DECISION DOCUMENTAL

Food and feed safety assessment of maize event MIR604

OECD: SYN-IR6Ø4-5

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 January 27, 2009, an application from Syngenta Agro S.A. was received, to carry out the human and animal food safety evaluation of the transformation event OECD: SYN-IR6Ø4-5 (MIR604) maize resistant to certain Coleopteran insects.

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 (MIR 604) maize, resistant to Coleopteran 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 MIR604 maize plants have been genetically modified to express the *mCry3A* proteins (modified version of Cry3A of *Bacillus thuriengiensis subsp. Terebrionis*, which confers resistance to some coleopterans) and PMI (modified version of the phosphomannose isomerase of E. coli strain K-12, which provides the ability of using mannose as an energetic carbon source). The transformation was measured by *Agrobacterium tumefasciens*, containing the pZM26 plasmid.

2 – Genetic stability and event molecular characterization

The main genes of the MIR604 event are: *mcry3A* which expresses the mCry3A protein and the *pmi* gene, which expresses the PMI protein (MIR604). To characterize the DNA insert in the MIR604 event and to confirm the presence of integrity and stability of the inserts in the final product, a molecular characterization was done using the Southern blot technique in six generations of the maize corn event MIR604. The results of the analysis confirmed that the MIR604 contains a single copy of the *mcry3A* and *pmi* genes, a single copy of the MTL and ZmUbilnt promoters and does not have sequences of vector out of integration borders. The stability of the insert has been determined using six backcross generations. All plant DNA has been analyzed through PCR (TaqMan®) showing the presence of a single copy of *mcry3A* and *pmi* genes. The hybridization pattern of the different generations of the event shows that the T-DNA insert through plasmid pZM26 is stable over several generations. All new genes are inherited in a predictable manner according Mendelian genetic principles.

3 – Products, pattern and levels of expression

The products of novel expression correspond to mCry3A and PMI (MIR604) proteins. The mCry3A protein is entirely made up of 528 amino acids and has approximately 67 kDa molecular weight. The initial methionine corresponds to the methionine in position 48 of the native. It has a protease Cathepsin-G recognition site, which speeds up the processing of the toxin and its activity.

The expression of this gene is controlled by the MTL promoter, which provides a preferential expression on roots. The PMI (MIR604) protein has suffered two conservative changes during the transformation event. It catalyzes the reversible inter-conversion of mannose-6-phosphate to fructose-6-phosphate (plant cells transformed with the *pmi* gene may survive and grow in an environment having mannose as the only source of energy).

The complete PMI (MIR604) protein is made up of 391 amino acids and has approximately 42.8 kDa molecular weight. The expression of this gene is controlled by promoter ZmUblint, which provides a constitutive expression in the tissues of the whole plant.

The concentration of proteins in different plant tissues was determined through the ELISA technique (Enzyme-linked immunosorbent assay method).

mCry3A:

- Leaves of 3 - 23 µg/g in fresh weight; 4 - 94 µg/g in dry weight.

- Leaves of 2 - 14 µg/g in fresh weight; 7 - 62 µg/g in dry weight.

- Complete plant 0.9 - 11 µg/g in fresh weight; 3 - 28 µg/g in dry weight.

- Grain (maturity) 0.6 – 1.4 µg/g in fresh weight; 0.8 - 2 µg/g in dry weight.

- Grain (senescence) 0.7 µg/g in fresh weight; 4 - 94 µg/g in dry weight.

- Pollen LOD = 0.07 µg/g in fresh weighty; in dry weight.0.15 µg/g.

PMI (MIR604):

- Leaves non-detectable ND-0.4 µg/g in fresh weight; ND -2.1 µg/g in dry weight.

- Roots of 0.03 – 0.2 µg/g in fresh weight; 0.1 - 1 µg/g in dry weight.

- Complete plant 0.02 – 0.3 µg/g in fresh weight; 0.04 - 2 µg/g in dry weight.

- Grain (maturity) 0.06 – 0.4 µg/g in fresh weight; 0.07 – 0.5 µg/g in dry weight.

- Grain (senescence) 0.14 µg/g in fresh weight; 0.17 µg/g in dry weight.

- Pollen 1.9 – 2.6 µg/g en in fresh weight; in dry weight 3.9 – 5.2 µg/g.

The stability of the expression was evaluated in 4 backcross generations. The results do not show any tendency and the values were located within the range of natural variation, indicating that the expression of these proteins is stable throughout the generations.

4 – Compositional Analysis

The applicant submitted information about the compositional analysis in grain and green tissue of hybrid corn plants containing the MIR604 event, compared to non-transgenic corn (isogenic line and commercial hybrid). Studies were submitted upon which a compositional analysis was conducted during the 2002 and 2003 seasons, in 6 US corn localities. 58 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 broiler chicken to evaluate diets containing MIR604 event grain, 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 MIR604 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 MIR604 corn is basically and nutritionally equivalent to its non-transgenic counterpart and to conventional hybrids.

5 – Allergenicity

Homology with known allergens:

In the bioinformatic analysis (FARRP allergen database) for the PMI protein, homology of 8 amino acids contiguous to the α-parvalbumin from rana species was found. A study was conducted using serum from the single individual know to be allergic to the α-parvalbumin to demonstrate cross-reactivity. The result did not show PMI recognition for the specific serum; therefore, the sequence identity of both proteins is not biologically relevant and has no implication from the allergenicity point of view. The mCry3A protein demonstrated no general sequence homology or immunologically relevant, when compared to allergens or pharmacologically active proteins.

Thermo stability

Results demonstrate that mCry3A protein is unstable at temperature of 65°C and completely inactive when exposed at 95°C during 30 minutes.

In the case of PMI, results demonstrate that after 30 minutes of exposure to temperatures of 65°C or higher, the protein is inactivated.

In Vitro digestibility:

The *in vitro* digestibility studies (FGS) support the conclusion that it is highly unlikely that such proteins present stability characteristics to the degradation in simulated gastric fluids associated to the allergenicity potential.

Results show that proteins are rapidly degraded (2 minutes) in simulated mammal gastric fluids; thus, according to the assessment of the evidence, it is concluded that it is highly unlikely that the MIR604 corn event expresses allergenic substances.

6 – Toxicity

The bioinformatic studies conducted for the proteins (mCry3A and PMI), to determine amino acid sequence homology with known protein toxins, demonstrated that there are no significant biological similarities.

The 14 day acute toxicity studies conducted in mice supplying mCry3A and PMI proteins, demonstrated that there are no acute toxic effects and no adverse effects related to the treatment when administered to mice high doses (2377 mg/kg pc mCry3A and 5050 mg/kg pc PMI).

None of the introduced proteins show toxic characteristics, that is why; there are no reasons to conduct sub chronic or chronic toxicity tests. Based on the above, and on the results obtained from the feeding studies conducted on broiler chicken with event grains, it is concluded that the MIR604 corn event is unlikely to present toxicological risks for humans and animals.

7 – 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,

• The studies submitted to evaluate the potential allerginicity demonstrate that no known allergenic substances are expressed for the mCry3A. For the PMI protein, similarities to the α-parvalbumin were found; however, studies conducted using an immunoreactive serum, demonstrated that the protein has no allergenic properties.

It is concluded that the MIR604 corn event is substantially equivalent 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 MIR604 corn for human and animal consumption.

8 – 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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