

Decision document

Evaluation of Event A5547-127 (Soybean tolerant to Glufosinate Ammonium ACS-GM006-4) for human and animal consumption

SUMMARY AND BACKGROUND

The process of food risk assessment of transformation events due to modern biotechnology is carried out by the National Service of Agrifood Health and Quality (SENASA), which is the 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 activity. This office 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 production, industrialization, consumption, research and development of genetically modified organisms.

An application from Agrevo Company was received in July, 2001, to carry out the human and animal food safety evaluation of the transformation event A5547-127, resistant soybean to glufosinate ammonium herbicide.

The application was reviewed in order to confirm the compliance with all the criteria proposed in SENASA Resolution N° 412/02, regulation that lays down the criteria and requirements for the human and animal food safety evaluation in genetically modified organisms.

Pursuant to this, more information is requested regarding item 5 of annex II, requesting a statement by which the company commits itself to recover from the market the product directly derived from the event, considered as appropriate by SENASA based on reasonable reasons. At the same time it is requested to submit human allergenicity studies and feeding in broiler chickens. The applicant submitted the requested information.

In February, 2004, the applicant sent a note requesting to suspend the evaluation of the referred event due to commercial reasons. However, in July 2006, the company asked to resume the evaluation since it decided to continue with the commercial development of the event.

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

EVALUATION

Soybean A5547-127 was evaluated following the guidelines shown in SENASA Resolution N° 412/02, on the "Basis and Criteria for the Evaluation of Food Products derived from Genetically Modified Organisms", the "Requirements and Standards Procedure for the Evaluation of Human and Animal Safety of Food Products derived from Genetically Modified Organisms", and the "Requested Information" for such evaluation. Such evaluation was carried out using the information submitted in the application, together with the additional information requested and consultations with experts to establish the safety for human and animal consumption.

1 – History of use and specification of the transformation event

The initial use of cultivated soybean in America in early 1800 was for the production of soybean sauce. Towards late 1800 soybean was grown mainly as a forage crop. In 1904 it was demonstrated the nature of soybean as a source of protein and oil. Soybean protein has the eight essential amino acids necessary for the human nutrition which are not produced by the body.

Towards mid 1930, soybean meal became an acceptable protein concentrate in poultry and cattle feed. The stabilization in the flavor of soybean oil gave way to its use in human foods. In 1982-1983, 76% of edible oil products were derived from soybean, compared to only 11% for combined oils derived from cotton, maize and peanut.

The uses of soybean can be classified in oil products, whole soybean products and soybean protein products. Glycerol, fatty acids and sterols derive from soybean oil. Refined soybean oil has several edible, technical and industrial uses. The same thing happens with lecithin, also derived from soybean oil. Whole soybean is used to produce products such as sprouts, baked soybean, toasted soybean, soybean meal and traditional soybean food products (soybean milk, soybean sauce and tofu). The protein soybean has a wide range of technical uses, food uses and feed uses (American Soybean Association, 1994).

Bayer Crop Science has developed varieties of soybeans tolerant to the herbicide whose active principle is glufosinate ammonium. The commercial name of the seed is Soy LibertyLink®. The varieties of

LL soy are based on well characterized transgenic lines, known as transformation event A5547-127, designated by the OECD code of unique identification ACS-GMØØ6-4.

Resistance to Glufosinate Ammonium is conferred by the expression of the gene phosphinothricin acetyl transferase (*pat*), derived from *S. viridochromogenes*. Glufosinate Ammonium (GA) is within the fosfotricine herbicides. It is a non systemic, non selective herbicide which provides an effective post emergent control of many broadleaf weeds. GA controls weeds through the inhibition of glutamine-synthetase (GS), which produces the accumulation of phytotoxic levels of ammonia in the plant. Glutamine synthetase is responsible for the synthesis of amino acid glutamine as from glutamic and ammonia acid. It is the only enzyme in the plant that can detoxificate the ammonia released by photo breathing, the reduction of nitrate and the degradation of amino acids. Ammonia, still a plant nutrient and a metabolite, is toxic in excess and produces the death of the plant cell.

Although glutamine synthetase of *S. viridochromogenes* is sensitive to an L- phosphinothricin, the bacteria produce an inactivating enzyme, PAT. PAT catalyzes the conversion of L- phosphinothricin to N-acetyl-L- phosphinothricin in the presence of acetyl CoA as a co-substrate. N-acetyl-L- phosphinothricin does not inactivate GS, and therefore it does not have an herbicide activity. As a consequence, plants that express the PAT enzyme, as a result of the insertion of a *pat* gene derived from *S. viridochromogenes*, are resistant to phosphinothricin herbicides.

