DECISION DOCUMENT

Evaluation of Food and Feed Safety of Soybean Event
FG72 x A5547-127 (OECD: MST-FGØ72-2 x ACS-GMØØ6-4)

Dirección de Calidad Agroalimentaria

Coordinación de Biotecnología y Productos Industrializados

Evaluation of Food and Feed Safety of Soybean Event FG72 x A5547-127

SUMMARY AND BACKGROUND

The process of Food and Feed Risk Assessment of biotechnology-derived transformation events is done by the National Service of Agrifood Health and Quality (SENASA), regulatory authority under the Agroindustry Ministry.
The Directorate of Agrifood Quality of SENASA is the area responsible for carrying out this function, counting with a scientific team and advice from a Technical Advisory Committee consisting of experts from various disciplines representing different sectors linked to production, processing, consumption, research and development of genetically modified organisms.
On July 16th, 2015 an application from Bayer CropScience was received, to carry out the evaluation of food and feed safety of the transformation event FG72 x A5547-127 (OECD: MST-FGØ72-2 x ACS-GMØØ6-4) "glyphosate, isoxaflutole and glufosinate ammonium tolerant soybean."
The review of the application for the purpose of verifying compliance with Resolution No. 412/02 SENASA (rules establishing the criteria and requirements for assessing food and feed safety genetically modified organisms), is carried out.
The submitted information was analyzed in the first instance by the specific technical team, and then was subjected to evaluation by the Technical Advisory Committee. Finally the Directorate of Agrifood Quality assessed again, in a third instance, and concludes in this document.

Therefore, the Directorate of Agrifood Quality (DICA) as a result of the food and feed evaluation process by the Coordination of Biotechnology and Industrial Products and the advice from the Technical Committee on the use of Genetically Modified Organisms (GMOs) of SENASA (Act of 17/12/2015) concluded that products derived from materials containing the transformation event A5547-127 x FG72 are suitable for human or animal consumption, are of no added or increased risk as a result of transgenesis beyond those inherent to the food and feed in question, and meet the criteria and requirements set out in SENASA Resolution N° 412/2002 and Codex Alimentarius FAO/WHO.

EVALUATION

FG72 x A5547-127 soybean tolerant to herbicides glyphosate, glufosinate ammonium and isoxaflutole was evaluated following the guidelines set out in SENASA Resolution N° 412/02 on the "Principles and Criteria for the Evaluation of Food and Feed Derived from Genetically Modified Organisms", the "Requirements and Standards of Practice for the evaluation of Food and Feed Safety of Food and Feed derived from Genetically Modified Organisms" and the "Required Information" for such evaluation. The

Resolution provides the criteria set by Codex Alimentarius FAO/WHO. The evaluation was performed using the information provided in the application, along with additional information requested and consultations with experts, to determine food and feed suitability for human and animal consumption.

1 - History of consumption and transformation event specifications

The initial use of the soybeans grown in America in the early 1800s was for the production of soy sauce. By the end of 1800 soybean was cultivated primarily as a forage crop. In 1904, a study conducted by George Washington Carver at the Tuskeegee Institute demonstrated the nature of soy as a source of protein and oil. The eight essential amino acids necessary for human nutrition that are not produced by the body, are found in soy proteins.
The change in the soybean crop for feed grain use rather than forage was initially caused by the use of the oil in the manufacture of soaps. By mid-1930 soybean meal had become an acceptable protein concentrate in food for poultry and livestock. Flavor stabilization in soybean oil gave another impetus for use in human food. The destruction of a large amount of cotton productions by weevils was also a reason for finding a second source of vegetable oil. Between 1982 and 1983, 76% of edible oil products were derived from soybeans, compared to only 11% for the combined oils derived from cotton, corn and peanuts (Mounts et al., 1987). Soybean meal is a key source of protein concentrate for animal feeding. The production of edible protein products is small when compared with the use of soybean meal as a concentrate for food.
The event FG72 x A5547-127 confers soybean tolerance to glyphosate, isoxaflutole and glufosinate ammonium herbicides. This stacked event was obtained by conventional breeding of parental FG72 and A5547-127 events.
The genetic modification in FG72 soybean consists in the integration into *Glycine max* genome of an insert containing the expression *cassettes* of *hppdw336* and *2mepsps* genes as the only modification. The 2mEPSPS protein has two point mutations from its natural variant of maize EPSPS which gives a lower sensitivity to inhibition caused by glyphosate, without modifying their enzymatic activity, while HPPDW336 protein has a substitution (glycine for tryptophan) that provides tolerance to inhibition by isoxaflutole.
The genetic modification in the parental event A5547-127 consists in the integration into *Glycine max* genome of an insert containing the expression *cassette* of *pat* gene, resulting in the expression of the PAT protein as the only modification. The PAT protein is an acetyltransferase that specifically catalyzes the acetylation of phosphinothricin and dimetilphosphinotricin. It metabolizes glufosinate to an acetylated inactive derivative, thus conferring herbicide tolerance to glufosinate ammonium.

