Decision document

Evaluation of Event MIR162
For human and animal consumption

Directorate of Agricultural Quality

Coordination of agro-industrialized products

SENASA

Content:

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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 (MAGyP).

The Directorate of Agrifood Quality of SENASA is the responsible area to carry out this task. It has a specific scientific team and the advice of a Technical Advisory Committee composed by experts from different scientific fields, representing different sectors related to production, industrialization, consumption, research and development of genetically modified organisms.

On February 7, 2008, an application from Syngenta was received, to carry out the evaluation of human and animal food safety of transformation event MIR162, corn resistant to certain insects.

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

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

EVALUATION

MIR162 corn was evaluated following the guidelines laid down in SENASA Resolution Nº 412/02, on the "Criteria for the Evaluation of Food Derived from Genetically Modified Organisms", the "Procedure Requirements and Standards for the Assessment of Human and Animal Food Safety of Food Derived from Genetically Modified Organisms", and the "Requested Information" for such evaluation. The evaluation was carried out using the information provided in the application together with additional information requested and consultation to experts, to establish the safety for human and animal consumption.

1 – History of use and specification of transformation event

Corn is the third most important crop worldwide, after rice and wheat. I was used domestically in pre-Columbian America more than 8000 years ago. It is commercially cultivated in several countries of the world.

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

Syngenta has developed corn event MIR162 (OECD: SYN-IR162-4), produced by mediated genetic transformation from *Agrobacterium tumefasciens*. Two novel proteins are expressed: Vip3Aa20, insecticidal protein for certain lepidopteran insects, and PMI, phosphomannose isomerase which acts as a selectable marker allowing the use of mannose as a carbon source.

Event MIR162 confers corn highly specific resistance to certain lepidopteran insects, and it is specially important to complement event Bt11 on field in the control of *Diatraea saccharalis,* since MIR162 expresses insecticidal protein Vip3Aa20 which belongs to the group of "vegetative insecticidal proteins" (Vip), which has a different action mechanism from events that express Cry protein, and therefore the possibility of occurrence of populations of resistant insects to *Diatraea* is reduced. It also controls other important pests such as *Helicoverpa zea* (corn earworm) and *Spodoptera frugiperda* (fall armyworm). Such endotoxin is produced by *B. thuringiensis*.

Pmi gene comes from Escherichia coli strain K12 and encodes PMI enzyme, which catalyzes the isomerization of mannose-6-phosphate to fructose-6-phosphate, giving plant cells carrying the *pmi* gene the possibility to survive and grow in media that have mannose as the only primary carbon source.

2 – Genetic stability and event molecular characterization

The company submitted a PCR analysis in plants of three backcross generations of MIR162 corn to determine inheritance proportions of *vip3Aa20* and *pmi* genes. For each tested generation, the expected segregation frequency was 1:1. Both genes segregate according to Mendelian rules of inheritance for a single genetic locus.

Moreover, the applicant presents a Southern blot analysis, where it is determined the stability of the DNA insert during conventional breeding of MIR162 corn, using different backcross generations. Expected hybridization results were reached for *vip3Aa* gene in several generations of MIR162 corn, showing stability of the insert in MIR162 corn over multiple generations.

3 – Products and levels of expression

The products of the novel expression are Vip3Aa20 protein, which differs in two amino acids (in positions 129 and 284) with respect to Vip3Aa1 protein encoded by native gene vip3Aa1 of *B. thuringiensis* strain AB88, and the PMI protein, encoded by *pmi* gene from Escherichia coli.

Vip3Aa20 protein is a vegetative insecticidal protein (Vip) which controls several lepidopteran pests in corn. It has approximately 89 kDa molecular weight and is composed by 789 amino acids in length.

Pmi gene encoding for phosphomannose isomerase protein (PMI) is of approximately 42,8 kDa and 391 amino acids. PMI catalyzes the reversible interconversion of mannose-6-phosphate and fructose-6-phosphate and it can be used as a selectable marker for the transformation of many plant species.

