Disse studiene viser at proteinet ikke fører til påvisbare helseeffekter på dyrene.

Faggruppen konkluderer med at det er lite sannsynlig at eksponering for 2mEPSPS-proteinet i seg selv, og i de mengder som tilføres via fôr fra den genmodifisert bomull fører til allergi eller toksiske effekter.

Faggruppe for genmodifiserte organismer konkluderer med at bomullsfrøolje og fôrvarer fra GHB614 er vesentlig lik olje og fôrvarer fra umodifiserte bomullsfrø, og finner at bruk av olje og fôrvarer fra den transgene bomullslinjen ikke utgjør noen større helserisiko enn kommersiell olje og fôrvarer fra umodifiserte bomullsplanter.

Søknaden gjelder godkjenning av bomullslinjen GHB614 for import, prosessering, mat og fôr. Faggruppen har derfor ikke vurdert mulige miljøeffekter knyttet til dyrking av bomullslinjen. Det er ingen indikasjoner på økt sannsynlighet for spredning, etablering og invasjon av bomullslinjen i naturlige habitater eller andre arealer utenfor jordbruksområder som resultat av frøspill i forbindelse med transport og prosessering. Bomull dyrkes ikke i Norge, og det er ingen stedegne eller introduserte viltvoksende arter i den europeiske flora som bomull kan hybridisere med. Det er derfor ikke risiko for utkryssing med dyrkede sorter eller ville planter i Norge.

Samlet vurdering

Faggruppen finner det lite trolig at den omsøkte bruken av bomullslinjen GHB614 vil medføre endret risiko for helse og miljø i forhold til annen bomull.

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Appendix II

The EFSA Journal (2009) 985, 2-24

SCIENTIFIC OPINION

Application (Reference EFSA-GMO-NL-2008-51) for the placing on the market of glyphosate tolerant genetically modified cotton GHB614, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Bayer CropScience¹

Scientific Opinion of the Panel on Genetically Modified Organisms

(Question No EFSA-Q-2008-016)

Adopted on 05 March 2009

PANEL MEMBERS*

Hans Christer Andersson, Salvatore Arpaia, Detlef Bartsch, Josep Casacuberta, Howard Davies, Patrick du Jardin, Lieve Herman, Niels Hendriksen, Sirpa Kärenlampi, Jozsef Kiss, Gijs Kleter, Ilona Kryspin-Sørensen, Harry Kuiper, Ingolf Nes, Nickolas Panopoulos, Joe Perry, Annette Pöting, Joachim Schiemann, Willem Seinen, Jeremy Sweet and Jean-Michel Wal.

SUMMARY

This document provides a scientific opinion of the Scientific Panel on Genetically Modified Organisms (GMO Panel) of the European Food Safety Authority (EFSA) on genetically modified (GM) cotton GHB614 (Unique Identifier BCS-GHØØ2-5) developed to provide tolerance to glyphosate-based herbicides.

In delivering its scientific opinion, the EFSA GMO Panel considered the application EFSA-GMO-NL-2008-51, additional information supplied by the applicant and scientific comments submitted by Member States. The scope of application EFSA-GMO-NL-2008-51 is for food and feed uses, import and processing of cotton GHB614 and all derived products, but excludes cultivation in the EU. The EFSA GMO Panel assessed cotton GHB614 with reference to the intended uses and appropriate principles described in the guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. The scientific assessment included molecular characterisation of the inserted DNA and expression of target proteins. A

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* (minority opinion) This opinion is not shared by 0 members of the Panel. / (conflict of interest) 0 members of the Panel did not participate in (part of) the discussion on the subject referred to above because of possible conflicts of interest.

comparative analysis of agronomic traits and composition was undertaken, and the safety of the new protein and the whole food/feed were evaluated with respect to potential toxicity, allergenicity and nutritional quality. An assessment of environmental impacts and the post-market environmental monitoring plan were undertaken.

Cotton GHB614 is derived from the cotton variety Coker 312 that was transformed by *Agrobacterium*-mediated gene transfer technology. Cotton GHB614 expresses a modified *epsps* (*2mepsps*) maize gene leading to the production of a modified 5-enolpyruvyl-shikimate-3-phosphate synthase (2mEPSPS) enzyme that confers tolerance to glyphosate-based herbicides.

The molecular characterisation data established that a single insert with one copy of the intact modified *epsps* (*2mepsps*) expression cassette is integrated in the cotton genomic DNA. Appropriate analyses of the integration site including sequence determination of the inserted DNA and flanking regions and bioinformatics analysis have been performed. Bioinformatics analysis of junction regions demonstrated the absence of any potential new open reading frames coding for known toxins or allergens. The expression of the gene introduced by genetic modification has been sufficiently analysed and the stability of the genetic modification has been demonstrated over several generations. The EFSA GMO Panel is of the opinion that the molecular characterisation of the DNA insert and flanking regions of cotton GHB614 does not raise any safety concern, and that sufficient evidence for the stability of the genetic modification was provided.

Based on comparative analyses, the EFSA GMO Panel concluded that cotton GHB614 is compositionally and agronomically equivalent to the non-GM counterpart and other conventional cotton except for the introduced trait. The risk assessment included an analysis of data from analytical studies, bioinformatics, and *in vitro* and *in vivo* studies. The EFSA GMO Panel considers it unlikely that the overall allergenicity of the whole plant is changed by the genetic modification and concludes that cotton GHB614 is as safe as the non-GM counterpart and other conventional cotton.

The application EFSA-GMO-NL-2008-51 concerns food and feed uses, import and processing. Therefore, there is no requirement for scientific information on possible environmental effects associated with the cultivation of cotton GHB614. The EFSA GMO Panel agrees that unintended environmental effects due to the establishment and spread of cotton GHB614 will not be different from that of conventionally bred cotton.

Considering the intended uses of cotton GHB614, the monitoring plan provided by the applicant is in line with both the EFSA GMO Panel guidance document on the risk assessment of GM plants and the opinion of the EFSA GMO Panel on post-market environmental monitoring. However, the EFSA GMO Panel is aware that, due to physical characteristics of cotton seeds and methods of transportation, accidental spillage is unavoidable. Therefore, the EFSA GMO Panel recommends that specific measures are introduced to actively monitor the occurrence of feral cotton plants in areas where seed spillage is likely to occur.

In conclusion, the EFSA GMO Panel considers that the information available for cotton GHB614 addresses the scientific comments raised by Member States and that cotton GHB614 is as safe as its conventional counterpart with respect to potential effects on human and animal health and the environment. The EFSA GMO Panel thus concludes that cotton

GHB614 is unlikely to have any adverse effect on human and animal health or on the environment in the context of its intended uses.

Key words: GMOs, cotton, GHB614, BCS-GHØØ2-5, glyphosate tolerant, 2mEPSPS, food/feed safety, animal and human health, environment, import, processing, Regulation (EC) No 1829/2003

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BACKGROUND

On 25 January 2008, the European Food Safety Authority (EFSA) received from the Netherlands an application (Reference EFSA-GMO-NL-2008-51), for authorisation of cotton GHB614 (Unique Identifier BCS-GHØØ2-5), submitted by Bayer CropScience within the framework of Regulation (EC) No 1829/2003 on genetically modified (GM) food and feed. 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 Member States and the European Commission, and made the summary of the dossier available to the public on the EFSA website. EFSA initiated a formal review of the application to check compliance with the requirements laid down in Articles 5(3) and 17(3) of Regulation (EC) No 1829/2003. On 11 March 2008, EFSA declared the application as formally valid in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003.

