

## **DECISION DOCUMENT**

**Food and Feed Safety Assessment of Soybean Event  
MON 87701 x MON 89788  
(OECD: MON-877Ø1-2 x MON- 89788-1)**



**Directorate of Agrifood Quality**

**Office of Biotechnology and Industrialized Agrifood Products**

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|--|---|
| SUMMARY AND BACKGROUND.....  | 3 |
| EVALUATION .....   | 3 |
| 1 – History of use and specification of the transformation event.....  | 3 |
| 2 – Genetic stability and molecular characterization of the event..... | 4 |
| 3 – Products, pattern and levels of expression .....                   | 4 |
| 4 – Compositional analysis.....  | 5 |
| 5 – Allergenicity .....  | 6 |
| 6 – Toxicity.....  | 6 |
| 7 – Metabolic interactions .....                                       | 6 |
| 8 – Conclusion.....  | 7 |
| 9 – Regulation and recommendations .....                               | 7 |

## SUMMARY AND BACKGROUND

The process of food and feed risk assessment of a transformation event, product of modern biotechnology, is done by the National Service for Agrifood Health and Quality (SENASA), regulatory agency depending on the Ministry of Agriculture, Livestock and Fisheries.

The Directorate of Agrifood Quality of SENASA is the area responsible for carrying out this function, counting for it with a Scientific Team and the advice of a Technical Advisory Committee composed of experts in several scientific disciplines that represent the different sectors linked to production, industrialization, consumption, research and development of genetically modified organisms.

On December 14<sup>th</sup> 2010, the request by Monsanto Argentina S.A.I.C. was received for the assessment of food and feed safety of the transformation event MON 87701 x MON 89788 (OECD: MON-877Ø1-2 x MON- 89788-1), soybean resistant to certain lepidopteran insects and tolerant to glyphosate.

A review of the application was done in order to corroborate the compliance with Resolution SENASA N° 412/02, legislation that establishes the criteria and requirements of food and feed risk assessment of genetically modified organisms.

The submitted information was analyzed in the first instance by the specific Technical Team, and then subject to the evaluation of the Technical Advisory Committee. Finally, in a third instance, the Directorate of Agrifood Quality evaluated it again and concluded in the current decision document.

## EVALUATION

Soybean MON 87701 x MON 89788, resistant to certain lepidopteran insects and tolerant to glyphosate, was evaluated following the guidelines from Resolution SENASA N° 412/02 on the “Fundamentals and Criteria for Evaluation of Food Derived from Genetically Modified Organisms”, the “Requirements and Standards of Procedures for the Food and Feed Risk Assessment of Food Derived from Genetically Modified Organisms”, and the “Required Information” for this assessment. The aforementioned resolution considers the criteria provided by *Codex Alimentarius* FAO/OMS. The evaluation was carried out using the information in the submission, together with the additional information requested and consultation with experts, to establish the safety for human and animal consumption.

### 1 – History of use and specification of the transformation event

Soybeans are the main dietary source of protein for human and animals and the second leader source of vegetable oil produced worldwide after palm oil. Soybean oil represents 71% of edible fats and oils consumed around the globe. Soybeans were domesticated in Asia more than 3,000 years ago. They are commercially cultivated in many countries, have an extensive record of safety consumption and no cases of intoxication or allergies linked to their reasonable consumption have been reported.

Soybeans MON 87701 x MON 89788 have been obtained by conventional crossing of carrier lines of simple events MON 87701 and MON 89788 to express protein Cry1Ac (from *Bacillus thuringiensis*) that confers resistance to certain lepidopteran insects, and protein CP4 EPSPS (from *Agrobacterium spp.*) that confers tolerance to glyphosate.

## 2 – Genetic stability and molecular characterization of the event

Simple events MON 87701 and MON 89788 were obtained through the transformation of meristematic tissues mediated by *Agrobacterium tumefaciens* and contain the genes *cry1Ac* and *cp4 epsps* which express the proteins Cry1Ac and CP4 EPSPS, respectively.

