



Decision Document

DD1999-33

**Determination of the Safety of Monsanto Canada Inc.'s
Roundup Ready™ Corn(*Zea mays* L.) Line GA21**

This Decision Document has been prepared to explain the regulatory decision reached under the guidelines Dir94-08 *Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits* and its companion document Dir94-11 *The Biology of Zea mays L. (Corn/Maize)* and the guidelines Dir95-03 *Guidelines for the Assessment of Livestock Feed from Plants with Novel Traits*.

The Canadian Food Inspection Agency (CFIA), specifically the Plant Biotechnology Office (PBO) of the Plant Health and Production Division and the Feed Section of the Animal Health and Production Division, has evaluated information submitted by Monsanto Canada Inc. This information is in regard to the glyphosate tolerant corn line GA21. The CFIA has determined that this plant with a novel trait does not present altered environmental interactions or pose concerns for the safety of livestock consuming feed derived from this PNT, when compared to currently commercialized corn varieties in Canada.

Unconfined release into the environment and use as livestock feed of the corn line GA21 is therefore authorized as of April 23, 1998. Any other *Zea mays* lines and intraspecific hybrids resulting from the same transformation event and all their descendants, may also be released into the environment and used as livestock feed, provided no inter-specific crosses are performed, provided the intended use is similar, provided it is known following thorough characterization that these plants do not display any additional novel traits and are substantially equivalent to currently grown corn, in terms of their potential environmental impact and livestock feed safety.

The GA21 corn line is subject to the same phytosanitary import requirements as its unmodified counterparts.

Please note that, while determining the livestock feed and environmental safety of plants with novel traits are critical steps in the commercialization of these plant types, other requirements still need to be addressed, such as the evaluation of food safety (Health Canada).

(publié aussi en français)

**March
1999**

This bulletin is published by the Plant Health and Production Division, Canadian Food Inspection Agency. For further information, please contact the Plant Biosafety Office, Plant Health and Production Division or the Feed Section, Animal Health and Production Division at the following address:

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I. Brief Identification of Plant with Novel Traits (PNT)

Designation(s) of the PNT:	GA21
Applicant:	Monsanto Canada Inc.
Plant Species:	<i>Zea mays</i> L.
Novel Traits:	Herbicide Tolerance (glyphosate).
Trait Introduction Method:	Microprojectile bombardment of plant cells.
Proposed Use of PNT's:	Production of <i>Z. mays</i> for seed oil for human consumption and seed oil, meal and silage for livestock feed. These materials will not be grown outside the normal production area for corn in Canada.

II. Background Information

Monsanto Canada Inc. has developed a corn line, derived from the inbred corn lines AT, containing an mEPSPS gene, coding for 5-enolpyruvyl shikimate-3-phosphate synthase, which imparts novel tolerance to glyphosate the active ingredient in Roundup® herbicide. This herbicide tolerance trait allows for the control or suppression of economically important weeds in corn production.

The development of the glyphosate tolerant corn line was accomplished with recombinant DNA technology. A maize derived mEPSPS gene, was inserted into a cell culture line and transformants were selected on their ability to withstand glyphosate. The modified maize gene imparts reduced sensitivity to Roundup herbicide at the site of the herbicide's activity. The expression of the gene is directed to the chloroplast, the site of the herbicide activity, by the addition of sequences coding for a plant derived transit peptide.

Monsanto Canada Inc. has provided data on the identity of line GA21, a detailed description of the transformation method, data and information on the gene insertion site, copy number and levels of expression in the plant, the role of the inserted gene and regulatory sequences in donor organisms and the full nucleotide sequence. The novel protein was identified, characterized and compared to the original plant protein, including an evaluation of its potential toxicity to livestock and non-target organisms. References to relevant scientific publications were included.

These materials have been field tested in Canada under confined conditions in Ontario in 1997. Field trials have also been conducted in the USA.

Agronomic characteristics of corn hybrids derived from GA21 such as seed dormancy, vegetative vigour (plant height), plant vigour, time to maturity, flowering period, susceptibilities to various *Z. mays* pests and pathogens, and seed production were compared

to those of unmodified *Z. mays* counterparts.

The Plant Biotechnology Office (PBO) of the Plant Health and Production Division, CFIA, has reviewed the above information, in light of the assessment criteria for determining environmental safety of plants with novel traits, as described in the regulatory directive Dir94-08. The PBO has considered:

- potential of the PNTs to become weeds of agriculture or be invasive of natural habitats,
- potential for gene flow to wild relatives whose hybrid offspring may become more weedy or more invasive,
- potential for the PNTs to become plant pests,
- potential impact of the PNTs or their gene products on non-target species, including humans, and
- potential impact on biodiversity.

The Feed Section of the Animal Health and Production Division, CFIA, has also reviewed the above information with respect to the assessment criteria for determining the safety and efficacy of livestock feed, as described in Dir95-03. The Feed Section has considered:

- potential impact to livestock and
- potential impact on livestock nutrition.