EFSA made the valid application available to Member States and the European Commission, and consulted nominated risk assessment bodies of Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC following the requirements of Articles 6(4) and 18(4) of Regulation (EC) No 1829/2003, to request their scientific opinion. Member State bodies had three months after the date of receipt of the valid application (until 11 June 2008) within which to make their opinion known.

The Scientific Panel on Genetically Modified Organisms (GMO Panel) of EFSA carried out a scientific assessment of the GM cotton GHB614 for food and feed uses, import and processing, in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003, taking into consideration the scientific comments of Member States and the additional information provided by the applicant (requested on 3 September 2008 and 12 November 2008).

In giving its scientific opinion on cotton GHB614 to the European Commission, Member States and the applicant, and in accordance with Articles 6(1) and 18(1) of Regulation (EC) No 1829/2003, EFSA has endeavoured to respect a time limit of six months from the receipt of the valid application. As additional information was requested by the EFSA GMO Panel, the time limit of six months was extended accordingly, in line with Articles 6(1), 6(2), 18(1), and 18(2) of Regulation (EC) No 1829/2003.

TERMS OF REFERENCE

The EFSA GMO Panel was requested to carry out a scientific risk assessment of cotton GHB614 for food and feed uses, import and processing in accordance with Articles 6(6) and 18(6) of Regulation (EC) No 1829/2003. Where applicable, any conditions or restrictions which should be imposed on the placing on the market and/or specific conditions or restrictions for use and handling, including post-market monitoring requirements based on the outcome of the risk assessment and, in the case of GMOs or food/feed containing or consisting of GMOs, conditions for the protection of particular ecosystems/environment and/or geographical areas should be indicated in accordance with Articles 6(5)(e) and 18(5)(e) of Regulation (EC) No 1829/2003.

The EFSA GMO Panel was not requested to give a scientific opinion on information required under Annex II to the Cartagena Protocol. Furthermore, the EFSA GMO Panel did also not consider proposals for labelling and methods of detection (including sampling and the

identification of the specific transformation event in the food/feed and/or food/feed produced from it), which are matters related to risk management.

ACKNOWLEDGEMENTS

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ASSESSMENT

1. Introduction

Cotton GHB614 (Unique Identifier BCS-GHØØ2-5) is assessed with reference to its intended uses and the appropriate principles described in the guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of GM plants and derived food and feed (EFSA, 2006a).

Cotton (*Gossypium hirsutum* L.) varieties derived from the GHB614 event express a modified 5-enopyruvyl-shikimate-3-phosphate synthase (2mEPSPS) of maize origin that is insensitive to broad-spectrum, post-emergent, foliar applied herbicides containing the active ingredient glyphosate.

2. Molecular characterisation

2.1. Issues raised by Member States

Issues raised by Member States are addressed in Annex G of the EFSA overall opinion.

2.2. Background data

2.2.1. Transformation process and vector constructs

Cotton tissue from variety Coker 312 was transformed by *Agrobacterium tumefaciens* using the binary vector system. The disarmed *A. tumefaciens* strain harboured the transformation vector pTEM2. This vector contained the T-DNA region, with the left and right borders (LB and RB) delimiting a single gene cassette for expression of the modified *epsps* gene, named *2mepsps*. This gene of maize origin codes for an EPSPS protein with two amino acid substitutions conferring insensitivity to glyphosate. The amino acid substitutions in 2mEPSPS are the same as in the modified EPSPS in the previously assessed event maize GA21 (EFSA, 2007a). *2mepsps* transcription is driven by the *Ph4a748At* (histone H4) gene promoter originating from *Arabidopsis thaliana*. High level constitutive expression is expected, especially in meristematic (rapidly growing) green tissues. The promoter is followed by the first intron of gene II of the histone H3.III variant of *A. thaliana* and by an optimized transit peptide (constructed from *Zea mays* and *Helianthus annuus* DNA sequences). Termination of transcription uses the 3' untranslated region of the histone H4 gene of *A. thaliana*.

The vector backbone, i.e. the sequences of pTEM2 located outside of the T-DNA and which are not aimed at integration, contains replication origins for plasmid maintenance in both *Escherichia coli* (ORI ColE1) and *A. tumefaciens* (ORI pVS1), a selectable marker gene conferring resistance to streptomycin and spectinomycin (*aadA*) for propagation and selection of the plasmid in *E. coli* and *A. tumefaciens*, a DNA region consisting of a fragment of the neomycin phosphotransferase coding sequence of the *nptII* gene from transposon Tn903 and residual sequences of *A. tumefaciens* origin (plasmid pTiAch5 flanking the left and right borders).

2.2.2. Transgenic constructs in the genetically modified plant

The DNA sequences actually inserted in the GHB614 event were characterized by Southern analysis and by PCR amplification of both the insert and the flanking regions.

The number of T-DNA copies was determined by Southern hybridization using a combination of 9 restriction enzymes and 5 probes corresponding to the full length T-DNA and to four internal fragments corresponding to the different components of the transgene cassette. The data demonstrate the presence of a single T-DNA insert, as well as its integrity as compared with the original transgene cassette in vector pTEM2.

PCR amplification of the single inserted T-DNA allowed sequence determination of the entire 3978 bp insert and established a perfect match with the corresponding sequence in the vector pTEM2. The 5' and 3' flanking sequences (738 bp and 214 bp respectively) were also PCR amplified and sequenced. Characterization of the wild type target locus was achieved by amplifying a 994 bp fragment from wild type cotton using primers derived from the 5' and 3' flanks of the T-DNA. Sequence alignment between the pre-insertion locus (from wild type cotton) and the insertion locus (from event GHB614) identified a 17 bp deletion at the junction between the T-DNA and genomic DNA.

Examination of the gene insertion site was performed by searching nucleotide sequence databases with the pre-insertion locus (947 bp) as query sequence (blastn algorithm). No similarity with known functional genes in plants or other organisms could be identified. Bioinformatic tools for the prediction of functional genes were used for analyzing the pre-insertion locus and a hypothetical protein coding gene preceded by putative promoter elements could be found on the reverse strand of the 5' flanking region. Protein database searches (blastx) identified several conserved polypeptides in plants, but with no known function. It seems likely that the T-DNA of cotton GHB614 was inserted near a protein coding gene of unknown function. However, there are no indications from comparative agronomic performance and compositional analyses of any unintended effect caused by the insertion.

The absence of vector backbone sequences in the GHB614 event was studied by Southern analysis, using overlapping probes covering the entire vector DNA. The absence of hybridization signals, with the appropriate controls, indicated that no vector sequence was integrated into the plant genome besides the T-DNA. Sequence analysis of the T-DNA insert and of its flanking regions in the plant confirmed that no vector sequence out of the T-DNA region was present in the transgene locus.

2.2.3. Information on the expression of the insert

2.2.3.1. Expression of the introduced gene

2mepsps is the only gene potentially expressed from the transgene cassette in event GHB614.

The leaves of the cotton plant are the principal organs exposed to herbicide applications and commercial-level herbicide tolerance depends upon the function of the 2mEPSPS enzyme in the leaves. As a constitutive promoter with high activity in the leaves and meristematic tissues, the *Arabidopsis* histone H4 promoter was chosen to drive the expression of the *2mepsps* gene.

