DECISION DOCUMENT

Food and feed safety assessment of maize event Bt11xMIR162xMIR604xGA21 OECD: (SYN-BTØ11-1 x SYN-IR162-4 x SYN-IR6Ø4-5 x MON-ØØØ21-9)

(Includes all possible intermediate combinations)

Directorate of Agrifood Quality

Office of Biotechnology and Industrialized Agrifood Products

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SUMMARY AND BACKGROUND

The food risk assessment process of transformation events due to modern biotechnology is carried out by the National Service of Agrifood Health and Quality (SENASA), regulatory body under the scope of the Ministry of Agriculture, Livestock and Fisheries.

The Directorate of Agrifood Quality of SENASA is the area responsible for the performance of this task, relying on a scientific team and the advice of a Technical Advisory Committee made up of experts from different scientific fields, representing different sectors related to production, industrialization, consumption, research and development of genetically modified organisms.

On August 31st, 2009, an application from Syngenta Agro S.A. was received, to carry out the human and animal food safety evaluation of the transformation event Bt11 x MIR162 x MIR604xGA21) OECD (SYN-BTØ11-1 x SYN-IR162-4 x SYN-IR6Ø4-5 x MON-ØØØ21-9) maize resistant to certain Coleopteran and Lepidoptera insects and tolerant to glyphosate and glufosinate ammonium.

The application was reviewed in order to confirm its compliance with all the criteria laid down in SENASA Resolution Nº 412/02, regulation that sets forth the criteria and requirements for the evaluation of human and animal food safety of genetically modified organisms.

The information submitted was analyzed at a first instance by the specific technical team, then subjected to the evaluation by the Technical Advisory Committee and finally the Directorate of Agrifood evaluated it at a third instance and concluded in the present document.

EVALUATION

The Bt11xMIR162xMIR604xGA21 maize, resistant to certain Coleopteran and Lepidoptera insects, was evaluated following the guidelines laid down in SENASA Resolution Nº 412/02 on the "Bases and Criteria for the Evaluation of Food Derived From Genetically Modified Organisms" and the "Requirements and Rules of Procedure for the Evaluation of Human and Animal Safety of Food Derived from Genetically Modified Organisms" and the "Requested Information" for such evaluation. The above mentioned Resolution includes the criteria established in the *Codex Alimentarius* FAO/WHO. The evaluation was conducted using the information provided in the application, together with additional information requested and expert consultations, to establish the safety for human and animal consumption.

1 – History of use and specification of transformation event

Corn is the third most important cereal crop worldwide, after rice and wheat. It was domesticated by pre-Columbian America over 8000 years ago. It is commercially grown in several countries of the world.

Corn has a wide history of safe use and no cases of intoxication or allergies have been reported due to its reasonable consumption.

The Bt11xMIR162xMIR604xGA21 maize plants have been obtained through conventional crossing to express the proteins mCry3A (modified version of Cry3A); PMI (MIR604) (modified version of the phosphomannose isomerase of E. coli); Cry1Ab (modified version of the *Bacillus thuringiensis* endotoxin Subsp:*kurstaki* HD-1); PAT (phosphinothricin acetyltransferase, protein that confers tolerance to glufosinate ammonium); VIP3Aa20 (*Bacillus thuringiensis* endotoxin that confers resistance to certain lepidopteran insects); mEPSPS (modified version of the EPSPS protein proper of the corn genome) and PMI (phosphomannose isomerase of E. coli strain K12).

2 – Genetic stability and event molecular characterization

The main genes of the Bt11xMIR162xMIR604xGA21 are: ***mcry3A***, which expresses the mCry3A protein; ***pmi* (MIR604),** which expresses the PMI (MIR604) protein; ***cry1Ab***, which expresses the Cry1Ab protein (modified version of the *Bacillus thuringiensis* var. *kurstaki* HD-1); ***pat***, which expresses the PAT protein (phosphinothricin acetyltransferase, protein that confers tolerance to glufosinate ammonium); ***vip3Aa20***, which expresses the VIP3Aa20 protein (Bacillus thuringiensis endotoxin that confers resistance to certain lepidopteran insects); ***pmi***, which expresses the PMI protein (phosphomannose isomerase of E. coli) and the ***mepsps*** gene, which expresses the modified version of the native EPSPS protein from corn.

Molecular tests were assessed to confirm the integrity of the DNA insert in the Bt11xMIR162xMIR604xGA21. The molecular organization of the insert was confirmed through Southern blot analysis, which demonstrated that the integrity of the insert of each individual event is maintained by the stacked. These analyses show the expected gene inheritance. Hybridization patterns were analyzed both for individual events and for the stacked hybrid.

The stability studies and results of each event were presented in the assessment of individual events.

