

Decision Document

Assessment of event LY038 X MON810 (High Lysine Corn and Lepidoptera insects resistant) for human and animal consumption

Summary and Background

The food risk assessment process of transformation events, as the result of modern biotechnology, is carried out by the National Service for Agrifood Health and Quality (SENASA), regulatory agency depending on the Secretariat of Agriculture, Livestock, Fisheries and Food (SAGPyA).

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

On October 22nd, 2004, an application was received from Monsanto S.A.I.C., to carry out the assessment of human and animal food safety of the transformation event LY038 x MON810, high lysine corn with Lepidoptera insects resistance.

This application was reviewed in order to confirm the compliance with the criteria laid down by SENASA Resolution N° 412/02, regulation which establishes the human and animal food safety evaluation criteria and requirements in genetically modified organisms. According to this, it is requested to fulfill point 5 of Annex II, requesting the statement by which the entity commits to recover from the market, the product derived directly from the event in case SENASA demands it, based on reasonable reasons. The applicant submits the requested information. Also, the applicant is requested to update point 6 of said Annex, which refers to the approval backgrounds of same event in other countries.

The information submitted is evaluated first by the technical team of the Agrifood Quality Directorate, then subjected to the evaluation of the Technical Advisory Committee and finally concluded in the present document.

Evaluation

Corn LY038 x MON810 was evaluated according to the guidelines established in SENASA resolution N° 412/02, on the "Principles and Criteria for the Evaluation of Foods derived from Genetically Modified Organisms", the "Requirements and Procedures for the Evaluation of human and animal food safety of foods derived from Genetically Modified Organisms", and the "Required Information" for such evaluation. This was performed using information provided in the application together with additional information requested and consultation with experts to establish the human and animal

food safety.

1 – Use history and specification of the transformation event

Corn is the third most important cereal crop in the world after rice and wheat. It is commercially grown in several countries of the world. In Mexico, it has been produced for about 8000 years and in Europe for 500 years. The current production is mainly concentrated in hybrid corn varieties. The world corn production is around 600 million tons per year. The United States and China are the major corn producing countries with the 43,2 % and the 17,9 % of the world production respectively (OECD, 2002).

Corn has a history of safe human consumption and no cases of intoxication or allergy have ever been reported due to its reasonable consumption. Most common uses for corn are detailed below:

- As an ingredient for animal feed.
- Dry milling to obtain oil and flour.
- Wet milling to obtain oil, dextrin, syrup and dextrose.
- Fermentation and distillation to obtain alcohol, lactic acid, acetone, etc.

Monsanto has developed corn LY038 x MON810, which accumulates free lysine in the grain and which is resistant to the attack of certain Lepidoptera insects during the production period. Corn LY038 x MON810 was produced by the crossing of traditional LY038 and MON810 events, both obtained by the use of particle acceleration method.

The insertion of cordapA gene in corn allows to obtain a better grain to be used as an ingredient in animal feed, mainly poultry (broilers and turkeys) as well as swine, due to the fact that it reduces or eliminates dietary supplement with synthetic lysine. The level of free lysine in high lysine corn is around between 1000-2000 ppm, when the conventional corn grain has around 100 ppm. The content of lysine in the protein mold of conventional corn grain is between 2500 and 2800 ppm on dry basis. Therefore, it is estimated that the high lysine corn grain contains between 3500 and 4800 ppm of total lysine. The insertion of Cry1Ab gene expresses a protein that belongs to the entomotoxines Cry family, which affects some Lepidoptera insects. These proteins are present in phytosanitary products used in Argentina as insecticide since 1971, without negative effects.

It has to be noticed that parental events LY038 as well as MON810 have been evaluated by SENASA to be apt for human and animal consumption.

2 – Genetic stability of the event

The information duly received of the transformations events LY038 and MON810 shows the genetic stability in lines as in hybrids, in the conversion and improvement programs.

The company submitted a molecular study of the combined event LY038 x MON810 describing that line LY038 x MON810 has each of the events LY038

and MON810 separately, without other gene fragment or part and showing Mendelian segregation for each event.

3 – Expression products

The protein of new expression is cDHDPS, produced by the event LY038 and Cry1Ab, produced by the event MON810.

The DHDPS, which expresses mainly in the grain, acts in a critical and limiting step of the biosynthesis rate of lysine, which is in turn controlled by the product inhibition. The enzyme catalyses the condensation of L-aspartate –4-semialdehyde and pyruvate to form 2,3-dihydrodipicolinate that is later converted to Lysine through a series of subsequent enzymatic reactions. The variant of DHDPS enzyme from *C. Glutamicum*, on the contrary, (cDHDPS) is not sensitive to inhibition by high concentrations of free lysine. Due to this, the grain that expresses cDHDPS accumulates more free lysine compared to conventional grains.