Expression level (data provided on a fresh weight basis) was measured by 2mEPSPS protein specific ELISA. Tissue samples were harvested from greenhouse grown cotton, at the 2-3 and 4-6 leaf stages of growth, pre-flowering and at flowering. It was found that 2mEPSPS protein ranged between 0.45 - 11.16 µg/g of leaves, 0.99 - 4.04 µg/g of roots, 1.58 - 1.94 µg/g of stems, depending on the growth stage of the plant, and was 5.47 ± 0.22 µg/g of apices, 5.35 ± 0.25 µg/g of squares (flower buds) and 0.16 ± 0.01 µg/g of pollen. 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 of cotton event GHB614. From published experience with the promoter and intron used, GHB614 plants were expected to show high levels of 2mEPSPS protein in rapidly growing plant parts, and lesser amounts in the other organs. Indeed, the following order of 2mEPSPS expression was found: leaf, apex >> roots, squares >> stems, seeds >> pollen.

The 2mEPSPS protein was also tested in seeds and processed seed fractions from unsprayed and sprayed plants produced in field trials in the US. The average 2mEPSPS protein content per test site in the field trial ranged from 15.8 µg/g to 25.5 µg/g in unsprayed fuzzy seed (overall average value of 19.2 ± 3.1 µg/g) and from 16.2 µg/g to 30.5 µg/g in sprayed fuzzy seed (overall average value of 21.2 ± 4.0 µg/g). The amount of 2mEPSPS protein was measured in 9 fractions of cottonseed, only 3 fractions contained detectable amounts of 2mEPSPS protein (delinted cottonseed: 102 ± 2 µg/g; hulls: 6.93 ± 0.40 µg/g; defatted meal: 0.26 ± 0.10 µg/g); the other fractions contained 6.63 µg/g.

2.2.3.2. Putative cryptic open reading frames

Open reading frame (ORF) and gene search tools were applied to predict the presence of potential newly created coding sequences both in the 5-prime flanking genomic/insert DNA junction region and in the insert/3-prime flanking genomic DNA junction region. Fourteen newly created ORFs were found that span the 5-prime and 3-prime junctions. In the unlikely event that the putative ORFs would be translated, bioinformatics analysis indicated that their putative translation products have no homology with any known toxins or allergens.

2.2.4. Information and stability of inserted DNA

The trait is inherited as a single dominant gene. Stability of the inserted DNA was demonstrated by Southern blot analysis of plants of multiple generations (from self-crosses and backcrosses into two genetic backgrounds), from different locations and environmental growth conditions. All tested samples showed the expected restriction enzyme digestion products.

Phenotypic stability was demonstrated by Mendelian inheritance of the herbicide tolerance trait over multiple generations and field locations, as well as throughout the development of commercial lines based upon cotton event GHB614.

2.3. Conclusion

The molecular characterisation data establish that the genetically modified cotton GHB614 contains one copy of an intact expression cassette with a modified maize *epsps* gene. No other parts of the plasmid used for transformation are present in the transformed plant. Bioinformatics analysis of the 5' and 3' flanking regions did not reveal any putative peptides

that would cause safety concerns. The stability of the inserted DNA and the herbicide tolerance trait were confirmed over several generations and a Mendelian inheritance pattern demonstrated.

3. Comparative analysis

3.1. Issues raised by Member States

Issues raised by Member States are addressed in Annex G of the EFSA overall opinion.

3.2. Evaluation of relevant scientific data

Having considered the information provided in the application and the Member States comments, the EFSA GMO Panel requested from the applicant further information with respect to the identity and breeding scheme of the non-GM comparator used in the agronomic / compositional analysis. The EFSA GMO Panel asked the applicant to check the consistency of some agronomic data presented in the application. The applicant provided the additional information as well as corrected agronomic data that the EFSA GMO Panel found adequate.

3.2.1. Choice of comparator and production of material for the compositional assessment

For compositional studies, cotton GHB614 was compared to its parent variety Coker 312. Data from the scientific literature regarding the natural ranges of key compounds in conventional cotton were also considered in the comparative assessment. Field trials with cotton GHB614 and its non-GM comparator Coker 312 were performed in the major cotton growing regions of the US in 2005 (9 sites) and 2006 (8 sites). In the year 2006, 8 trials were conducted at the same locations used the year before. Trials comprised 3 treatments at each location and 3 replications per treatment. The 3 treatments consisted of: (a) non-GM cotton grown using conventional herbicide weed control, (b) GM cotton grown using conventional herbicide weed control, and (c) GM cotton grown with glyphosate-based herbicide weed control.

3.2.2. Compositional analysis

Whole, linted cottonseed was used as suitable raw agricultural commodity for comparative compositional analysis. The seeds were analysed for key nutrients, anti-nutrients, and toxicants as defined by the OECD consensus document for cotton (OECD, 2004). Thus besides proximates (moisture, total fat, total protein, ash, total carbohydrates), acid detergent fibre (ADF), and neutral detergent fibre (NDF), the samples were analysed for 18 amino acids, 10 fatty acids (C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:0, C22:0, and C24:0), minerals (calcium, phosphorus, magnesium, potassium, iron, zinc), vitamin E, anti-nutrients (cyclopropenoid fatty acids and phytic acid) and the toxicant gossypol (free and total gossypol).

The statistical analysis of compositional data collected each year was carried out on a per location basis, using data from 3 replicates per location, and on the combined data from all sites each year. For most constituents, compositional differences between GHB614 cotton and its non-GM comparator occurred occasionally but not consistently over years and locations.

No change of the total amino acid composition was caused by the newly expressed protein-in GHB614 cotton.

For C16:1 (palmitoleic acid), C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid) and C18:3 (linolenic acid) compositional differences were observed at 8, 11, 13, 12 and 17 out of the 17 field trial locations. However, differences were very small and are therefore not considered biologically relevant.

In case of the anti-nutritional cyclopropenoid fatty acids (CPFAs), the t-tests at the majority of per-location analyses found significantly lower values for sprayed and unsprayed cotton GHB614 versus the non-GM control. The estimated differences between the CPFAs mean values for the control and the GHB614 groups were all very small and are therefore not considered biologically relevant. There were no differences in the levels of free and bound gossypol.

All constituent levels for cotton GHB614 and the non-GM control fell inside the ranges of natural variability as reported in literature.

Besides the raw agricultural commodity, the chemical compositions of cottonseed linters, hulls, delinted seeds, meal, toasted meal, crude oil and refined, deodorised oil produced from cotton GHB614 and the non-GM counterpart harvested from one field trial were compared, and the analytical results assessed in light of the reference ranges in plant constituents reported in the literature. No nutritionally relevant differences were found. The obtained results support the conclusion with regard to compositional equivalence drawn for the raw agricultural commodity. No gossypol was detected in refined cottonseed oil obtained from cotton GHB614. The tendency of a slightly decreased content of CPFAs in cotton GHB614— as observed for whole linted cottonseed – was confirmed for the crude and refined oil.

The EFSA GMO Panel considered the observed compositional differences between cotton GHB614 and its non-GM comparator in the light of the field trial design and the natural ranges of the studied compounds reported for conventional cotton varieties. The EFSA GMO Panel concluded that cotton GHB614 (treated and untreated with the target herbicide) is compositionally equivalent to the non-GM counterpart and other conventional cotton, except for the introduced trait.