All new genes are inherited in a predictable manner according Mendelian genetic principles.

3 – Pattern and levels of expression

Concentration levels of transgenic proteins were determined through ELISA techniques in various plant tissues and growth stages of plant crops which were grown in a same locality at the same time. All protein concentrations were similar between the stacked hybrid and the four individual events.

4 – Compositional Analysis

The applicant submitted information about the compositional analysis (2006 season in 6 US corn locations) in grain and green tissue of hybrid corn plants containing the Bt11xMIR162xMIR604xGA21 event, compared to non-transgenic corn (isogenic line and commercial hybrid).

65 components in forage and grain were analyzed and compared statistically. The statistical significance of the genotype effect for each analyte was determined through a standard Fisher test (a probability of the F test inferior to 5% indicates that the difference between the genotypes was statistically significant). The studies show that, even though some significant statistical differences were found, all the values obtained were within the range and close to the average of the scientific literature (OECD and ILSI); thus the differences were not considered biologically relevant.

A 49 day study was carried out in 540 broiler chicken to evaluate diets containing the Bt11xMIR162xMIR604xGA21 grain event, compared to the isoline and a commercial hybrid. The results of this study demonstrated that there were no adverse dietary effects on chicken that consumed diets prepared with Bt11xMIR162xMIR604xGA21 corn grain, compared to diets prepared with non-transgenic corn grain, be it for the direct effect of transgenic proteins on the diet or as a result of unintentional compositional changes in the grain that might have generated toxic effects or altered its nutritional value.

It can be concluded then that the Bt11xMIR162xMIR604xGA21 corn is basically and nutritionally equivalent to its non-transgenic counterpart and to conventional hybrids.

5 – Allergenicity

Homology with known allergens:

The allergenicity evaluations of each of the proteins introduced were submitted with the individual events and are in force. The results of the bioinformatic analysis that were submitted show the absence of general sequence homology or immunologically relevant, when compared to pharmacologically active allergens or proteins.

The characteristics of molecular weight, concentration, simulated digestibility and thermo stability of the new proteins were timely submitted for each individual event. No evidence

was found for any of the proteins identifying them as potential allergens. These characteristics were not modified by the accumulation of events; therefore, according to the assessment of the evidence, it is concluded that it is highly unlikely that the Bt11xMIR162xMIR604xGA21 maize event expresses allergenic substances.

6 – Toxicity

The acute toxicity and bioinformatic studies of the proteins were timely evaluated in parental events. A 49-day feed study conducted on chickens was evaluated using the stacked corn event in their diets and demonstrated that there are no dietary adverse effects. It is concluded that the Bt11xMIR162xMIR604xGA21 maize event is unlikely to present toxicological risks for humans and animals.

7 – Metabolic interactions

The evaluated studies indicate that interaction effects (synergistic, antagonistic and additive) are unlikely to exist among the proteins of the stacked events. Other evaluations evidence that there are no phenotypic, compositional, nutritional and bioefficacy changes and that proteins do not share metabolic routes or modes of action. According to what was previously exposed, it is concluded that interaction mechanisms among the genetic elements that affect the expression of new proteins are unlikely to exist.

8 – Conclusion

After performing a complete food risk assessment to the material submitted by SYNGENTA S.A. and taking into account that:

• Inheritance studies performed indicated that there is Mendelian segregation,

• Proteins of new expression in grain are expressed in low levels,

• It is substantially and nutritionally equivalent to its non-transgenic counterpart,

• No evidence of similarity or homology with known proteins was found,

• No evidence of expression of allergenic substances known for proteins expressed in the stacked event was found,

• Studies that show that there are no interaction effects among the proteins of the events when accumulated were assessed.

It is concluded that the Bt11xMIR162xMIR604xGA21 maize event is substantially similar to its conventional counterpart; therefore, it is as safe as and not less nourishing than conventional commercial hybrid corns.

According to the foregoing, and based on the scientific knowledge available as well as the internationally accepted requirements and criteria, there are no objections to approve the Bt11xMIR162xMIR604xGA21 maize and all the possible combinations of simple events for human and animal consumption.

9 – Resolutions and recommendations

- SENASA Resolution Nº 1265/99.

- SENASA Resolution Nº 412/02.

- Principles for the risk analysis of food derived from modern biotechnology (CAC/GL 44-2003).

- Guidelines for the safety evaluation of food derived from Recombinant DNA plants (CAC/GL 45-2003).

- Consensus Document's for the work on the Safety of Novel Foods and Feeds (OECD).

- Resolution of the Ministry of Agriculture, Livestock and Fisheries N° 701/2011.

- ILSI 2007 database.

- Database of allergens (FARRP database).

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