In both events it was confirmed that the insertion was done in a unique locus and that it contains a unique copy of the expression cassette. Sequences from the plasmid backbone or from the selection marker were not detected. Also, it was confirmed that inserts are intact, that they keep the predicted organization and that the 5' and 3' flank sequences correspond to soybean genome native sequences. It gets demonstrated by bioinformatic analysis the unlikelihood of any endogenous ORF having been interrupted after the transformation.

Molecular analysis aimed at confirming the integrity of the DNA inserted in soybean MON 87701 x MON 89788 were assessed. The molecular organization of the insert was confirmed by Southern blot method, resulting in the integrity of the insert of each individual event being retained by the stacked event.

Taking into consideration that the stacked event was developed through a conventional crossing process, there are no scientific reasons why this crossing could result in the sequences of both inserts being more unstable than those of simple events.

## 3 – Products, pattern and levels of expression

The protein Cry1Ac produced in the event MON 87701 is a 133 kDa protein and has an identity of more than 99% with the *Bacillus thuringiensis sbp kurstaki* protein and with the one present in the B.t. insecticide formulations used in agriculture. It differs only in four amino acids with the protein Cry1Ac present in the Bollgard cottons (MON 531 event). It was demonstrated that the Cry1Ac protein is particularly active against lepidopteran insects. In Argentina, the production of Cry1Ac protein in the stacked event MON 87701 x MON 89788 is effective for the control of pests such as *Anticarsia gemmatalis*, *Epinotia aporema*, and *Rachiplusia nu*. By means of bioassays of the Cry1Ac protein with 72 insect species, which represent 37 families from 9 orders of insects, one of worm and two mites species, it was shown that no species out of the order Lepidoptera were sensitive. The *cry1Ac* gene expresses itself in the plant's aerial tissues under the regulation of a promoter which directs the expression to aerial tissues.

The protein CP4 EPSPS coded by the gen *cp4 epsps* derived from *Agrobacterium sp.* strain CP4 consists of one polypeptide of 455 amino acids and 47.6 kDa. The *cp4 epsps* gene expresses itself in all the plant's tissues due to its constitutive promoter, and the product of the expression is the CP4 EPSPS protein. This protein has a similar structure

and is functionally identical to the endogenous EPSPS enzymes of the plant, but with a reduced glyphosate affinity.

The average level of Cry1Ac protein was between 4.8 µg/g and 7.9 µg/g of dry weight of harvested seed, and between 30 µg/g and 81 µg/g of dry weight of forage. The average level of the CP4 EPSPS protein was between 100 µg/g and 160 µg/g of dry weight of harvested seed, and between 95 µg/g and 120 µg/g of dry weight of forage. These data were obtained from samples from US and Argentina.

#### **4 – Compositional analysis**

The applicant submitted information on the analysis of 64 analytes (7 in forage and 57 in seed) of soybean grains and green tissues samples collected in field trials carried out in 5 locations in Argentina during the 2007/2008 season, and in 5 US locations during the 2007 season. The study compares the soybean that contains the event MON87701 x MON89788, the A5547 conventional soybean (as similar genetic background), and non-transgenic materials (range of commercial varieties).

Data collected were statistically evaluated and compared (ANOVA). Some statistically significant differences were observed when comparing the stacked event with the conventional control (A5547 line) and with the commercial varieties (range of commercial materials). However, all the values obtained were in the range established in the scientific literature (ILSI 2006), except for raffinose and stachyose, only in the range of the commercial varieties.

In light of the afore stated, it is considered that the variability was not altered by the transformation event.

The company carried out a 42-days study in broiler chickens with the aim of assessing the effects of diets containing grains of the event MON 87701 x MON 89788 and comparing them with diets containing the conventional isolate (A5547) and the commercial varieties. The results of the study showed that there were no adverse events observable in chickens that received diets based on soybeans of the stacked event MON 87701 x MON 89788, compared with those that received diets based on non-transgenic soybeans, being this for the direct effect of the transgenic proteins in the diet or as a result of non-intentional compositional changes in the grain that could have generated toxic effects or altered their nutritional value.