3.2.3. Agronomic traits and GM phenotype

The applicant provided information on agronomic performance and phenotypic characteristics derived from several field trials in the US performed in 2004 and 2005. Treatments consisted of: (a) non-GM cotton grown using conventional herbicide weed control, (b) GM cotton grown using conventional herbicide weed control, and (c) GM cotton grown with glyphosate-based herbicide weed control. 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, cotton seed and fibre quality.

The EFSA GMO Panel noted that differences were observed in some instances with regard to several characteristics related to yield, lint percentage, and reproduction. However, these differences did not occur consistently in the various studies and, therefore, were not considered to be related to the genetic modification.

The EFSA GMO Panel concludes that cotton GHB614 (treated and untreated with the target herbicide) is not agronomically different from other currently grown non-GM cotton varieties, with the exception of the newly introduced trait.

3.3. Conclusion

Compositional and agronomic analyses carried out on both glyphosate-treated and conventionally treated cotton GHB614, its non-GM comparator Coker 312 and other conventional cotton varieties treated with conventional herbicides indicated that cotton GHB614 is compositionally and agronomically equivalent to the non-GM counterpart and other conventional cotton, except for the introduced trait. The comparative analysis of cotton GHB614 therefore provided no indication for unintended effects resulting from the genetic modification.

4. Food/Feed safety assessment

4.1. Issues raised by Member States

Issues raised by Member States are addressed in Annex G of the EFSA overall opinion.

4.2. Evaluation of relevant scientific data

The EFSA GMO Panel has considered the information provided in the application and requested from the applicant further information with regards to the results of an acute oral toxicity study in mice using the 2mEPSPS protein, as well as the statistical analysis of data obtained in a broiler feeding study with seeds from cotton GHB614 and the identity of the non-GM comparator used in this study. Additional bioinformatics studies on potential homology of the 2mEPSPS protein to known toxic and allergenic proteins using up-to-date databases were also requested. The requested information was provided.

4.2.1. Product description and intended use

The scope of application EFSA-GMO-NL-2008-51 includes the import and processing of cotton GHB614 and its derived products for use as food and feed. Thus, the possible uses of cotton GHB614 include the production of refined oil from seeds and cellulose from linters for use as food or food ingredient, and use of cottonseed meal, hulls and linters in animal feed.

The genetic modification of cotton GHB614 is intended to improve agronomic performance only and is not intended to influence the nutritional properties, processing characteristics and overall use of cotton as a crop.

4.2.2. Effect of processing

Cotton GHB614 has been found to be compositionally equivalent to the non-GM comparator and other conventional cotton varieties except for the introduced trait (see Section 3.2.2).

The applicant provided data on the chemical compositions of cottonseed linters, hulls, delinted seeds, meal, toasted meal, crude oil and refined, deodorised oil obtained by

processing of cotton GHB614 and the non-GM counterpart. The amount of 2mEPSPS present in those materials is summarised in section 2.2.3.1. No nutritionally relevant differences were found (see Section 3.2.2). Taking into account the compositional analysis of whole linted cottonseed providing no indication of relevant compositional changes (see Section 3.2.2), the Panel has no reason to assume that the characteristics of cotton GHB614 and derived processed products would be different from those of the respective products derived from conventional cotton. Considering the toxicological profile and allergenic properties (see Sections 4.2.3, 4.2.4 and 4.2.5) the potential presence of the 2mEPSPS protein in processed products does not raise concern.

4.2.3. Toxicology

4.2.3.1. 2mEPSPS protein used for safety assessment

Due to the low expression level of the 2mEPSPS protein in cotton GHB614 and the very difficult task to isolate a sufficient quantity of purified protein from the genetically modified cotton, the safety studies with the newly expressed protein were conducted with a 2mEPSPS protein expressed in a recombinant *Escherichia coli* strain. The structural and functional equivalence of the 2mEPSPS protein produced by *E. coli* to that produced in cotton GHB614 was shown by N-terminal sequencing (Edman degradation), mobility in SDS-PAGE, Western analysis, HPLC/electrospray mass spectrometry (LC/MS) of peptides from a trypsin digest, glycosylation analysis and determination of 2mEPSPS enzymatic activity. Based on the identified similarity in structure and function between these proteins, the GMO Panel accepts the use of the 2mEPSPS protein derived from *E. coli* for the safety testing of the 2mEPSPS protein present in cotton GHB614.

4.2.3.2. Toxicological assessment of expressed novel protein

EPSPS enzymes occur in conventional plants, fungi and microorganisms and are thus consumed as part of the normal diet by humans and animals. No adverse effects associated with the intake of these proteins have been identified. The amino acid sequence of the 2mEPSPS protein is identical to that of the modified EPSPS (mEPSPS) protein expressed in GM maize event GA21, which has been previously evaluated by the EFSA GMO Panel and regarded as safe as its non-GM counterparts for human and/or animal consumption (EFSA, 2007a).

The 2mEPSPS protein expressed in cotton GHB614 (molecular mass ca. 47 kDa) is a modified version of the endogenous maize EPSPS protein. The amino acid sequence of the protein expressed in cotton GHB614 differs from that of the maize protein in 2 of the total of 445 amino acids. Threonine in position 102 of maize EPSPS has been replaced by isoleucine in 2mEPSPS, and proline in position 106 by serine, resulting in tolerance of the plants to glyphosate.

(a) Acute toxicity testing

In an acute oral toxicity study using mice, the 2mEPSPS protein produced in *E. coli* did not induce adverse effects after administration by gavage at a single dose of 2000 mg/kg bodyweight (bw). In addition, no systemic effects were induced when the protein was administered intravenously up to the highest dose of 10 mg/kg bw.

(b) Degradation in simulated digestive fluids

The digestibility of the 2mEPSPS protein was studied *in vitro* in simulated gastric fluid (SGF). No intact protein and no fragments were detectable after incubation for 30 seconds in pepsin-containing SGF at pH 1.2 as demonstrated by SDS-PAGE and protein staining.

Rapid degradation (within seconds) of the 2mEPSPS protein also occurred in simulated intestinal fluid (SIF) containing pancreatin at pH 7.5 as demonstrated by Western analysis.

(c) Bioinformatic studies

Bioinformatics-supported comparisons of the amino acid sequence of the 2mEPSPS protein expressed in cotton GHB614 with amino acid sequences contained in protein databases (dated 2004 and 2006) using the blastp algorithm indicated significant homology only with other EPSPS-related proteins. No sequence homology between the 2mEPSPS protein and known toxic proteins was found. On request of the EFSA GMO Panel the applicant provided an additional analysis using up-to-date protein databases (dated 2007 and 2008) and the FASTA sequence alignment tool, which confirmed the results of the previous study.

4.2.3.3. Toxicological assessment of new constituents other than proteins

No new constituent other than the 2mEPSPS protein is expressed in cotton GHB614 and no relevant changes in the composition of cotton GHB614 were detected in the comparative compositional analysis (see Section 3.2.2).

4.2.4. Toxicological assessment of the whole GM food/feed

On the basis of the comparative analysis the EFSA GMO Panel concluded that cotton GHB614 is compositionally and agronomically equivalent to the non-GM comparator and other conventional cotton varieties except for the introduced trait. In addition, this analysis as well as the molecular characterisation provided no indications of unintended effects of the genetic modification. According to the EFSA GMO Panel guidance document, animal safety studies with the whole food/feed are not required (EFSA, 2006a).

4.2.5. Allergenicity

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

4.2.5.1. Assessment of allergenicity of the newly expressed proteins

The *epsps* gene encoding the EPSPS protein was originally derived from maize, a source which is not regarded as a common allergenic food.

