

DECISION DOCUMENT

**Evaluation of the food safety of the cotton event
GHB614 x LLCotton25**



Agri-food Quality Office

Biotechnology and Industrialized Products Coordination

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Evaluation of the food safety of the cotton event GHB614 x LLCotton25

SUMMARY AND BACKGROUND

The process of assessment of food safety risk of transformation events, which is the product of modern biotechnology, is performed by the National Service for Agri-food Health and Quality [Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA)], regulatory organism depending on the Ministry of Agriculture, Livestock and Fisheries.

The Agri-food Quality Office of SENASA is the area in charge of fulfilling this function through a scientific team and the guidance of a Technical Advisory Committee comprised by experts in different scientific disciplines, who represent the different sectors relating to the production, industrialization, consumption, research and development of genetically modified organisms.

On August 19th, 2010, a request from the company, Bayer CropScience S.A., was received for performing the assessment of human and animal food safety of the transformation event GHB614 “glyphosate-tolerant cotton” and on November 9th, 2011, a request was received from the same company for evaluating the transformation event GHB614xLLCotton25 “glyphosate- and glufosinate ammonium-tolerant cotton”.

The request is revised so as to verify its compliance with the Resolution of SENASA Resolution N° 412/02, which establishes the assessment criteria and requirements for human and animal food safety of genetically modified organisms.

The submitted information was initially analyzed by the specific technical team; afterwards it was submitted to the Technical Advisory Committee for evaluation. Finally, the Agri-food Quality Office performed a new analysis, for the third time, ending in this document.

As a result of the food safety evaluation process conducted by the Biotechnology and Industrialized Products Coordination and the guidance of the Technical Committee on the use of Genetically Modified Organisms of SENASA (minute dated 03/06/2014), the Agri-food Quality Office [Dirección de Calidad Agroalimentaria (DICA)] has come to the conclusion that products derived from materials containing the transformation event GHB614xLLCotton25 and simple parental events are fit for human and animal consumption and do not pose added or increased risks resulting from transgenesis other than the risks inherent to the food in question, and meet the criteria and requirements established in SENASA Resolution N° 412/2002 and the Codex Alimentarius FAO/WHO.

EVALUATION

Cotton GHB614xLLCotton25 tolerant to glyphosate and glufosinate ammonium, as well as its parental events, were evaluated following the guidelines set forth in SENASA Resolution N° 412/02 on “Rationale and Criteria for Assessing Foods Deriving from Genetically Modified Organisms”, the “Requirements and Standard Procedures for the Assessment of Human and Animal Food Safety of Foods deriving from Genetically Modified Organisms”, and the “Information Required” for such evaluation. The mentioned Resolution provides for the criteria laid down by the Codex Alimentarius FAO/WHO. The assessment was conducted using the information provided in the application, as well as additional information required and consultations to experts, so as to determine the food safety for human and animal consumption.

1 – History of Safe Use and Specification of the Transformation Event

Cotton is a widespread crop and has an extensive history of safe use. It is neither considered dangerous nor pathogenic for humans, although under natural conditions, it produces two types of molecules with potential toxicity: gossypol and cyclopropenoid fatty acids.

Cotton has been grown for hundreds of years with the purpose of obtaining its fiber. Its seed has been used as food for man and cattle in the United States and in few other countries. Nowadays the main products obtained from the cotton seed with feeding purposes are: oil, cottonseed meal and flour, hulls, linters, non-deslinted seeds and deslinted seeds.

GHB614xLLCotton25 cotton plants are the product of the conventional crossing of events GHB614 and LLCotton25 to express the following proteins:

-2mEPSPS: endogenous maize (*Zea mays*) EPSPS protein modified in two amino acids; confers tolerance to glyphosate.

-PAT (phosphinothricin N-acetyl transferase), which confers tolerance to herbicides containing glufosinate ammonium.

2 – Molecular Characterization and Genetic Stability of the event.

