

DECISION DOCUMENT

**Food and feed safety assessment of maize event
Bt11 x MIR162 x GA21
OECD:SYN-BTØ11-1 x SYN-IR162-4xMON-ØØØ21-9
(Includes all possible intermediate combinations)**



Directorate of Agrifood Quality

Office of Biotechnology and Industrialized Agrifood Products

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Safety evaluation for consumption of Bt11xMIR162xGA21 maize event

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 27, 2009, an application from Syngenta Agro S.A. was received, to carry out the food and feed safety evaluation of the transformation event SYN-BTØ11-1 x SYN-IR162-4xMON-ØØØ21-9 “maize resistant to lepidopteran insects and tolerant to glyphosate and ammonium glufosinate.”

The application was reviewed in order to confirm its compliance with all the criteria laid down in SENASA Resolution N° 412/02, regulation that sets forth the criteria and requirements for the evaluation of food and feed 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 Bt11xMIR162xGA21 maize, resistant to lepidopteran insects and tolerant to glyphosate and ammonium glufosinate, was evaluated following the guidelines laid down in SENASA Resolution N° 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.

Bt11xMIR162xGA21 maize plants are the result of the combination of the Bt11, MIR162 and GA21 events to express the following proteins:

Bt11 Event: expresses the protein **Cry1Ab** which controls certain lepidopteran insect pests (*Diatraea saccharalis*) and **PAT** (*phosphinothricin acetyltransferase*), which confers tolerance to herbicides that contain ammonium glufosinate.

MIR162 Event: expresses the protein **Vip3Aa20**, highly specific controlling *Helicoverpa zea*, *Spodoptera frugiperda*, *Agrostis ipsilon* and **PMI** (Phosphomannose Isomerase), selectable marker that allows the use of mannose as a source of carbon.

GA21 Event: expresses the protein **mEPSPS** modified in two amino acids of the endogenous protein EPSPS of *Zea mays* that confers glyphosate tolerance.

The Bt11xMIR162xGA21 event has been obtained by conventional crossing of the parental events.

2 – Genetic stability and molecular characterization

The main genes of the Bt11xMIR162xGA21 event are:

The Bt11 maize contains the gene *cry1Ab* codifying the protein Cry1Ab (truncated version of a δ -endotoxin of *Bacillus thuringiensis* var. *kurstaki* strain HD1), and the *pat* gene, from the *Streptomyces viridochromogenes* expressing the PAT protein (phosphinothricin acetyltransferase).

The MIR162 maize contains the gene *vip3Aa20* (insecticidal protein from Vip3Aa19) codifying protein Vip3Aa20 isolated from *Bacillus thuringiensis* ssp.

The GA21 maize contains the gene *mepsps* that expresses the enzyme mEPSPS (5-enolpyruvylshikimate-3-phosphate synthase).

To characterize the DNA in the Bt11xMIR162xGA21 event and to confirm the presence

and integrity of the inserts of each individual event in the final product, molecular tests were run based on Southern blot analysis of Bt11xMIR162xGA21 maize and individual lines Bt11, MIR162 and GA21. These confirmed that the lines obtained through conventional breeding maintain the number, structure and organization of the inserts.

The molecular characterization show stability of the insert in the plant genome of each individual event (Bt11, MIR162 and GA21) utilized for the production of the transgenic hybrid Bt11xMIR162xGA21.

The Bt11xMIR162xGA21 maize hybrid has been obtained by conventional crossing between the lines carrying the individual events. Each event segregates according to Mendelian rules through various generations, independently from one another.

3 – Products, pattern and levels of expression

The products of novel expression are the Cry1Ab, PAT, VIP3Aa20, PMI and mEPSPS proteins.

The Cry1Ab protein is identical to the N-terminal part of the native δ -endotoxin. It has insecticidal activity for certain types of lepidopteran insects such as *Helicoverpa zea*, *Spodoptera frugiperda* and *Diatraea saccharalis*.

The PAT protein (phosphinothricin-N-acetyltransferase), isolated from the microorganism from the soil *Streptomyces viridochromogenes* strain Tu494, confers tolerance to the ammonium glufosinate herbicide. This protein is made up of 183 amino acids. The phosphinothricin inhibits the activity of the glutamine synthetase (GS) attaching in a competitive manner instead of the habitual substrate, the glutamate (glutamic acid). It prevents the synthesis of L-glutamine, which is not only an important chemical precursor for the synthesis of nucleic acids and proteins but also it works as a mechanism to incorporate ammoniac in plants. The phosphinothricin-based treatment causes the accumulation of ammoniac and it stops the photosynthesis, probably due to the lack of glutamine.

The Vip3Aa20 protein differs in two amino acids (in positions 129 and 284) with respect to Vip3Aa1 protein encoded for the native gene vip3Aa1 of *B. thuringiensis* strain AB88. The Vip3Aa20 protein is an insecticidal protein (Vip) that controls several lepidopteran pests in corn, it has approximately 89 kDa molecular weight and is made up of 789 amino acids in length.

The phosphomannose isomerase protein PMI, of approximately 42,8 kDa and 391 amino acids, catalyzes the reversible interconversion of mannose-6-phosphate and it can be used as a selectable marker. The evaluation of the expressions of Cry1Ab, PAT, Vip3Aa20, PMI and mEPSPS proteins in Bt11xMIR162xGA21 corn plants was conducted in several plant tissues and stages of crop cycle. The determinations were carried out using the Enzyme-

linked immunosorbent assay (ELISA) method. Samples were taken from plants carrying the individual event and the triple stacked, grown in the same location, simultaneously. The results are shown in table 1.