Bioinformatics-supported comparisons of the amino acid sequence of the 2mEPSPS protein with sequences of known allergens using databases dated 2004 and 2006 and the blastp algorithm were performed. A search for overall similarity indicated no similarity of 2mEPSPS with known allergenic proteins applying a criterion of 35% identity over a window of 80 amino acids. A search for identical sequences of at least 8 contiguous amino acids using the FindPatterns algorithm also showed no similarities between the 2mEPSPS protein expressed in cotton GHB614 and known allergens. Additional studies using up-to-date databases (dated 2007 and 2008) and the FASTA and, respectively, the FindPatterns algorithm, were provided on request of the EFSA GMO Panel and confirmed the previous results. Moreover, the protein is not glycosylated.

As described above, 2mEPSPS was rapidly degraded under simulated gastric and intestinal conditions (see Section 4.2.3.2.).

Based on this information, the EFSA GMO Panel considers that it is unlikely that the newly expressed 2mEPSPS protein in cotton GHB614 is an allergen.

4.2.5.2. 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 transgene 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 to the EFSA GMO Panel since cotton is not considered to be a common allergenic food. Furthermore, the main cottonseed product in human food, cottonseed oil, is highly purified and contains negligible levels of proteins, if any. Edible oils that are refined, bleached and deodorised do not appear to pose a risk to allergic individuals, as they contain virtually no proteins. The applicant states that no toxic or allergic effects on workers handling cotton GHB614 in the field since its first field release in 2002 have been reported.

Based on the available information, the EFSA GMO Panel concludes that it is unlikely that the overall allergenicity of the whole GM cotton GHB614 has been changed.

4.2.6. Nutritional assessment of GM food/feed

A 42-day feeding study using broiler chickens (Ross #708) was performed. Three groups of 140 animals consisting of 14 pens (7 pens/gender) with 10 animals each were fed diets containing toasted meal obtained from seeds of cotton GHB614 sprayed with glyphosate-based herbicides, the non-GM counterpart Coker 312 or another conventional non-GM variety, both treated with a different herbicide. The inclusion rate of cottonseed meal in the starter, grower and finisher diets was 10%. Although some statistically significant differences were noted among several determinations, mostly at specific time points, there were no relevant differences in body weight gain, feed consumption and feed conversion rate. There were also no relevant differences in weights of chilled carcass, abdominal fat pad, leg, thigh, wing and breast in animals fed meal derived from cotton GHB614 compared with animals fed meal from the non-GM conventional cotton varieties.

Thus, the broiler feeding study supported the results of the comparative compositional analysis which showed that seed from cotton GHB614 is compositionally and therefore nutritionally equivalent to the non-GM comparator and other conventional cotton varieties.

4.2.7. Post-market monitoring of GM food/feed

The risk assessment concluded that no data have emerged to indicate that cotton GHB614 is any less safe than its non-GM comparator. In addition, cotton GHB614 is, from a nutritional point of view, equivalent to conventional cotton. Therefore, and in line with the EFSA GMO Panel guidance document (EFSA, 2006a), the EFSA GMO Panel is of the opinion that post-market monitoring of the GM food/feed is not necessary.

4.3. Conclusion

The 2mEPSPS protein expressed in cotton GHB614 differs from the EPSPS protein present in conventional maize in 2 amino acids. The protein shows no homology to known toxic proteins and/or allergens. The 2mEPSPS protein was rapidly degraded in simulated gastric and intestinal fluid. This protein is also expressed in maize GA21 which has been previously assessed for its safety by the EFSA GMO Panel.

The comparative analysis showed no biologically relevant compositional, agronomic, and phenotypic changes of cotton GHB614 in relation to conventional cotton except for the introduced trait. A nutritional feeding study using broiler chickens indicated that seed from cotton GHB614 is nutritionally equivalent to seed from the non-GM counterpart and other conventional cotton. The study therefore supports the conclusion of the compositional and agronomical comparison that the genetic modification resulted in no unintended effects. The EFSA GMO Panel considers that no additional animal safety or nutritional wholesomeness study is needed. Based on the available information, the EFSA GMO Panel concludes that it is unlikely that the overall allergenicity of the whole cotton GHB614 has been changed. The EFSA GMO Panel is of the opinion that cotton GHB614 is as safe as its non-GM counterpart and other conventional cotton varieties.

5. Environmental risk assessment and monitoring

5.1. Issues raised by Member States

Issues raised by Member States are addressed in Annex G of the EFSA overall opinion.

5.2. Evaluation of relevant scientific data

5.2.1. Environmental risk assessment

The scope of application EFSA-GMO-NL-2008-51 includes import and processing for food/feed uses of cotton GHB614 and does not include cultivation. Considering the proposed uses of cotton GHB614, the environmental risk assessment is concerned with the indirect exposure through manure and faeces from gastrointestinal tracts mainly of animals fed on cotton GHB614 and with the accidental release into the environment of cotton GHB614 seeds during transportation and processing.

As the scope of the present application excludes cultivation, concerns regarding the use of glyphosate-based herbicides on cotton GHB614 apply only to imported and processed cotton products that may have been treated with these herbicides in countries of origin. The risk assessment of residues of this active ingredient falls within the scope of Directive 91/414/EEC concerning the placing of plant protection products on the market.

5.2.1.1. Potential unintended effects on plant fitness due to the genetic modification

Gossypium herbaceum and *G. hirsutum* are highly domesticated crops that have been grown in Southern Europe since the 19th century, giving rise to feral plants which can occasionally be found in the same area (Davis, 1967). The main cultivated cotton (*G. hirsutum*) is an annual self-pollinating crop. In the absence of insect pollinators (such as wild bees, honeybees, bumblebees), cotton flowers are self-pollinated, but when these pollinators are present low percentages of cross-pollination occur (McGregor, 1959; Moffett and Stith, 1972; Moffett et al., 1975; Van Deynze et al., 2005).

Pollen and seed dispersal are potential sources of vertical gene flow to cross-compatible wild cotton relatives, other cotton varieties and to occasional feral cotton plants. However, in Europe, there are no cross-compatible wild relatives with which cotton can hybridise. Because cotton pollen is very large (120 and 200 microns), heavy and sticky, wind-mediated dispersal of pollen to other cotton varieties is negligible (Vaissière and Vinson, 1994). In addition, cross-pollination percentages rapidly decrease with increasing distance from the pollen source (Umbeck et al., 1991; Kareiva et al., 1994; Llewellyn and Fitt, 1996; Xanthopoulos and Kechagia, 2000; Van Deynze et al., 2005; Zhang et al., 2005; Hofs et al., 2007; Llewellyn et al., 2007). Seeds are thus the only survival structures.

The seed-mediated establishment of cotton and its survival outside of cultivation in Europe is mainly limited by a combination of absence of a dormancy phase, low competitiveness, and susceptibility to diseases and cold climate conditions (Eastick and Hearnden, 2006). Adequate soil moisture is an additional factor affecting the survival of feral cotton seedlings. Since general characteristics of cotton GHB614 are unchanged relative to its conventional counterpart, the inserted herbicide tolerance trait is not likely to provide a selective advantage outside of cultivation in Europe. If accidental release into the environment occurs, cotton GHB614 plants will only have a selective advantage in the presence of glyphosate-based herbicides which are not currently used on cultivated cotton or in most areas where the GM cotton might be spilled. It is thus considered very unlikely that cotton GHB614, or its progeny, will differ from other cotton varieties in their ability to survive until subsequent seasons or to establish feral populations under European environmental conditions. The risk of GM cotton becoming feral along transportation roads, or a weed on dairy farms where raw cotton seed is used as feed has been shown to be negligible in north-east Australia (Addison et al., 2007).