The protein Cry1Ab expressed by cry1Ab gene, which was isolated from *Bacillus thuringiensis* subsp. *kurstaki*. This protein has insecticide activity over certain lepidoptera insects which have specific receptors for this Cry1Ab protein (delta-endotoxine) in mean intestine, and are therefore susceptible when consuming it.

The mean cDHDPS protein levels reported in grains, pollen, forage and fresh root were 41; 0,75; 0,19 and 0,12 µg/g dry weight (DW), respectively. For same tissues, the mean Cry1Ab protein levels were 0,43; 1,5; 23; 22 and 120 µg/g dry weight, respectively. These values are similar to those produced by parental events.

4- Nutritional characteristics

The applicant provided information on compositional analysis in grain and green tissue of hybrid corn plants containing event LY038 x MON810, its negative segregant control LY038(-) and 15 commercial hybrids, which were grown in four different trials in four different field sites with three replicates of each material in Argentina. All samples were analyzed to determine their nutritional components.

In addition, the applicant determined the amount of free lysine and six lysinerelated metabolites.

In all, 85 analytic components were reported (10 for green tissue and 75 for grain).

The results presented show that the amount of lysine and free lysine and its main associated catabolite, saccharopine, was statistically different in LY038 x MON810 compared to its control LY038(-) for all the data comparisons in all sites included combined sites, and fell outside the 99% interval of tolerance for commercial hybrids. It was reported that mean content of free lysine in LY038 ranged between 1323.4 and 1651.7 µg/g dry weight depending on the

site, while in the control LY038(-), it was from 17.4 to 44.5 µg/g dry weight.

Studies presented by Monsanto report that grain and forage corn LY038 x MON810 are compositionally equivalent to conventional corn except for the intentional increase levels of free lysine and total lysine in grain and increase levels of lysine associated catabolites: saccharopine and α -aminoadipic acid. These metabolites are normal components of the lysine metabolic pathway in plants and animals and can be measured in some common foods. Therefore, the levels of metabolites α -aminoadipic acid and saccharopine in LY038 x MON810 are not harmful for human and animal health. This conclusion was confirmed through a 90 day feeding study conducted in rats with LY038 corn grain and a 42 day feeding study conducted in broilers, with corn grain LY038 x MON810, carried out with big exposure margins compared to the most conservative exposition estimate in humans, where no unexpected effects were found.

In conclusion, the LY038 x MON810 grain and forage composition is considered equivalent to commercial corn except for the expected increase levels of lysine and free lysine in grain and increase levels of lysine catabolites: saccharopine and α -aminoadipic acid. Corn grain and forage of LY038 x MON810 high lysine are compositionally equivalent to those produced by negative segregant control LY038(-) and other hybrids corn commercially grown, except for the intentional changes included. The compositional study of grain and forage LY038xMON810 shows that there is no evidence of interaction between high lysine characteristic (LY038 event) and insect resistance (MON810) when combined in the same plant using conventional improvement methods.

5- Allergenicity

No protein allergens or substances have been identified neither in donor organisms nor in the receptive organism.

The applicant submitted a bio informatic analysis where the allergenic and toxicological potential or the pharmacologically active activity of the protein sequence cDHDPS from LY038 were evaluated. The comparison was conducted against AD4 allergens, TOXIN5, and ALLPEPTIDES databases using bioinformatic tools. The information demonstrates that cDHDPS is unlikely to share structurally or immunologically relevant similarities with known allergens. The process used to determine the percentage of amino acid identities between the expressed protein and the known allergens that can be found in protein databases is the following:

more than 35 % of identity in the amino acid sequence of the protein expressed, using an 80 amino acid window; or an identity of 6 contiguous amino acids. If such values are obtained, this would indicate a significant homology with some allergen.

Studies comparing the protein sequence Cry1Ab to known allergenic protein and gliadine were also submitted, which were grouped in an AD HOC database (AD3-1). The informatic program FASTA was used for the comparison and linedup of sequences. There were no relevant similarities found between

allergenic protein or gliadines and the Cry1Ab protein of MON810 event.

Additionally, no similarities were found between this protein Cry1Ab and proteins from the allergens and gliadines database. The lack of structural similarities and short amino acid windows suggests that it is highly unlikely that Cry1Ab has epitopes of immunologic importance.

Therefore, it is concluded that it is unlikely that corn LY038 and MON810 express allergenic substances for humans/and/or animals.