Both proteins are expressed in tissues of the whole plant. The expression of both proteins was measured and verified in the following tissues: leaves, roots, stem, grains, stigmas, pollen and whole plant, with average levels of protein shown as follows:

µg Vip3Aa20/g dry weight (average)				
Tissue	V9-V12	Anthesis	Seed maturation	Senescence
Leaf	97.26 (76.12 – 119.12)	107.74 (97.10 – 118.80)	121.79 (77.25 – 159.66)	21.31 (12.93 – 30.28)
Root	31.80 (28.10 – 35.65)	28.34 (26.30 – 30.20)	20.29 (9.87 – 27.48)	21.66 (11.58 – 32.13)
Stalk (marrow)	N/A	31.71 (29.43 – 36.48)	58.21 (52.74 – 63.68)	N/A
Grains	N/A	N/A	43.56 (40.47 – 50.50)	34.24 (30.90 – 37.67)
Stigma	N/A	97.40 (60.54 – 149.00)	N/A	N/A
Pollen	N/A	47.13 (41.45 – 53.52)	N/A	N/A
Complete plant	91.53 (88.68 – 96.51)	67.61 (61.68 – 72.63)	49.04 (34.84 – 63.14)	34.30 (21.12 – 55.17)

Vip3Aa20 concentrations in negative control samples are <LOD or <LOQ and are not included in this table.
N/A= Not analysed in this stage

µg PMI/g dry weight (average)				
Tissue	V9-V12	Anthesis	Seed maturation	Senescence
Leaf	11.12 (8.26 – 16.76)	9.75 (6.92 – 14.68)	5.77 (4.57 – 7.55)	<0.26 (<LOD – LOQ)
Root	4.32 (3.17 – 7.08)	3.49 (2.51 – 5.22)	1.99 (1.08 – 3.09)	1.51 (0.47 – 2.53)
Stalk (marrow)	N/A	2.01 (1.53 – 2.40)	2.75 (2.36 – 3.19)	N/A
Grains	N/A	N/A	1.93 (1.33 – 2.54)	0.75 (0.54 – 0.97)
Stigma	N/A	20.70 (12.60 – 27.16)	N/A	N/A
Pollen	N/A	5.29 (3.82 – 7.62)	N/A	N/A
Complete plant	8.74 (7.49 – 9.75)	7.10 (6.32 – 7.61)	3.76 (2.16 – 5.37)	2.36 (1.85 – 3.09)

"<" (less than) indicates that the estimated LOQ or LOD value was used to represent samples when the average value was estimated.

PMI concentrations in negative control samples are <LOD or <LOQ and are not included in this table.
N/A= Not analysed in this stage

4 – Compositional analysis

The applicant submitted information about the compositional analysis in grain and green tissue of hybrid corn plants containing event MIR162 compared to the composition of grain and forage of a non transgenic corn of near isogenic line. The levels of 65 key components in forage and in grain were measured. Results of compositional analysis of hybrid corn with event MIR162 that were submitted by the applicant were evaluated by means of an statistical analysis ANVA (analysis of variance), determining a standard test F (F < 5%) to measure the level of signification between the mean values of MIR162 and its non transgenic comparative. Results of the analysis did not show significant biological differences of the 65 analytes measured in grain and forage in relation to the isoline.

Significant differences (1 in forage and 14 in grain) were evaluated separately in order to consider the deviations and determine their biological importance. These analytes and their significant differences are: neutral detergent fibre (11,57%) in forage; and ashes, FDN, and starch (9,33, 5,32, y 2,82% respectively), calcium, iron, and phosphorus (7,95, 5,05, y 6,25%, respectively), vitamin A (β-carotene), vitamin B_6 (pyridoxine), vitamin E (α-tocopherol) and vitamin B_9 (folic acid) (5,78, 6,55, 5,46 and 1,63% respectively), linoleic fatty acids and linoleic (0,65 y 3,21%, respectively), ferulic acid and ρ-fumaric acid (9,31 y 11,44% respectively) in grain.