Data presented in the application gathered over a series of field trials across the US in 2004 and 2005 indicate that cotton GHB614 has no altered reproductive, dissemination or survivability characteristics compared to its conventional counterpart. In addition to the data presented by the applicant, the EFSA GMO Panel is not aware of any scientific report of increased fecundity, persistence (volunteerism) or ferality of GM cotton in regions where it is cultivated (Eastick and Hearnden, 2006; Bagavathiannan and Van Acker, 2008). There is no information to indicate change in survival capacity (including over-wintering). Furthermore,

there is no evidence that the herbicide tolerance trait introduced by the genetic modification results in increased persistence and invasiveness of any crop species, except in the presence of glyphosate-based herbicides. Thus escaped plants and genes dispersed to other cotton plants would result in plant populations no different from existing populations and would not create additional agronomic or environmental impacts. In addition, the applicant states that cotton GHB614 will be imported as mostly non-viable seed. Therefore, the likelihood that some imported seed could escape and germinate is very low.

The EFSA GMO Panel is of the opinion that, even in case of accidental release into the environment, cotton GHB614 is very unlikely to show any enhanced fitness and would behave as conventional cotton.

5.2.1.2. Potential for gene transfer

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

(a) Plant to bacteria gene transfer

Based on current scientific knowledge and previous scientific opinions (EFSA, 2004) or statements (EFSA, 2007b), horizontal gene transfer from GM plants to microorganisms under natural conditions is extremely unlikely (Keese, 2008).

Transgenic DNA is a component of many food and feed products derived from GM cotton. Therefore, microorganisms in the digestive tract of humans and animals (domesticated animals and other animals feeding on fresh and decaying GM plant material) may be exposed to transgenic DNA.

In the case of accidental release and establishment of cotton GHB614 in the environment, exposure of microorganisms to transgenic DNA derived from GM cotton plants would take place during natural decay of GM plant material and/or pollen in the soil of areas where GM plants establish.

The modified *epsps* gene derives from wild type *epsps* maize gene. Taking into account the origin and nature of the *2mepsps* gene and the lack of selective pressure for this gene in the intestinal tract and/or the environment, the likelihood that horizontal gene transfer would result in increased fitness of microorganisms is very limited. For this reason, it is very unlikely that the *2mepsps* gene from cotton GHB614 would become transferred and established in the genome of microorganisms in the environment (including plant-associated microorganisms e.g., rhizobia) or human and animal digestive tract. In the very unlikely event that such a horizontal gene transfer would take place, no adverse effects on human and animal health and the environment are expected.

(b) Plant to plant gene transfer

Considering the intended uses of cotton GHB614 and physical characteristics of cotton seeds, a possible pathway of gene dispersal is from seed spillage and pollen of occasional feral GM cotton plants originating from accidental seed spillage during transportation and/or processing.

G. herbaceum is reported (Zohary and Hopf, 2000) to be a traditional fiber crop in the Eastern Mediterranean area already in the pre-Columbus period (before 1500 AD). The genus *Gossypium* consists of at least four species: *Gossypium arboreum*, *Gossypium barbadense*, *G. herbaceum* and *G. hirsutum*. In Southern Europe, *G. herbaceum* and *G. hirsutum* have been grown since the 19th century giving rise to occasional feral plants in the same area (Davis, 1967; Tutin et al., 1992), but no sexually compatible wild relatives of *G. hirsutum* have been reported in Europe. Therefore, the plant to plant gene transfer from cotton GHB614 is restricted to cultivated and occasional feral populations. The EFSA GMO Panel also takes into account the fact that the present application does not include cultivation of cotton GHB614 within the EU so that the likelihood of cross-pollination between the imported cotton GHB614, other cotton crops and occasional feral cotton plants is considered to be extremely low. Even in case feral populations of cotton GHB614 were established or transgene flow occurred to cultivated and feral cotton, a selective advantage would only occur if the complementary glyphosate-based herbicides were applied.

5.2.1.3. Potential interactions of the GM plant with non-target organisms

Due to the intended uses of cotton GHB614, which exclude cultivation and due to the low level of exposure to the environment, potential interactions of the GM plant with non-target organisms were not considered an issue by the EFSA GMO Panel.

5.2.1.4. Potential interactions with the abiotic environment and biogeochemical cycles

Due to the intended uses of cotton GHB614, which exclude cultivation and due to the low level of exposure to the environment, potential interactions with the abiotic environment and biogeochemical cycles were not considered an issue by the EFSA GMO Panel.

5.2.2. Monitoring

Objectives of a monitoring plan according to Annex VII of Directive 2001/18/EC are (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the environmental risk assessment are correct, and (2) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment which were not anticipated in the environmental risk assessment.

Monitoring is related to risk management, and thus a final adoption of the monitoring plan falls outside the mandate of EFSA. However, the EFSA GMO Panel gives its opinion on the scientific quality of the monitoring plan provided by the applicant (EFSA, 2006a,b). The only significant exposure to the environment of the GM cotton would be through manure and faeces from the gastrointestinal tracts mainly of animals fed on the GM cotton or through accidental spillage of GM seeds during transportation and processing. The EFSA GMO Panel is aware that, due to physical characteristics of cotton seeds and methods of transportation, accidental spillage is unavoidable. Therefore, the EFSA GMO Panel recommends that appropriate management systems are introduced to actively monitor the occurrence of feral cotton plants in areas where seed spillage and plant establishment are likely to occur as proposed in the EFSA GMO Panel guidance document (EFSA, 2006a) and the scientific opinion of the EFSA GMO Panel on post-market environmental monitoring (EFSA, 2006b).

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

The general surveillance plan proposed by the applicant includes (1) the description of an approach involving operators (federations involved in cotton import and processing), reporting to the applicants, via a centralised system, any observed adverse effect(s) of GMOs on human health and the environment, and (2) a coordinating system newly established by EuropaBio for the collection of the information recorded by the various operators. The applicant will submit a general surveillance report on an annual basis and a final report at the end of the consent. In case of confirmed adverse effects, the applicant will immediately inform the European Commission and Member States.

The EFSA GMO Panel is of the opinion that the scope of the monitoring plan provided by the applicant is in line with the intended uses of cotton GHB614 since the environmental risk assessment did not cover cultivation and identified no potential adverse environmental effects. The EFSA GMO Panel agrees with the reporting intervals proposed by the applicant in the general surveillance plan.

5.3. Conclusion

Cotton GHB614 is being assessed for import and processing for food/feed uses and thus there is no requirement for scientific information on environmental effects associated with cultivation. Considering the intended uses, the environmental risk assessment is concerned with indirect exposure through manure and faeces from gastrointestinal tracts mainly of animals fed on the cotton GHB614 and with accidental spillage of GHB614 seeds during transportation and processing. The EFSA GMO Panel considered the environmental issues raised by Member States in the above sections of Chapter 5 and concludes as follows: *G. hirsutum*, which has no cross-compatible wild relatives in Europe, is a cultivated plant in Europe since the 19th century and occurs only occasionally as feral plants in Europe.