The main genes of event GHB614xLLCotton25 are:

The *bar* gene, which derives from *Streptomyces hygroscopicus*, expresses protein PAT (phosphinothricin N-acetyl transferase).

The *2mEPSPS* gene, which expresses the enzyme 2mEPSPS (5-enolpyruvylshikimate-3-phosphate synthase); corn native gene with 2 mutations, which results in introduction of 2 amino acid substitutions in the enzyme sequence.

In order to characterize the DNA of event GHB614xLLCotton25 and confirm the presence and integrity of the inserts of each of the simple events, the molecular analyses based on the *Southern blot* assays of cotton GHB614xLLCotton25 and the single events

GHB614 and LLCotton25 were evaluated. This assessment showed that the event obtained from conventional crossing keeps the number, structure and organization of the inserts.

This indicates that the inserts of each single event have kept stable in the genome of the plant after conventional crossing.

3 – Products, pattern and expression levels

The products of new expression are proteins 2mEPSPS and PAT.

The expression of proteins 2mEPSPS and PAT was assessed in multiple vegetal tissues and stages of the cultivation cycle of GHB614xLLCotton25 cotton plants and plants containing the simple events. Determinations were made using the enzyme-linked immunosorbent assay (ELISA). The samples were taken from plants which contained the stacked event in the different tissues, samples of leaves, charts and grains, grown in the same location (Research Triangle Park, North Carolina, U.S.A., in 2008).

The expression levels of the 2mEPSPS protein are: 26.9 in leaf and 17.6 in grain; the PAT protein levels are: 7.45 in leaf and 276 in grain (values expressed in µg/g)

4 – Compositional Assessment

A study was presented, which involved the compositional assessment of cottonseed GHB614xLLCotton25 and non-transgenic cotton of the FM958 isoline, sown in 2008 in 7 field assays distributed in southern U.S.A. (in the states of Georgia, Arkansas, Mississippi, Louisiana and Texas). In each site, the varieties had been sown in a completely randomized block design with three repetitions per genotype. The assays were analyzed using the ANOVA model and the treatment, the site and the interaction between them were considered as factors. The differences were estimated with a 95% confidence interval.

In seeds, 50 analytes were measured and 8 statistically significant differences were identified among the genotypes (it was considered statistically different only if the GMO treated and not treated with herbicides differed from non-GM isoline), among which are: ashes, calcium, potassium, iron, free gossypol, total gossypol, phytic acid and dihydro-sterculic acid. The differences observed were small in all cases and had little biological relevance. In addition, the mean values were within the ones reported in the literature for commercial cottons.

Nutritional Suitability

Two studies on the feeding of broiler chickens were assessed:

*Forty-two day study with broiler chickens ROSS#708 which demonstrates that no dietary adverse effects were observed in those chickens that ate diets elaborated with cottonseed of event GHB614 (10% of the dietary content) as compared with chickens that had diets prepared with conventional cottonseeds (2 control groups, the non-transgenic counterpart and a non-transgenic commercial variety).

*Thirty-three day study with broiler chickens ROSS#508 which demonstrates that no differences were observed between the groups of chickens that ate diets prepared with cottonseeds of event LLCotton25 (10% of the dietary content), from crops treated and not treated with glufosinate, seeds of the FiberMax™ commercial variety and seeds of the Coker312 isogenic variety.

From the assessment of the results of these studies and after determining that there are no biologically significant differences, it may be concluded that cotton events GHB614 and LLCotton25 are substantially and nutritionally equivalent to their non-transgenic counterparts and to conventional commercial varieties. In addition, the stacked event GHB614xLLCotton25 is also substantially and nutritionally equivalent to the non-transgenic counterparts and the conventional commercial varieties since there is no evidence indicating any dietary adverse effect when stacking simple events by means of conventional crossing.

5 and 6 – Allergenicity and Toxicity

2mEPSPS

Protein 2mEPSPS derives from the EPSPS protein of maize and only has 2 modified amino acids, and therefore, an identity higher than 99.5%.