6- Toxicity

In the same study mentioned before, the applicant submitted a bioinformatic analysis where the toxicological potential or the pharmacological active activity of cDHDPS protein sequence in LY038 and Cry1Ab in MON810 are evaluated. The result of this analysis indicates that there are no structurally relevant biological similarities or sequence homology in cDHDPS and Cry1Ab (40% coincidence or more) when compared to allergens amino acid, toxins or pharmacologically active proteins.

The company also submitted an acute oral toxicity study in mice, in which the protein used was produced by E. Coli. This protein demonstrated to be identical to the protein produced in LY038 corn plant. The study was conducted using two groups of 10 mice CD-1 per sex, which received a single gavage dose of either 800 mg/kg body weight of test protein (cDHDPS) or control (bovine serum albumin - BSA) respectively. The study lasted 14 days after dosage in which animals were daily observed and weekly weighed.

Under the conditions of this study, neither mortalities nor adverse reactions were reported.

Monsanto informed that food risk assessment in cDHDPS was carried out comparing daily probable consumption in humans and animals against NOEL (no-observed-effect level) derived from the acute toxicology study in mice. This comparison is considered enough for the protein safety assessment since, except some antinutrients, most known toxins show an acute toxicity mechanism.

On the other hand, the applicant submitted information about a 90 day feeding study conducted in rats in which animals received corn grain event LY038 and corn grain LY038 and control corn grain LY038(-). The parameters evaluated were the following: clinical observations and survival, body weight, food consumption, hematology (blood serum and urinalysis) and anatomic pathology (macroscopic tests, organ weight data, microscopic tests).

All animals survived for necropsy. No clinical effects related to the evaluation material were observed. No effects were observed on body weight, food consumption, clinical pathology parameters (hematology, blood serum and urinalysis) or organ weight. No abnormalities were observed in micro or macroscopic tests in tissues related to LY038 material in evaluation.

The warming effect in Maillard reaction was also evaluated, reaching the conclusion that due to low concentration of lysine, the additional formation of glycation endproducts such as AGEs (Advanced glycation end products) and free radicals is not relevant compared to the lysine content in traditional corn. It was also submitted an acute oral toxicity study of Cry1Ab protein, conducted in mice. Protein Cry1Ab was orally supplied to a group of ten mice, male and female. Additionally, a group of ten mice were also supplied with the same mean without the Cry1Ab protein. The doses of Cry1Ab supplied to mice were 0,400, 1000 and 4000 mg/kg. The group of control mice received bovine serum albumin (BSA) with the 4000 mg/kg dose. At the moment of sacrifice, 7 days later, no statistic differences were found in mortality, weight, weight gain or food consumption between the BSA control group and the Cry1Ab protein groups.

As a result, it is concluded that corn event LY038 x MON810 does not pose toxic risks to be consumed by humans and animals.

7- Conclusion

After carrying out a comprehensive food risk assessment on the material submitted by Monsanto, and having into account that:

- LY038 x MON810 corn studies describe that it has each event LY038 and MON810 separately, and that it has no other gene fragment or part,
- LY038 x MON810 grain and forage composition is considered equivalent to commercial corn except for the intentional increase levels of free lysine and lysine in grain and increase levels of lysine associated catabolites: saccharopine and α -aminoadipic acid,
- No evidence of metabolic interaction between the event LY038 and MON810 was found,
- Bioinformatic analyses of cDHDPS and Cry1Ab protein sequences in LY038 x MON810, showed no structural similarities biologically relevant or sequence homology in them compared to allergens, toxins or pharmacologically active proteins.

It is concluded that corn event LY038 x MON810 is similar to its conventional counterpart except by the free lysine increase and two of its associated catabolites.

8- Rules and recommendations:

- SENASA Resolution N° 1265/99
- SENASA Resolution N° 412/02
- Principios para el análisis de riesgos de alimentos obtenidos por medios biotecnológicos modernos (CAC/GL 44-2003)

- Directrices para la realización de la evaluación de la inocuidad de los alimentos obtenidos de plantas de ADN Recombinante (CAC/GL 45-2003)

- Nutritional and Safety Assessments of Foods and Feeds Nutritionally Improved through Biotechnology (ILSI International Food Biotechnology Committee)

- Consensus Documents for the work on the Safety of Novel Foods and Feeds (OECD)

9- Note

Nevertheless, it is important to inform different stakeholders in the production, commercialization and consumption chain as well as public institutions, food authorization and inspection services that the benefit of free lysine increase has been only demonstrated for chicken broilers provided corn does not undergo any cooking.

According to what has been previously expressed and taking into consideration the current scientific knowledge and international accepted requirements, there are no obstacles for the approval of corn LY038 x MON810 for human and animal consumption. In case corn is intended for human consumption, it is recommended not to state any nutritional property which differentiates it from conventional corn not genetically modified.

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