III. Description of the Novel Trait

1. Glyphosate Tolerance

- A modified wild type maize gene which imparts field level tolerance to glyphosate, the active ingredient in Roundup herbicide, was introduced into the GA21 corn line.
- A plant-derived coding sequence expressing an optimized chloroplast transit peptide was fused to the modified maize gene. This peptide facilitates the import of the newly translated modified maize EPSPS enzyme into the chloroplast, the site of both the shikimate pathway and glyphosate mode-of-action.
- The inserted gene codes for a version of the native 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS), an enzyme involved in the shikimate biochemical pathway for the production of the aromatic amino acids. The wild type version of this maize enzyme is sensitive to glyphosate. The herbicide disrupts this essential pathway, leading to growth suppression or death of the plant. A modified version of the plant enzyme was created with site directed mutagenesis. The altered enzyme is glyphosate tolerant and allows production of aromatic amino acids in the presence of the herbicide. The expression of the novel enzyme in the plant was quantified.
- The expressed novel enzyme is a single polypeptide with a 99.3% sequence identity to the wild type.

- The modified maize EPSPS (mEPSPS) was detected by western blot. Western blot data indicated that the EPSPS expression in the transformed plants was an order of magnitude higher than the non-transformed plants. A gene coding for the mEPSPS was expressed in bacteria and the resulting enzyme compared to the plant derived mEPSPS by Western blot. The enzymes expressed from the two sources were shown to be identical. The microbial derived enzyme was used to evaluate the enzyme kinetics, to perform toxicology studies and as a standard in the determination of protein expression from the modified plant.
- The expression of the mEPSPS gene was evaluated in forage and in grain from plants at 5 field locations. Pooled grain samples from 9-16 ears were analysed. Expression of EPSPS protein averaged 3.2 µg/ fresh weight and ranged from 1.4-4.9 µg/g fwt . Expression of the wild-type EPSPS in grain was undetectable at all sites. Expression of EPSPS protein in forage (entire plant minus the roots) averaged 118.7 µg/g fwt. and ranged from 46.6-210.4 µg/g fwt.. The wild type EPSPS was detected at 4 out of 5 sites but not at levels high enough to quantify.
- The mEPSPS protein was not stable against digestion. The enzyme was completely digested within 15 seconds in simulated gastric fluid (pepsin) and within 1 minute in simulated intestinal fluid (pancreatin). Results were shown on western blots.
- No toxicity from the protein was apparent in animal studies. A database of protein toxin sequences was assembled from public databases and compared to the amino acid sequence of the mEPSPS. Using the "FASTA" computer program, no biologically relevant sequence similarities were detected between mEPSPS and known toxins. A database of 219 allergen and gliadin sequences was constructed from public domain databases. No significant sequence similarities between mEPSPS and known allergens and gliadins were discovered. Data were presented that showed that the protein was not glycosylated, a property common to many allergens. EPSPS is an enzyme that is ubiquitous in nature, and therefore would not be expected to be toxic or allergenic.

2. Development Method

The GA21 corn line was transformed with a DNA fragment containing only the modified corn EPSPS gene fused to an optimized chloroplast transit peptide and promoter sequences, respectively. The DNA fragment was introduced by microprojectile bombardment into cultured plant cells. Glyphosate tolerant transformed cells were selected, then cultured in tissue culture medium for plant regeneration.

3. Stable Integration into the Plant's Genome

Southern blot analysis of the transformed corn line indicate the presence of a single insertion, with three complete internal copies of the mEPSPS gene.

Southern blot data from two generations of GA21 was presented. After four generations of breeding the Southern blots showed identical banding patterns. The line continued to display tolerance to glyphosate during breeding.

IV. Assessment Criteria for Environmental Safety

1. Potential of the PNT to Become a Weed of Agriculture or be Invasive of Natural Habitats

The biology of corn (*Zea mays*), described in Dir94-11, shows that unmodified plants of this species are not invasive of unmanaged habitats in Canada. Corn does not possess the potential to become weedy due to traits such as lack of seed dormancy, the non-shattering aspect of corn cobs, and poor competitive ability of seedlings. According to the information provided by Monsanto Canada Inc., GA21 and derived corn hybrids were determined not to be different from their counterparts in this respect.

The CFIA evaluated data submitted by Monsanto Canada Inc. on the reproductive and survival biology of corn hybrids derived from GA21, and determined that early stand establishment, vegetative vigour, time to maturity and seed production were within the normal range of expression of these traits currently displayed by commercial corn hybrids. No genes were inserted for cold tolerance or winter survival.

No competitive advantage was conferred to these plants, other than that conferred by resistance to glyphosate herbicide. Resistance to Roundup herbicide will not, in itself, render corn weedy or invasive of natural habitats since none of the reproductive or growth characteristics were modified.

The above considerations, together with the fact that the novel traits have no intended effects on weediness or invasiveness, led the CFIA to conclude that the GA21 corn line has no altered weed or invasiveness potential compared to currently commercialized corn.