This evaluation was carried out based on the type and importance of the analyte, its statistical difference and database for the species and for commercial hybrids in the market.

As a result of the evaluation, the average values (which could be quantified) including those where significant differences were found, were located within the range of natural variation according to what it was reported in literature, considering them non biologically significant.

Moreover, a 44 day food study was carried out in broiler chicken, where it was demonstrated that there were no adverse dietary effects in broiler chicken which consumed diets prepared with MIR162 corn grain, compared to those which consumed diets prepared with non transgenic corn grain.

It can be concluded then that MIR 162 corn is basically and nutritionally equivalent to its non transgenic counterpart and to conventional hybrids.

5 – Allergenicity

In order to evaluate the allergenic potential of proteins Vip3Aa20 and PMI, the following tests were carried out:

Source of the protein:
Protein Vip3Aa was initially isolated from strain AB88 of *Bt*, therefore it does not derive from a known producing source of allergenic proteins.

Amino acid sequence homology with know allergens:
A bioinformatic analysis was submitted, where it was determined that protein Vip3Aa20 neither shared general sequence homology (sequential peptide of 80 amino acids) with any known allergenic protein nor significant amino acid sequence identity was identified (eight contiguous amino acids) with known or presumed allergenic proteins which could imply an allergenic potential.
In the case of protein PMI, through a bioinformatic analysis, it was detected a similarity of 8 identical amino acids between PMI and α-parvalbumin of frog *Rana sp.* Studies carried out with PMI and serum from an allergic patient to the protein expressed by the frog did not result in an allergic reaction, therefore this similarity is not considered as biologically significant.

Stability to heat treatment:
Analysis presented show that Vip3Aa20 is unstable under temperature of 65 ºC and higher.
In the case of PMI, there is inactivation after 30 minutes of exposure to temperatures higher than 65ºC.

In Vitro digestibility:
Analysis were submitted where it was evaluated the susceptibility of proteins to the degradation in a simulated gastric fluid (SGF) containing pepsin. Results show that Vip3Aa20 as well as PMI are rapidly degraded in simulated gastric fluids of mammals.

Therefore, it is concluded that it is highly unlikely that corn event MIR162 expresses allergenic substances for humans and/or animals.

8 – Toxicity

Potential toxicity of protein Vip3Aa20 was evaluated carrying out a thorough bioinformatic search to determine whether the amino acid sequence of Vip3Aa20 has a significant homology with protein sequences identified as toxins and performing a 14 day analysis of acute oral toxicity in mice.
Vip3Aa20 does not share a significant homology with known toxins (different to protein toxins Vip) and did not show adverse effects related to the treatment when administered to mice in high doses (1250 mg/kg body weight).
Bioinformatic analysis with PMI do not show significant homologies with known toxins either.

Consequently, it is concluded that corn event MIR162 does not pose toxicological risks to be consumed by humans and animals.

9- Conclusion

After carrying out a complete food risk evaluation to the material submitted by Syngenta, and having into account that:

- Inheritance studies performed indicated that there is Medelian segregation,
- Proteins of the new expression are in low levels in grain,
- It is basically and nutritionally equivalent to its non transgenic counterpart,
- No evidence of similarity or homology with toxic proteins was reported,
- Analysis submitted to evaluate the potential allergenicity shows that no allergenic substances are expressed,

It is concluded that corn event MIR162 is similar to its conventional counterpart; therefore it is as safe as and not less nourishing than conventional commercial hybrid corns.

According to the above mentioned, and based on the scientific knowledge available and the requirements and criteria internationally accepted, there are no objections to approve MIR162 corn for human and animal consumption.

10- Resolutions and recommendations:

- SENASA Resolution Nº 1265/99
- SENASA Resolution Nº 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)

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Ing. Agr. JUAN C. BATISTA
DIRECTOR de CALIDAD AGROALIMENTARIA
SENASA