2- Event Characterization

The features introduced in soybean FG72 x A5547-127 are tolerance to glyphosate, isoxaflutole and glufosinate ammonium herbicides. Its function is to provide soybean plants the ability to survive in the presence of such herbicides, which confers an advantage over sensitive weeds that compete with them.

3 - Introduction Method

The genetic modification present in FG72 soybean consists in the integration into *Glycine max* genome of an insert containing the expression *cassettes* of *hppdpfw336* and *2mepsps* genes. This modification was generated by direct transfer of a fragment from pSF10 vector.
The transformation method used for the generation of soybean event A5547-127 was biobalistics. DNA preparation and transfer (particle bombardment) was performed following Christou *et al*. methods. No DNA carrier was used in the process.

4 - Genetic Stability of the Event

A series of Southern blot hybridizations were evaluated for stability and integrity of the DNA inserts in parental strains of stack event FG72 x A5547-127, showing the structural stability of both parental lines FG72 and A5547-127 in soybean FG72 x A5547-127.
Stability and single dominant Mendelian heritability of FG72 event was compared up to T9 generation and A5547-127 up to T5 generation.

5 - Characteristics and biological activity

2mEPSPS
The EPSPS protein is a key enzyme in the shikimate metabolic pathway. The 2mEPSPS protein contains two amino acid substitutions compared to the native corn protein (EPSPS). These modifications result in lower insensitivity to the presence of glyphosate without losing its original activity. Given the high homology between both (2mEPSPS and EPSPS), 2mEPSPS protein is considered to have the same biosafety profile as the native corn protein.

PAT
It is an acetyl transferase that specifically catalyzes the acetylation of phosphinothricin and dimetilphosphinotricin. It metabolizes glufosinate into an inactive acetylated derivative, thus conferring tolerance to herbicides based on glufosinate ammonium.

HPPDW336
The donor organism of the gene that confers tolerance to HPPD inhibitor herbicides (such as isoxaflutole) is a bacterium commonly found on surfaces of plants, vegetal material, soil and water. It can be isolated from soil, water, plants, animals and hospitable environment. It is nonpathogenic, non-allergenic and non-toxic to human and animal health. The metabolic pathway in which the enzyme HPPD is involved in plants is related to several anabolic processes. Their reaction product is homogentisate which is the aromatic precursor of vitamin E, a membrane-associated antioxidant, and plastoquinone.
HPPD enzyme is inhibited by the presence of isoxaflutole. In order to avoid this effect one amino acid substitution (glycine for tryptophan) was introduced at position 336, conferring isoxaflutole insensitivity to the enzyme and retaining its original activity.

6 - Expression Levels

Analysis was performed by ELISA to quantify the expression of 2mEPSPS, HPPDW336 and PAT protein in leaves, roots, seed and forage. The samples were obtained from three trials with four replications each, carried out during 2012 in United States locations. Assays were performed with and without application of isoxaflutole.
HPPDW336, 2mEPSPS and PAT proteins were detected in all the tissues samples analyzed and in all stages of development.

7 - Compositional Analysis

To determine the centesimal composition of FG72 x A5547-127 soybean, field trial studies conducted in the 2012 campaign in areas of common US crop productions, were evaluated.
Each field trial consisted of 6 entries replicated 4 times (24 plots in total) in a randomized complete block design (DBCA). All plots were located in the same field and subjected to the same growth conditions.
The experimental design included treated and untreated plots with herbicides.
Composition analysis to determine the levels of key nutrients and antinutrients in grain and forage were performed, including: proximal composition, fiber components, micronutrients, minerals, vitamins, isoflavones, antinutrients: raffinose, stachyose, phytic acid, trypsin inhibitor and lectins, total amino acids and total fatty acids.
The data analysis was performed by comparing the observed values for soybean FG72 x A5547-127 with the conventional counterpart, commercial varieties and ranges obtained from literature (OECD 2001, ILSI 2007).
Statistical analysis of the results shows that soybean FG72 x A5547-127 is substantially equivalent to its non-GM counterpart and that its components are within the range of values reported in the literature and commercial varieties for each case.
Where the values were not within the mentioned ranges, these were not consistent across locations and therefore, the differences were not considered biologically relevant.

