

NOVEL FOOD INFORMATION - FOOD BIOTECHNOLOGY

GLYPHOSATE TOLERANT SOYBEAN 40-3-2

Health Canada notified Monsanto Canada Inc. (April, 1996) that it had no objection to the food use of transgenic soybean line GTS 40-3-2, which has been developed to be tolerant to broad-spectrum glyphosate-containing herbicides, specifically Roundup®. The Department conducted a comprehensive assessment of glyphosate tolerant line 40-3-2 according to its *Guidelines for the Safety Assessment of Novel Foods* (Sept. 1994). These guidelines are based upon internationally accepted principles for establishing the safety of foods derived from genetically modified organisms.

On May 19, 2000, Monsanto Canada submitted additional molecular characterization data to Health Canada regarding the GTS 40-3-2 line. Using more sensitive detection methods, Monsanto scientists detected additional non-functional DNA sequences not previously characterized in the original submission material in 1994. Based on the evaluation of the supplementary information provided by Monsanto, Health Canada has concluded that the additional molecular characterization data has no impact on the safety of glyphosate tolerant soybeans available on the Canadian marketplace.

BACKGROUND:

The following provides a summary regarding the Monsanto Canada Inc. notification to Health Canada and contains no confidential business information.

1. Introduction

The GTS 40-3-2 line of soybean (*Glycine max* L.) was developed through a specific genetic modification to be tolerant to the activity of glyphosate herbicide. The novel variety was developed from the A5403 soybean variety (Asgrow Seed Co.) by insertion of a gene encoding a glyphosate tolerant variant of the endogenous 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Glyphosate specifically binds to and inactivates EPSPS, which is involved in the biosynthesis of the aromatic amino acids tyrosine, phenylalanine

This Novel Food Information document has been prepared to summarize the opinion regarding the subject product provided by the Food Directorate, Health Protection Branch, Health Canada. This opinion is based upon the comprehensive review of information submitted by the petitioner according to the *Guidelines for the Safety Assessment of Novel Foods*.

(Également disponible en français)

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and tryptophan. This enzyme is present in all plants, bacteria, fungi, but not animals, which do not synthesize their own aromatic amino acids. Thus, EPSPS is normally present in food derived from plant and microbial sources. The modified soybean line permits farmers to use glyphosate containing herbicides, such as Roundup®, for weed control in the cultivation of soybean.

2. Development of the Modified Plant

The GTS 40-3-2 soybean line was created through direct DNA transformation by microparticle bombardment of plant cells with DNA-coated gold particles. The plasmid used for transformation contained the EPSPS encoding gene from the common soil bacterium, *Agrobacterium* sp. CP4. Data from 6 generations of backcrossing demonstrated stable inheritance of the novel trait.

The original molecular analysis of GTS 40-3-2 determined that one EPSPS DNA insert had integrated into the genome. Recent data submitted in May 2000 revealed that additional DNA sequences are present in GTS-40-3-2. Specifically, a 250 base pair (bp) segment of CP4 EPSPS DNA located adjacent to the termination element of the previously described EPSPS insert and a second insert comprising a 72 bp segment of CP4 EPSPS DNA were detected. Further analysis demonstrated that only the full length CP4 EPSPS mRNA and protein is expressed in GTS 40-3-2 and that the presence of the two additional DNA sequences does not result in the expression of any mRNA or protein. In summary, the newly detected sequences are constituents of the DNA that was used in the transformation of the soybean and are not different genes than those originally inserted into the soybean genome.

3. Product Information

The production of CP4 EPSPS in both seeds and leaves from transgenic GTS 40-3-2 was quantified using enzyme linked immunosorbent assay (ELISA) and was estimated to be 0.239 and 0.495 µg/mg fresh weight tissue, respectively. Naturally occurring soybean trypsin inhibitor, which acts to inhibit normal protein digestion in humans and animals, is the chief reason why raw soybean meal and flour have an anti-nutritive activity. There was no significant difference in the trypsin inhibitor activity between transgenic GTS 40-3-2 soybean and non-transgenic control soybeans. Likewise, there were no significant differences in the levels of plant lectins, as determined by hemagglutination assay, or isoflavone glucosides between transgenic and control soybean. These latter substances, which include genistin and daidzin, can exhibit estrogenic and hypocholesterolemic activities. Other than tolerance to glyphosate herbicides, the disease, pest and other agronomic characteristics of GTS 40-3-2 soybean were comparable to non-transgenic A5403 soybean.

The additional data submitted in May 2000 does not raise any concerns regarding the levels of trypsin inhibitor, lectins or isoflavone glucosides because the additional fragments of DNA were present within the soybeans used in all of the original safety testing.

3. Dietary Exposure

The genetic modification of GTS 40-3-2 soybean will not result in any change in the consumption pattern of soybean products in Canada. Consequently, the dietary exposure of Canadians to this product is anticipated to be the same as for other lines of commercially available soybean. Dietary exposure to EPSPS

is not novel in that all plants, bacteria and fungi produce this enzyme and the CP4 EPSPS will be ingested as inactive denatured protein since all soybean-derived human food products are heated prior to consumption.

5. Nutrition

The analysis of nutrients from transgenic GTS 40-3-2 soybean and non-transgenic soybean did not reveal any significant differences in the levels of protein, fat, fibre and starch. Comparisons of the amino acid composition of the raw soybeans and the fatty acid profiles of extracted oil from transgenic and control plants did not reveal any significant differences. The consumption of product from GTS 40-3-2 soybean will have no significant impact on the nutritional quality of the Canadian food supply. The additional data submitted in May 2000 does not raise any concerns regarding the nutritional quality of the soybean because the additional DNA fragments were present within the soybeans used in all of the original safety testing.

6. Safety

a) Potential Toxicity:

The amino acid sequence of CP4 EPSPS is closely related to the sequence of the endogenous soybean enzyme. An analysis of the amino acid sequence of the inserted CP4 EPSPS enzyme did not show homologies with known mammalian protein toxins and it is not judged to have any potential for human toxicity. Additionally, acute oral toxicity studies with purified CP4 EPSPS did not reveal any deleterious effects when mice were administered a dose of 572 mg/kg body weight, which was approximately 1300-fold greater than the highest anticipated potential consumption of CP4 EPSPS from soybean. The additional data submitted in May 2000 does not raise any concerns with regard to toxicity because the DNA fragments do not result in the expression of any new proteins.

b) Potential Allergenicity:

The CP4 EPSPS enzyme does not possess characteristics typical of known protein allergens. There were no regions of homology when the sequence of this introduced protein was compared to the amino acid sequences of known protein allergens. Unlike known protein allergens, CP4 EPSPS is rapidly degraded by acid and/or enzymatic hydrolysis when exposed to simulated gastric or intestinal fluids. The CP4 EPSPS is extremely unlikely to be allergenic. The additional data submitted in May 2000 does not raise any concerns with regard to allergenicity because the DNA fragments do not result in the expression of any new proteins.

CONCLUSION:

Health Canada's review of the information presented in support of the food use of glyphosate tolerant GTS 40-3-2 soybean concluded that soybean does not raise concerns related to safety. Health Canada is of the opinion that products from GTS 40-3-2 soybean are as safe and nutritious as those available from current commercial varieties. Based on the evaluation of the additional data submitted in May 2000, the approval status of GTS 40-3-2 remains unchanged.

Health Canada's opinion deals only with the food use of this glyphosate tolerant soybean. Issues related to



growing glyphosate tolerant soybean in Canada and its use as animal feed are addressed separately through existing regulatory processes in the Canadian Food Inspection Agency.