Type of tissue (Phenologic stage)	Hybrid	Cry1Ab	PAT	Vip3Aa20	PMI	mEPSPS
Leaves (Vegetative)	Simple event	117,26 - 180,55	N/A	172,1 - 202,72	5,64 - 10,13	N/A
	Stack	123,06 - 173,32	N/A	159,34 - 213,99	6,76 - 10,49	N/A
Leaves (Reproductive)	Simple event	30,22 - 40,12	0,51 - 0,81	112,36 - 154,79	6,03 - 7,41	30,39 - 40,44
	Stack	21,86 - 40,39	0,4 - 0,97	116,71 - 187,47	6,39 - 8,76	28,86 - 39,59
Leaves (Physiological maturity)	Simple event	4,38 - 34,78	N/A	N/A	0,028 - 5,32	N/A
	Stack	15,04 - 33,13	N/A	N/A	0,68 - 5,33	N/A
Roots (Vegetative)	Simple event	N/A	0,48 - 0,93	N/A	N/A	N/A
	Stack	N/A	0,46 - 1,10	N/A	N/A	N/A
Roots (Reproductive)	Simple event	10,33 - 15,53	0,35 - 0,79	9,54 - 51,60	1,45 - 3,65	11,81 - 18,98
	Stack	9,17 - 14,45	0,31 - 0,47	19,07 - 38,45	1,54 - 3,18	10,68 - 15,44
Roots (Physiological maturity)	Simple event	N/A	0,32 - 1,21	N/A	N/A	N/A
	Stack	N/A	0,428 - 1,50	N/A	N/A	N/A
Pollen	Simple event	0,06 - 0,07	< LOQ < LOD	103,72 - 111,18	4,25 - 5,30	65,79 - 116,33
	Stack	0,05 - 0,15	< LOQ < LOD	138,53 - 173,94	4,41 - 5,30	103,15 - 144,19
Grains (Physiological maturity)	Simple event	4,35 - 10,67	< LOQ < LOQ	56,41 - 108,27	1,11 - 2,58	5,35 - 8,76
	Stack	4,85 - 10,64	< LOQ < LOQ	59,18 - 102,10	1,21 - 2,61	3,53 - 8,57
Complete Plant (Reproductive)	Simple event	16,15 - 22,72	0,66 - 1,08	64,92 - 102,64	3,56 - 4,42	35,65 - 62,96
	Stack	13,91 - 20,42	0,65 - 0,86	67,56 - 86,51	3,52 - 4,22	36,64 - 54,73
Complete Plant (Physiological maturity)	Simple event	8,80 - 18,26	N/A	N/A	N/A	N/A
	Stack	5,17 - 17,49	N/A	N/A	N/A	N/A
Complete Plant (Senescence)	Simple event	4,41 - 13,36	N/A	N/A	N/A	N/A
	Stack	2,88 - 7,64	N/A	N/A	N/A	N/A

Ref.: Concentration of Cry1Ab, PAT, Vip3Aa20, PMI and mEPSPS proteins in Bt11, MIR162 and Bt11xMIR162xGA21 events, expressed as dry weight in mg/kg; LOD Limit of detection; LOQ Limit of quantification; N/A Information Not Analyzed.

Only 6 significant differences were found out of the 32 statistical analysis conducted between the concentrations of transgenic proteins expressed in the plant tissue carrying

individual events and the hybrid Bt11xMIR162xGA21. These differences are present only in certain stages and are not maintained through the crop cycle; thus they are not biologically relevant.

4 – Compositional Analysis

This evaluation was carried out based on the type and importance of the analyte, its statistical difference and the ranges published in the bibliography (ILSI, 2007) for the specie.

Studies were submitted analyzing the composition of the grain and forage of Bt11xMIR162xGA21 maize and of non-transgenic maize of the isogenic line grown in 2006 in 6 corn localities of North America. In each site, the hybrids were planted following a block design completely randomized with three repetitions for each genotype.

60 components of forage and grain were analyzed and compared statistically. In grain, significant statistical differences were found for the genotype effect on the levels of vitamin B1, vitamin B3, vitamin B6, in amino acids Isoleucine, Leucine, Tyrosine, Phenylalanine, Histidine, Arginine, Aspartic Acid, Threonine, Serine, Glutamic Acid, Proline, Glicine, Alanine and Valine; in stearic fatty acids 18:0, oleic 18:1 and phytic acid. All the analyzed values were within the range of variation and close to the average of the scientific literature (ILSI 2007); therefore, the differences were not considered biologically relevant.

After analyzing the results of this study and determining that there are no significant biological differences, it can be concluded that the Bt11xMIR162xGA21 is substantially 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 shows 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 Bt11xMIR162xGA21 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.

Syngenta submitted acute toxicity studies with rats for the five new proteins (Cry1Ab, PAT, Vip3Aa20, PMI and mEPSPS) present in the Bt11xMIR162xGA21 event, which shown that there are no adverse effects as a result of the incorporation of grain of this corn into the rat diet. It is concluded that the Bt11xMIR162xGA21 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 empowerment) 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 toxics proteins was found,
- No evidence of expression of allergenic substances known for proteins expressed in the stacked event was found,
- Studies that show^s that there are no interaction effects among the proteins of the events when accumulated were assessed.

It is concluded that the Bt11xMIR162xGA21 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

Bt11xMIR162xGA21 maize and all the possible combinations of simple events for human and animal consumption.

9 – Resolutions and recommendations

- SENASA Resolution N° 1265/99.
- SENASA Resolution N° 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).

Buenos Aires, 09/16/2011.