EPSPS is an enzyme which is widely expressed in nature (soybean, tomato, maize, etc.). It has a very specific and well-known biochemical role. In addition, 2mEPSPS is the same enzyme than the one expressed in corn event GA21, which means that it already has a history of safe consumption.

It has no similitude with the amino acid sequence of known allergens or toxins.

It has a high structural similitude with maize (*Zea mays*) wtEPSPS enzyme and other EPSPS enzymes, which are neither allergenic nor toxic.

It shares the same N-glycosylation sites than the endogenous *Zea mays* enzyme and both are directed to the same subcellular compartment. As a result, it is unlikely to have different allergenic characteristics by post-translational glycosylation as compared to the wild-type enzyme.

In an acute oral toxicity study there were no deaths or adverse clinical signs, in OF1 mice, at 2000 mg 2mEPSPS protein/kg body weight.

PAT

PAT protein is an enzyme which is widely distributed in nature and produced by microorganisms which are not known as pathogens for man and animals.

Its mode of action, acetylation, is very well defined.

The enzymatic properties are within the range of normal biological functions and it has high substrate specificity for herbicides containing glufosinate ammonium.

An *in vitro* dye study confirmed it has no N-glycosylation sites.

In an acute study with OF1 mice, PAT protein was administered intravenously and no adverse effects were observed even at high exposure levels. It has no amino acid sequence significantly homologous to known allergens or toxins.

In vitro digestibility studies in simulated, gastric and intestinal fluids showed a rapid degradation for both proteins of new expression.

There is no scientific evidence that these characteristics are modified by the stacking of events through conventional crossing, therefore, according to the analyzed evidence, it is concluded that it is highly unlikely that cotton event GHB614xLLCotton25 expresses allergenic or toxic substances.

7 - Metabolic interactions

Proteins 2mEPSPS and PAT have different modes of action and do not share metabolic pathways. The synthesis of the transgenic proteins in plants takes place independently and they can be found in different cellular compartments (protein 2mEPSPS is synthesized along with a signal peptide which directs its transit towards plastids). In addition, there are further evidences (no phenotypical, compositional or nutritional changes) which support that the presence of interaction mechanisms is slightly probable.

In view of the above, it is concluded that it is unlikely that there is interaction between the expressed proteins, or that there are interaction mechanisms between the genetic elements which affect the expression of the new proteins.

8 – Conclusion

Having fully analyzed the dietary risk in the information provided by the company, Bayer CropScience S.A., and taking into account that:

- Molecular characterization studies demonstrate that the stability of the inserts of each single event has been kept in the genome of the plant after conventional crossing.
- The proteins of new expression are expressed at low levels in the grain.
- It is substantially and nutritionally equivalent to its non-transgenic counterpart.
- No evidence of similitude or homology with known toxic proteins has been found either in the stacked event or the simple events.
- No evidence of expression of known allergenic substances has been found for the expressed proteins either in the stacked event or the simple events.
- Studies were evaluated indicating that there are no interaction effects between the proteins of the events when they are stacked.

It can be concluded that the cotton event GHB614xLLCotton25 and simple events are substantially equivalent to their conventional counterpart; therefore, they are as safe as and not less nutritive than the conventional commercial cotton varieties.

Based upon the above and the scientific knowledge currently available and the internationally accepted requirements and criteria, there are no objections for the approval of cotton event GHB614xLLCotton25 and simple parental events for human and animal consumption.

9 – Standards and recommendations

- SENASA Resolution N° 1265/99.
- SENASA Resolution N° 412/02.
- Principles for the Risk Analysis of Foods Derived from Modern Biotechnology (CAC/GL 44-2003).
- Guidelines for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (CAC/GL 45-2003).
- Consensus Documents for the work on the Safety of Novel Foods and Feeds (OECD).
- Resolution MAGyP N° 701/2011.
- ILSI 2007 Database.
- Allergens Database (FARRP Database).

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