If accidental spillage and subsequent release into the environment of cotton GHB614 seeds occurs, cotton GHB614 plants will only have a selective advantage in the presence of glyphosate-based herbicides which are not currently used on cultivated cotton or in most areas where the GM cotton might be spilled. Therefore, the EFSA GMO Panel is of the opinion that the likelihood of the establishment and spread of cotton GHB614 is very low and that unintended environmental effects due to this GM cotton will be no different from that of other cotton varieties. Furthermore, the scope of the monitoring plan provided by the applicant is in line with the intended uses of cotton GHB614 since this does not include cultivation.

The EFSA GMO Panel is aware that, due to physical characteristics of cotton seeds and methods of transportation, accidental spillage is unavoidable. Therefore, the EFSA GMO Panel recommends that, within general surveillance, appropriate management systems are introduced to actively monitor the occurrence of feral cotton plants in areas where seed spillage is likely to occur.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

The EFSA GMO Panel was requested to carry out a scientific risk assessment of the cotton GHB614 for food and feed uses, import and processing.

Cotton GHB614 has been modified to express a modified *epsps* maize gene providing tolerance to glyphosate-based herbicides. The EFSA GMO Panel is of the opinion that the molecular characterisation provided for cotton GHB614 is sufficient for the safety assessment. The bioinformatic analysis of the inserted DNA and flanking regions does not raise any safety concern. The expression of the gene introduced by genetic modification has been sufficiently analysed and the stability of the genetic modification has been demonstrated over several generations. The EFSA GMO Panel considers that the molecular characterisation does not indicate any safety concern.

Comparative analysis has shown that cotton GHB614 is compositionally and agronomically equivalent to conventional cotton, except for the introduced trait. The risk assessment included an analysis of data from analytical studies, bioinformatics, and *in vitro* and *in vivo* studies. Based on the available information, the EFSA GMO Panel concludes that it is unlikely that the overall allergenicity of the whole cotton GHB614 has been changed. The EFSA GMO Panel is of the opinion that cotton GHB614 is as safe as its non-GM counterpart and other conventional cotton varieties.

The application EFSA-GMO-NL-2008-51 concerns import, processing and food/feed uses. There is therefore no requirement for scientific information on possible environmental effects associated with the cultivation of cotton GHB614. Considering the scope of the application, not for cultivation, the EFSA GMO Panel is of the opinion that the likelihood of the spread and establishment of cotton GHB614 is very low and that unintended environmental effects due to this cotton will be no different from that of other cotton varieties. The scope of the monitoring plan provided by the applicant and the reporting intervals are in line with the intended uses of cotton GHB614. However, the EFSA GMO Panel is aware that, due to the physical characteristics of cotton seeds and methods of transportation, accidental spillage is unavoidable. Therefore, the EFSA GMO Panel recommends that, within general surveillance, specific measures are introduced to actively monitor the occurrence of feral cotton plants in areas where seed spillage is likely to occur.

In conclusion, the EFSA GMO Panel considers that information available for cotton GHB614 addresses the outstanding questions raised by the Member States and considers it unlikely that cotton GHB614 will have any adverse effect on human and animal health or on the environment in the context of its proposed uses.

DOCUMENTATION PROVIDED TO EFSA

1. Letter from the Competent Authority of the Netherlands, dated 25 January 2008, concerning a request for placing on the market of cotton GHB614 in accordance with Regulation (EC) No 1829/2003.
2. Acknowledgement letter, dated 30 January 2008, from EFSA to the Competent Authority of the Netherlands (Ref SR/KL/shv (2008) 2649496).

3. Letter from EFSA to applicant, dated 11 March 2008, delivering the 'Statement of Validity' for application EFSA-GMO-NL-2008-51, cotton GHB614 submitted by Bayer CropScience under Regulation (EC) No 1829/2003 (Ref SR/AC/shv (2008) 2757434).
4. Letter from EFSA to applicant, dated 3 September 2008, requesting additional information and stopping the clock (Ref PB/YD/md (2008) 3272819).
5. Letter from applicant to EFSA, dated 1 April 2008, providing the timeline for submission of response.
6. Letter from EFSA to applicant, dated 12 November 2008, requesting additional information and maintaining the clock stopped (Ref PB/YD/shv (2008) 3449720).
7. Letter from applicant to EFSA, dated 1 December 2008, providing additional information.
8. Letter from applicant to EFSA, dated 7 January 2009, providing the timeline for submission of response.
9. Letter from applicant to EFSA, dated 12 January 2009, providing additional information.
10. Letter from EFSA to applicant, dated 24 February 2009, restarting the clock (Ref PB/YD/shv (2009) 3698443).

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Appendix III

COTTON

General information

Cotton is mainly grown for its commodity product the cotton boll. The fibres on the cotton boll are separated from the cottonseeds by a cotton gin machine. The fibres, which consist almost completely 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, known as linters, 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 cottonseed oil is used in food and feed. Following oil extraction, the cottonseed can be processed into various other side-products that are also used in food and feed, such as cottonseed meal, various protein preparations, and cottonseed milk. Protein-rich cottonseed meal is mostly used as an animal feed ingredient. Another major processed product derived from cottonseed are fibre-rich hulls, which may also be used in animal feeds (Figure 4.2-1).

Processing for food and feed uses

Cottonseed

Fuzzy cottonseed may be dehulled, cooked, cracked, flaked and is processed into four major products: oil, meal, hulls, and linters, see Figure 4.2-1. Typical processing yields of fuzzy cottonseed is 45% meal, 26% hulls, 16% oil, 9% linters and 4% lost in processing (OECD, 2004). WCS contains high quality protein and oil. The processing steps which are used to produce the various cotton products are shown in figure 4.2-1. The processing of 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, cooled and grounded. Roasting, extruding, and cracking whole cottonseed has improved digestibility in some trials but under some conditional may also has increased the availability of free gossypol.

By-products of processing can be included in human diet, such as linters and oil, or in animal diet such as hulls and meal. The two main soluble proteins in cottonseed are albumin and globulin. The amounts of these proteins are three times higher than the fractions of insoluble proteins (prolamine and glutelin; Arieli, 1998). The rumen protein degradability values are usually over 70% in dairy cattle (Arieli, 1998).

WCS typically contains 1.5-2.0% gossypol, all in the unbound form, but levels can vary to as low as 0.4% in some commercial cultivars (Calhoun et al., 1995). The presence of gossypol and cyclopropenoid fatty acids (CPFA) in cottonseed limits its use as a protein supplement in animal feed, except for cattle, who are unaffected by these components because they are detoxified by digestion in the rumen.

Cottonseed oil

Several methods are used to extract cottonseed oil either by mechanical pressing, solvent (usually n-hexane) extraction or supercritical fluid extraction (Saxena et al., 2011). The various steps in refining the oil are alkali refining (removes free fatty acids, glycerol, metals, proteins), bleaching (removes metals and colour), winterization (low temperature causes stearin to precipitate), hydrogenation (hydrogenate carbon-carbon double bonds) and deodorization (removes volatile compounds e.g. free fatty acids and peroxide). Processing of the oil removes most of the gossypol and CPFAs. Cottonseed oil consists of 70% unsaturated fatty acids including 18% oleic acid, 52% linoleic acid, and 26% saturated fatty acids (primarily palmitic and stearic acids). The main fatty acid composition of refined cottonseed oil (in % of total fatty acids) is 16:0 palmitic acid (range 21.1-28.1%), 18:0 stearic acid (2.1-3.1%), 18:1 oleic acid (12.9-20.1%) and 18:2 linoleic acid (46.0-58.2) (OECD, 2004). Cottonseed oil is a high-value cooking or frying oil and is sometimes used to make margarine. The oil is also a source of vitamin E.