Two 90-days diet studies in Sprague Dawley rats that received processed soybean food MON 87701 (formulated at 30% weight/weight) and MON 89788 (portions formulated at 11% and 33% weight/weight) were also assessed. The diet based on soybeans of the event MON 89788 and MON 87701 did not result in any adverse effects on the health or growth of the rats.

It can therefore be concluded that soybeans of the event MON87701 x MON89788 is substantially and nutritionally equivalent to its non-transgenic counterpart and to conventional varieties.

## 5 – Allergenicity

### Homology with known allergenic proteins:

Considering that proteins do not come from sources recognized as allergenic (nor *Bacillus thuringiensis* or *Agrobacterium spp.* have a background as allergy-causing factors); that the results of the bioinformatic analysis of the new expression proteins and of the possible polypeptides generated by the translation of the other 5 reading frames (2 to 6) demonstrate the absence of homologies of general sequence or immunologically relevant (window of 80 amino acids and 8 consecutives) when they were compared with pharmacologically active allergens and proteins from the AD6 allergens database (Mc Clain and Silvanovich, 2006); that the new proteins are easily digested in gastric fluids; that thermal stability studies show a drastic reduction of the immunodetection after heat treatment (due to the denaturalization and/or formation of insoluble complexes); and that these characteristics did not alter due to the events stacking, it is concluded that it is highly unlikely that the soybean event MON 87701 x MON 89788 expresses allergenic substances.

## 6 – Toxicity

The new expression proteins come from *Bacillus thuringiensis* and *Agrobacterium spp.*, and no substances naturally present in these organisms with an adverse biological activity were identified.

The bioinformatic studies carried out on the sequence of the proteins expressed, using the FASTA tool and the TOXIN6 and PROTEIN databases, showed that no biologically relevant similarities were found between the sequences of the proteins and the possible polypeptides, and toxins or proteins with a known biologically adverse activity for humans and animals.

Acute oral toxicity studies in mice were carried out for each of the individual proteins. The results indicated that the treatments produced no observable effects on body weight, food consumption, survival, and clinical or pathological symptomatology.

It can be therefore concluded that it is highly unlikely that soybean event MON 87701 x MON 89788 presents toxicological risks for human and animals.

## 7 – Metabolic interactions

The evaluated studies indicate that the existence of effects of interaction (synergistic, antagonistic or enhancers) between the proteins when stacked is unlikely. Other

evaluated evidences demonstrate that there are no phenotypic, compositional, nutritional or bioefficacy changes, and that the proteins do not share metabolic pathways or modes of action.

Therefore, it is concluded that the existence of mechanisms of interaction between the genetic elements that affect the expression of the new proteins is unlikely.

## **8 – Conclusion**

After a comprehensive assessment on the food and feed risk to the information submitted by Monsanto Argentina S.A.I.C., and taking into account that:

- The inheritance studies carried out indicated that there is Mendelian segregation.
- The proteins of new expression in grain are expressed at low levels.
- It is substantially and nutritionally equivalent to its non-transgenic counterpart.
- No evidence of similarity or homology with known toxic proteins was found.
- No evidence of expression of known allergenic substances for the proteins expressed in the stacked event was found.
- Studies that indicate that there are no effects of interaction between the proteins when the events are stacked were evaluated.
- The feeding studies with broiler chickens and rats demonstrated that there are no adverse dietary effects.

It is concluded that soybean event MON 87701 x MON 89788 is substantially equivalent to its conventional counterpart; therefore it is as safe and nutritious as the conventional commercial soybean varieties.

According to what was previously described, and based on the currently available scientific knowledge and on the internationally accepted requirements and criteria, there are no objections for the approval, for human and animal consumption, of soybean MON 87701 x MON 89788 and its parental events.

## **9 – Regulation and recommendations**

- Resolution SENASA N° 1265/99
- Resolution SENASA N° 412/02
- Principles for the risk analysis of food derived from modern biotechnology means (CAC/GL 44-2003)
- Guidelines for the assessment of the safety of food derived from plants with recombinant DNA (CAC/GL 45-2003)
- Consensus Document's for the work on the Safety of Novel Foods and Feeds (OECD)
- Resolution MAGyP N° 701/2011
- Database ILSI 2007
- Allergens database (FARRP)

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