2 – Genetic stability of the event

The company submitted information on genetic stability of event A5547-127, where it is stated that such event was field tested to evaluate the proportion of segregation of this event, the presence of medelian segregation was demonstrated.

3 – Expressed material

The PAT protein is the new expressed material. ELISA analyses show that the PAT protein is present in low levels in forage, hay and seeds of transformed plants. The highest level of PAT protein in grain was 7147 ng PAT per gram of forage, and 4809 ng per gram of hay, but most of the samples ranged between 1000-2000 ng/g, the level of protein in seeds varied from 593 to 11557 ng/g dry weight, depending on the transformed genotype analyzed and the cultivation place.

Therefore, the levels of PAT protein indicate that its presence is low in the analyzed grains and forage.

4- Nutritional characteristics

The applicant submitted information about comparative analyses of soybean derived from event A5547-127, using nontransformed parental line A5547 as a marker. Line A5547 belongs to cultivars with degree of maturity V. Apart from A5547, comparisons were carried out with non transgenic soybean, from which there is information available in the bibliography.

Along the history of field tests with A5547-127, no differences were observed that could be attributed to pleiotropic effects caused by the insertion of *pat* gene. Soybean derived from event A5547-127 does not differ significantly from the parental line or from other conventional soybean in terms of nutritional, agronomic terms or reproductive characters.

It can be concluded that soybean LL tolerant to glufosinate ammonium, event A5547-127, is substantially and nutritionally equivalent to its non transgenic counterpart, the parental line of soybean A5547, and varieties of conventional soybeans.

5- Allergenicity

The company carried out a bioinformatics search for amino acid sequence homology of the PAT protein in the databases of known allergens. The total amino acid sequence was compared to the sequences of all known toxins and allergens cited in 7 public databases (SwissProt, trEMBL, GeneSeq-Prot, PIR, PDB, DAD and GenPept).

Based on these results, no similarity or homology of epitope sequences was found in PAT protein, subdivided in blocks of 8 amino acids known as belonging to toxic or allergenic proteins, taking a coincidence criterion of "100% similarity in a segment of 8 contiguous amino acids".

Therefore it is concluded that based on the available scientific knowledge on allergens, this event does not pose any additional risk if compared to conventional soybean, due to the fact that PAT protein does not present homology with known food allergens, it is heat and acid labile and it is rapidly degraded by human and animal gastric fluids.

6- Toxicity

The applicant submitted evidence, on the amino acid sequence of the phosphinothricin acetyl transferase protein coded for synthetic *pat* gene, totally analyzed and the protein is identical to the native protein of *Streptomyces viridochromogenes*.

PAT protein (derived from *pat* gene), has no characteristics applicable to allergens, since it is heat and protease labile, it does not contain glycosylation places and it is not modified post-transcriptionally in bacteria as well as soybean.

The global homology search for PAT protein did not show any similarity with known toxins or allergens. PAT protein only has a high similarity with other non toxic and non allergenic acetyl transferase proteins.

Digestibility studies of PAT protein were presented in human simulated gastric fluids and pig gastric juices, where a rapid degradation (seconds) of the PAT protein is confirmed.

The studies presented on acute oral toxicity of repeated doses in rats indicate that toxic effects associated to PAT protein in humans and mammals are highly unlikely.

Therefore, it is concluded that there is no scientific evidence that shows PAT protein as toxic for humans and animals.

7- Conclusion

After performing a complete food risk assessment to the material submitted by Bayer company, and having into consideration that:

- The inheritance studies showed mendelian segregation,
- PAT protein is found in low levels in grain,
- It is substantially and nutritionally equivalent to its non transgenic counterpart,
- No evidence of similarity or homology with toxic or allergenic proteins was found,
- There is scientific evidence that PAT protein is not toxic for humans and animals,

It is concluded that soybean event A5547-127 is similar to its conventional counterpart; therefore, it is as safe and wholesome as conventional varieties of soybean.

According to what has been previously exposed and having into account the current scientific knowledge available as well as the requirements and criteria internationally accepted, no objections can be found to approve Soybean A5547-127 for human and animal consumption.

8- Regulations and recommendations:

- SENASA Resolution N° 1265/99
- SENASA Resolution N° 412/02
- Principles for the risk analysis of food obtained by modern biotechnological means (CAC/GL 44-2003)
- Guidelines for the safety evaluation of food derived from plants of recombinant DNA (CAC/GL 45-2003)
- Consensus Documents for the work on the Safety of Novel Foods and Feeds (OECD)

BUENOS AIRES, April 30, 2008