Ministerio de Economía y Producción
Secretaría de Agricultura, Ganadería, Pesca y Alimentos
Servicio Nacional de Sanidad y Calidad Agroalimentaria

Decision Document

Assessment of corn event LY038 (High Lysine Corn) 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 an specific scientific team and the advise 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.
In October 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, high lysine corn.
This application was reviewed in order to confirm the compliance with the criteria laid down by SENASA Resolution Nº 412/02, regulation which establishes the human and animal food safety evaluation criteria and requirements in genetically modified organisms. According to this, more information is requested regarding point 4 of Annex II of the above mentioned Resolution, with reference to the presentation of a monitoring system project of the event genetic stability and its expression in order to verify the functional and structural identity of the event exactly as it was approved, during all the period of its commercialization and point 5 of such annex 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.
Later, additional information related to levels of lysine in proteins and free lysine is requested, as well as any research on pigs feeding. Likewise, the applicant presents more information in a feeding study on 90-day-rats.

The information is evaluated 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 was evaluated according to the guidelines established in SENASA resolution Nº 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 – 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 High Lysine corn LY038 which accumulates free lysine in the grain. Corn event LY038 was produced by the particle acceleration method, resulting in the introduction of the bacterial gene *cordapA* from *Corynebacterium glutamicum* which encodes the dihydrodipicolinate synthase (cDHDPS) enzyme. This enzyme expresses predominantly in the germ resulting in the accumulation of lysine in the grain.

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.

2 – Genetic stability of the event

Heritability and stability of the *cordapA* gene cassette in LY038 were evaluated through the segregation rates in 3 generations: the F1´generation, prior to excision of the npt *II* marker gene through cre-lox system; in F3 after the excision of marker gene and F4 generation with two rounds of backcrossing to conventional inbred lines.

The heritability studies performed showed no significant differences in the observed-to-expected segregation ratios for *cordapA* gene cassette. This observation of Mendelian segregation for the transgene is also consistent with the molecular analysis which suggested that the *cordapA* gene was stably integrated at a single site in the genome.

3 – Expression levels

The protein of new expression is cDHDPS. The DHDPS, the first enzyme in lysine pathway mediates a critical rate-limiting step in the lysine biosynthetic pathway which is in turn controlled by the inhibition of the product. 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.

Protein cDHDPS is mainly expressed in grains produced by plants.

The mean cDHDPS protein levels in grains, pollen, forage and root were 41; 0,75; 0,19 and 0,12 µg/g dry weight (DW), respectively.

4- Nutritional characteristics

The applicant provided information on compositional analysis in grain and green tissue of hybrid corn plants containing event LY038, negative segregant LY038(-) as control material 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 lysine-related 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 compared to its control LY038(-) for all the data comparisons in all sites included combined sites, and

fell outside the 99% interval tolerance for commercial hybrids. The amount of other amino acids related to the aspartate (Threonine, Isoleucine, Aspartate, and Methionine) metabolic pathway were not statistically different in LY038 compared to LY038 (-) for in none of the comparison groups. It was reported that mean content of free lysine in LY038 ranged between 1254.9 and 1614.1 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 are compositionally equivalent to conventional corn except for the intentional increased levels of free lysine and total lysine in grain and increased levels of lysine 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 are not harmful for human and animal health. This conclusion was confirmed through a 90 day feeding study conducted in rats and a 42 day feeding study conducted in broilers, with corn grain LY038, carried out with big exposure margins (>9000) compared to the most conservative exposition estimate in humans, where no unexpected effects were found.

In conclusion, the LY038 grain and forage composition is considered equivalent to commercial corn except for the intentional increased levels of free lysine and total lysine in grain and increased levels of lysine catabolites: saccharopine and α-aminoadipic acid. The composition of grain and forage from high lysine LY038 is equivalent to the ones produced by negative segregant control LY038(-) and other commercially grown hybrid corns, except for the intentional modifications included.

5- Allergenicity

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

The applicant presented 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 similarity search was conducted against AD4, TOXIN5, and ALLPEPTIDES databases using bioinformatic tools. The information demonstrates that cDHDPS is unlikely to share structurally or immunologically relevant similarities with known protein 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, over a 80 amino acid window; or an identity of 6 contiguous amino acids. In case of obtaining such values, this would indicate a significant homology with some allergen.

The company presented *in vitro* and *in vivo* studies with purified and characterized protein, where it has been demonstrated that (i) no toxicity was observed in cDHDPS protein, after an acute toxicity study in the protein administration by oral gavage, (ii) rapid degradation in simulated gastric fluids, (iii) absence of significant homology of cDHDPS with proteins known as toxic or allergenic, and (iv) characteristics of cDHDPS protein do not coincide with the profile of known allergens.

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

6- Toxicology

The assessment on the potential impact of enzyme cDHDPS on human health is based on the complete protein characterization and its similitude with other DHDPS enzymes commonly present in a wide range of food sources with safe history use.

In the same study mentioned before, the applicant presented a bioinformatic analysis where the toxic potential or the pharmacological active activity of cDHDPS protein sequence in LY038 is evaluated. The result of this analysis indicates no relevant biological similarities or sequence homology in cDHDPS (40% coincidence or more) compared to allergens, 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 equivalent to the protein produced in LY038. the study was conducted using two groups of 10 mice CD-1 per sex, which received a single gavage dose of either 1000 mg/kg body weight cDHDPS or BSA (bovine serum albumin) respectively. The study lasted 14 days 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 enough for the protein safety assessment due to the fact except some antinutrients, most known toxins inhibit an acute toxicity mechanism.

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

endproducts AGEs and free radicals is not relevant compared to the lysine content in traditional corn.

As a result it is concluded that corn event LY038 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:

- The heritability studies performed showed no significant differences in the observed-to-expected segregation ratios for *cordapA* gene cassette,

- LY038 grain and forage composition is considered equivalent to commercial corn except for the intentional increased levels of free lysine and total lysine in grain and increased levels of lysine catabolites: saccharopine and α-aminoadipic acid

- Bioinformatic analyses of cDHDPS protein sequence in LY038 showed no relevant biological similarities or sequence homology in cDHDPS compared to allergens, toxins or pharmacologically active proteins.

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

8- Rules and recommendations:

- SENASA Resolution Nº 1265/99
- SENASA Resolution Nº 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, 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 exposed, and taking into consideration the current scientific knowledge and international accepted requirements, there are no obstacles for the approval of corn LY038 for human and animal consumption. In the case of corn intended for human consumption it is recommended not to state any nutritional property which differentiates it from conventional corn not genetically modified.