8 - Allergenicity

The sequences of the 2mEPSPS, HPPDW336 and PAT proteins were compared using the BLASTP algorithm with all protein sequences present in the following databases: Uniprot_Swisprot, Uniprot_TrEMBL, PDB, DAD and Gen Pept.
The bioinformatics studies show that these proteins have no similarity to known allergens.
Moreover the 2mEPSPS proteins (47kDa), HPPDW336 (40 kDa) and PAT (21 kDa) do not have molecular weights that can be associated with either group of high or low molecular weight.
The three proteins 2mEPSPS, HPPDW336 and PAT are labile at the temperatures used for cooking and are not glycosylated; therefore, they do not have the typical characteristics of glycosylated allergens. The rapid degradation of 2mEPSPS, HPPDW336 and PAT proteins either in simulated gastric or intestinal fluids indicate that the probability of being absorbed through the gastrointestinal system while retaining its structure and/or activity, in extremely low. For the reasons mentioned, these proteins are not considered a risk to human and animal health.

9 - Toxicity

The study of the history of the 2mEPSPS, HPPDW336 and PAT proteins and bioinformatic analysis of their sequences, show no evidence of having potential toxic effects. To evaluate the possibility that 2mEPSPS, HPPDW336 and PAT proteins could have toxic effects, acute toxicity studies were conducted on animals. In these studies *E. coli* produced proteins purified to 99% were used.
The experimental design contemplated oral inoculation of 5 OF1 female mice with a dose of 2000 mg of protein per Kg of body weight (bw) for 2mEPSPS and HPPDW336. All animals survived and no clinical symptoms were observed, concluding that it is very unlikely that HPPDW336 or 2mEPSPS proteins are toxic even under conditions of maximum oral exposure to high doses as 2000 mg/kg bw.
Acute toxicity tests were also conducted in animals using intravenous inoculation. Five OF1 females were used in each group which were inoculated with a dose of 10mg/kg bw of 2mEPSPS and HPPDW336 proteins.
The results show that none of the proteins have a toxic effect on animals under the conditions tested.
To investigate whether PAT has the potential to generate an acute toxic response, an animal assay was performed in which these were injected with PAT protein intravenously. Groups of 5 animals were injected at a dose of 10 mg/kg of bw, concluding that it is very unlikely that PAT holds a toxic effect on humans or mammals even in conditions of maximum exposure as is an intravenous dose of 10 mg/kg bw.

10 - Nutritional Assessment

For parental FG72 soybean event and its derived products, broiler feeding studies were evaluated. Animals were studied in regard to their physiological behavior, food acceptance, weight and size gain and feed conversion and the studied were concluded with a necropsy of all animals. No evidence of pathogenic effects on the animals was observed.
Moreover, the potential toxic effects of the parental soybean event A5547-127 was analyzed through feeding Wistar rats with transgenic soybean and its conventional counterpart. No significant differences were found in the evaluated parameters.

11- Metabolic interactions

The FG72 and A5547-127 events were assessed individually and it was concluded that there are no statistically significant differences with their respective isolines.
Since proteins present in the event FG72 (2mEPSPS and HPPDW336) showed no evidence of interaction in the single event, it not expected to present it in the stack.
Moreover, it is not expected that there is interaction with the PAT protein present in A5547-127 event since there are no points in common in their metabolic pathways or modes of action.
It was determined that there are no statistically significant differences in expression patterns of the proteins between the stack and the parental events. Accordingly, there were no statistically significant differences in the composition of the stack FG72 x A5547-127 and the parental events.

12 - Conclusions

After completing a full evaluation of the information provided by the company Bayer CropScience, with respect to dietary risk of FG72 x A5547-127 soybean, and taking into account that:

- The soybean event FG72 x A5547-127 was obtained by conventional breeding of parental event already approved.
- Molecular and phenotypic studies showed its molecular integrity and its genetic and phenotypic stability as well as a simple dominance Mendelian inheritance pattern.
- The expressed proteins were evaluated (in the submission of the parental events) regarding toxicity and allergenicity and approved without identifying potential risks.
- The expression pattern of 2mEPSPS, HPPDW336 and PAT proteins is equivalent in the parental and in the stack event.
- The compositional analysis shows that it is substantially and nutritionally equivalent to its non-genetically modified counterpart and commercial varieties used as control.
- There are no technical or experimental evidence to indicate that the expressed proteins may have a metabolic interaction resulting in unexpected effects, with no changes in the pattern or expression levels as well as its phenotypic aspects.

It is concluded that soybean event FG72 x A5547-127 is substantially equivalent to its conventional counterpart, therefore, is as safe as and no less nutritional than soybean conventional commercial varieties.
According to the above described, and according to the scientific knowledge currently available and the requirements and criteria internationally accepted, there are no objections to the approval for human and animal consumption of soybean event FG72 x A5547-127.

13 - Regulations and Recommendations:

- SENASA Resolution N° 1265/99
- SENASA Resolution N° 412/02
- Principles for risk analysis of food derived from modern biotechnology (CAC / GL 44-2003).
- Guidelines for conducting the safety assessment 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).
- MAGyP Resolution N° 701/2011.
- ILSI Database 2007.
- Allergen Database (FARRP database)

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