A longer term concern, if there is general adoption of several different crop and specific herbicide weed management systems, is the potential development of crop volunteers with a combination of novel resistances to different herbicides. This could result in the loss of the use of these herbicides and any of their potential benefits. Therefore, agricultural extension personnel, in both the private and public sectors, should promote careful management practices for growers who use these herbicide-tolerant crops, to minimize the development of multiple resistance.

2. Potential for Gene Flow to Wild Relatives Whose Hybrid Offspring May Become More Weedy or More Invasive

The biology of corn, as described in Dir94-11, indicates that there are no wild relatives in Canada that can hybridize with *Zea mays*.

The CFIA therefore concludes that gene flow from GA21 to corn relatives is not possible in Canada.

3. Altered Plant Pest Potential

The intended effects of the novel trait is unrelated to plant pest potential, and corn is not a plant pest in Canada (Dir94-11). In addition, agronomic characteristics of the modified

corn hybrids were shown to be within the range of values displayed by currently commercialized corn hybrids, and indicate that the growing habit of corn was not inadvertently altered. Glyphosate is commonly used for chemical fallow production and resistant corn volunteer plants will not be controlled. Despite the tolerance to glyphosate, GA21 volunteers can still be managed by growers using alternative herbicides with different modes of action. Field observations did not indicate modifications of disease and pest susceptibilities.

The CFIA has therefore determined that the GA21 corn line does not display any altered pest potential.

4. Potential Impact on Non-Target Organisms

The detailed characterization of the novel gene and resulting enzyme, as briefly summarized in Part III of the present document, has led to the conclusion that the expression of the novel protein does not result in altered toxic or allergenic properties. The enzyme is rapidly inactivated in mammalian stomach and intestinal fluids by enzymatic degradation and pH-mediated proteolysis. Seed protein profiles and fatty acid composition fall within the range of those of the unmodified counterparts. A search of the FAST amino-acid sequence database revealed no significant homology of the novel proteins with known toxins or allergens entered in that database.

Based on the above, the CFIA has determined that the unconfined release of the GA21 corn line will not result in altered impacts on interacting organisms, including humans, compared to current corn varieties.

5. Potential Impact on Biodiversity

GA21 has no novel phenotypic characteristics which would extend its use beyond the current geographic range of corn production in Canada. Since corn does not outcross to wild relatives in Canada, there will be no transfer of novel traits to unmanaged environments. In addition the novel trait was determined to be safe to non-target organisms.

The CFIA has therefore concluded that the potential impact on biodiversity of GA21 will not be altered.

V. Nutritional Assessment Criteria for Use as Livestock Feed

1. Nutritional Composition of the PNT

Comparisons of protein, fat, fibre, ash, Ca, P, amino acids and fatty acids of corn grain and protein, fat, fibre, ash, Ca and P in whole plant material from the PNT line vs. its parent line and five or six commercial non-PNT varieties were made, in three separate trials. In both the grain and the whole plant, there were occasional significant differences in individual trials between the PNT and the non-PNT in grain protein content, but overall there were no differences among the varieties for any of the nutrients. There were no

differences in the other nutrients, in either grain or forage material.

Nutrient composition was within the published range for corn, in both the grain and the whole plant in the PNT. The CFIA has determined that line GA21 is substantially equivalent to traditional corn varieties.

2. Anti-Nutritional Factors

The parent plant *Zea mays* is not known for the production of anti-nutritional factors and the transformation event which produced GA21 would not be expected to induce their synthesis.

VI. Regulatory Decision

Based on the review of data and information submitted by Monsanto Canada Inc., and through comparisons of corn hybrids derived from GA21 with unmodified corn counterparts, the Plant Biotechnology Office of the Plant Health and Production Division, CFIA has concluded that the novel gene and its corresponding traits does not confer to these plants any characteristic that would result in intended or unintended environmental effects following unconfined release.

Based on the review of submitted data and information, the Feed Section of the Animal Health and Production Division has concluded that the novel trait do not in itself raise any concerns regarding the safety or nutritional composition of GA21. Grain corn, its byproducts and corn oil are currently listed in Schedule IV of the *Feeds Regulations* and are therefore approved for use in livestock feeds in Canada. GA21 and corn hybrids derived from it have been assessed and found to be substantially equivalent to traditional corn varieties. GA21 and its byproducts are considered to meet present ingredient definitions and are approved for use as livestock feed ingredients in Canada.

Unconfined release into the environment and use as livestock feed of the corn line GA21 is therefore authorized as of April 23, 1998. Any other *Zea mays* lines and intraspecific hybrids resulting from the same transformation event and all their descendants, may also be released into the environment and used as livestock feed, provided no inter-specific crosses are performed, provided the intended use is similar, provided it is known following thorough characterization that these plants do not display any additional novel traits and are substantially equivalent to currently grown corn, in terms of their potential environmental impact and livestock feed safety.

The GA21 corn line is subject to the same phytosanitary import requirements as its unmodified counterparts.

Please note that, while determining the livestock feed and environmental safety of plants with novel traits is a critical step in the commercialization of these plant types, other requirements still need to be addressed, such as the evaluation of food safety (Health Canada).