Cottonseed meal (CSM)

The cottonseed meal is the by-product of cottonseed oil extraction and is a protein-rich feed ingredient. The presence of gossypol and cyclopropenoid fatty acids (CPFA) in cottonseed limits its use as a protein supplement in animal feed, except for cattle, who are unaffected by these components because they are detoxified by digestion in the rumen. The rumen protein degradability values are usually over 70% in dairy cattle (Arieli, 1998). Calves, however, are susceptible to gossypol toxicity because of their incomplete rumen development.

Inactivation or removal of gossypol and CPFA during processing enables the use of low levels of cottonseed meal in feeds for fish, poultry, rabbit and swine (Heuzé and Tran, 2015).

Cottonseed hulls

Cottonseed hulls (CSH) are the by-product of the dehulling step of cottonseed oil extraction. The hull is mainly hemicellulose and lignin compounds with a nearly pure cellulose linter fibre attached. No pigment glands have been reported on the hull fibre or linter fibre fractions after processing. Hulls have less than 0.049 % free gossypol content (Forster and Calhoun, 1995).

Cottonseed hulls also contain condensed tannins, which are mainly bound to fibre and protein (Yu et al., 1996). Condensed tannins can have an anti-nutritional factor effect on ruminants, but at low concentrations they can improve efficiency of protein digestion by forming hydrogen-bonded complexes with proteins in the rumen (Yu et al., 1995).

Linters

The linted cottonseed remaining after the ginning process is called fuzzy or whole cottonseed, and the short fibers still adhering to the cotton seed after the ginning process are called linters. Unprocessed fuzzy cottonseeds are not suitable for food.

Cotton linters are short fibre removed from cottonseed during processing. Linters, like raw cotton, are 90-95% cellulose, with no lignin, and only a small amount of waxes, pectin, organic acids, and ash-producing inorganic substances. Linters are a major source of cellulose for both chemical and food uses. When linters are used in food products, they undergo processing (for example, alkaline washing at high temperatures), which would effectively denature and/or remove any protein present.

Linters are also used in absorbent cotton, medical pads, gauze, twine, wicks, carpet yarns, surgical, paper, and packing products; second-cut linters, in chemical cellulose for preparation of regenerated s, films, lacquers, explosives, plastics, and papers; and mill-run linters in chemical cellulose and padding products.

Endogenous toxin gossypol

Gossypol is a terpenoid phytoalexin pigment found naturally in many *Gossypium* species and is located in glands throughout the plant. Gossypol (Chemical Abstracts Service CAS Registry Number 303-45-7) is crystalline, intensely yellow, insoluble in water and soluble in organic solvents and fats. Free gossypol will covalently bind to cottonseed protein and reduce the protein quality due to binding to lysine. The availability of lysine is reduced when meal is fed to non-ruminants (OECD, 2004; EFSA, 2008).

Animal sensitivity to gossypol differs considerably between species and classes of animals. It is particularly toxic to non-ruminants. Acute toxicity has been shown in the heart, lung, liver, and blood cells, resulting in increased erythrocyte fragility (EFSA, 2008). Reproductive toxicity is seen particularly in males, where gossypol affects sperm motility and inhibits spermatogenesis. In females gossypol disrupts the oestrus cycles (EFSA, 2008).

According to EFSA (2008), the potential exposure to free gossypol, based on the maximum permitted concentration in cottonseed meal and recommended maximum inclusion rates in complete feed, would not be expected to result in adverse effects in ruminants, poultry or fish. However, not all monogastric livestock animals, e.g. pigs, have been fully investigated for potential reproductive effects occurring at low doses.

The current EU regulations (Annex I of Council Directive 2002/32/EC; as reported in EFSA, 2008) specifies maximum levels of free gossypol in various feed commodities and animal feeds with a moisture content of 12%:

- 5000 mg/kg in cottonseed
- 1200 mg/kg in cottonseed cake and cottonseed meal
- 20 mg/kg in complete formulated feeds for most monogastric animals, including piglets, fish and laying hens

- 500 mg/kg in complete formulated feeds for ruminants (cattle, sheep and goats)
- 100 mg/kg in complete formulated feeds for poultry (other than laying hens) and calves
- 60 mg/kg in complete formulated feeds for rabbits and pigs (except piglets)

The toxicity of the (–) enantiomer was the more toxic isomer in a study with broiler (Gamboa et al., 1997). There is also a relative good relationship between dietary free gossypol and tissue accumulation of gossypol enantiomers (Gamboa et al., 2001). Accumulation of total gossypol occurs at a faster rate in liver than in plasma or any other tissue. In this feeding study by Gamboa et al. (2001), one-day-old broilers were fed 0, 7, 14, 21 and 28 % cottonseed meal in their diets, corresponding to 0, 0.13, 0.26, 0.39 and 0.53 g/kg diet of free gossypol, for 21 days. An increment of 1 µg/g of dietary free gossypol resulted in an increment of 0.568 µg/g dry matter (DM) in liver, 0.065 µg/g DM in kidney, 0.018 µg/g DM in muscle, and 0.026 µg/mL in plasma. The proportion of (–)- gossypol was higher in plasma (26.7%) and kidney (25.6%) when compared to muscle (19.1%) and liver (16.0%).

The toxicity of (±) gossypol acetic acid has also been studied in *Cynomolgus* monkeys (Heywood, 1988). They were administrated 25 mg (±) gossypol/kg bw per day for thirteen weeks. At this gossypol concentration gossypol induced death, a variety of clinical signs, extensive biochemical changes and pathology in the heart, liver, kidney and testes. The toxicity of the enantiomeric form (–) gossypol was investigated in male *Cynomolgus* monkeys at dosages of 1.5, 4 or 5 mg/kg/day for 4 weeks. No animals died. Clinical signs involving the gastrointestinal tract, adverse effects on body weight gain, consistent biochemical changes in serum proteins, calcium, inorganic phosphorus and serum cholesterol were recorded at 4 mg/kg per day and above. Morphological change was not induced (Heywood, 1988).

Gossypol is less toxic to ruminants, but inhibition of spermatogenesis, embryo development and increased erythrocyte fragility occurred at doses of 6-18 mg/kg bw per day in cattle and cardiomyopathy in lambs at 2-3 mg/kg bw per day (EFSA, 2004).

Gossypol levels in the reported feeding trials

42-day nutritional assessment trial with broilers (see section 4.5.2)

Gossypol levels in toasted cottonseed meals (in percent)

	A	B	C
Free gossypol	0.010	0.010	0.010
- gossypol	0.360	0.400	0.420
+ gossypol	0.330	0.500	0.490
Total gossypol	0.690	0.900	0.910

Toasted cottonseed meals from **A**: Non-transgenic commercial cottonseed; **B**: Transgenic GHB614 cottonseed; **C**: Non-transgenic counterpart cottonseed

Gossypol in diets (in percent; given as range in starter, grower and finisher diets)

	A	B	C
Free gossypol	<0.010	<0.010	<0.010
- gossypol	0.02-0.03	0.06-0.07	0.05
+ gossypol	0.02	0.06-0.07	0.06
Total gossypol	0.04-0.05	0.13	0.11

Diets containing **A**: Non-transgenic commercial cottonseed; **B**: Transgenic GHB614 cottonseed; **C**: Non-transgenic counterpart cottonseed