



Canadian Food
Inspection Agency

Agence canadienne
d'inspection des aliments

Canada

Français	Contact Us	Help	Search	Canada Site
Table of Contents	What's New	Acts and Regulations	Site Map	
Food Safety	Animal Health	Plant Protection	Corporate Affairs	
Quick Pick By Commodity / Key Topic				

- [Main Page - Plant Biosafety Office](#)
- [Plant Health and Production Division](#)
- [The Regulation of Plants With Novel Traits in Canada](#)
- [Status of Regulated Plants with Novel Traits \(PNTs\) in Canada](#)
- [Import Permit Requirements for Plants with Novel Traits \(including Transgenic Plants\), and their Products \(D-96-13\)](#)
- [Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits \(Dir94-08\)](#)
- [Guidelines for the Environmental Release of Plants with Novel Traits within Confined Field Trials in Canada \(Dir2000-07\)](#)
- [Decision Documents](#)
- [Summary of Confined Field Trials](#)
- [Fee Submission Form](#)
- [Contacts](#)

**Canadian Food Inspection Agency,
Plant Health and Production Division,
Plant Biosafety Office**

Decision Document 98-22: Determination of the Safety of AgrEvo Canada Inc.'s Glufosinate Ammonium Tolerant Corn (*Zea mays*) lines, T14 and T25

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 of the Plant Health and Production Division and the Feed Section of the Animal Health and Production Division, with input from the Plant Health Risk Assessment Unit, has evaluated information submitted by AgrEvo Canada Inc. regarding corn lines T14 and T25. These plants were transformed with a gene conferring tolerance to the glufosinate ammonium herbicide Liberty™. The CFIA has determined that these plants with a novel trait should not pose concern to environmental or livestock feed safety, when compared to currently commercialized corn varieties.

Unconfined release into the environment and use as livestock feed of AgrEvo's corn lines T14 and T25 is therefore authorized. Any other *Zea mays* lines and intra specific hybrids resulting from the same transformation events and all their descendants are also approved 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 provided that the resulting lines are substantially equivalent to currently grown corn in terms of their potential environmental impact and livestock feed safety.

Table of Contents

- I. [Brief Identification of the Plants with Novel Traits \(PNT's\)](#)
- II. [Background Information](#)
- III. [Description of the Novel Traits](#)
 1. [Glufosinate Ammonium Tolerance](#)
 2. [The Ampicillin Resistance Gene](#)
 3. [Development Method](#)
 4. [Stable Integration into the Plants' Genome](#)
- IV. [Assessment Criteria for Environmental Safety](#)
 1. [Potential of the PNT to Become a Weed of Agriculture or Be Invasive of Natural Habitats](#)
 2. [Potential for Gene Flow to Wild Relatives Whose Hybrid Offspring May Become More Weedy or Invasive](#)
 3. [Altered Plant Pest Potential](#)
 4. [Potential Impact on Non-Target Organisms](#)
 5. [Potential Impact on Biodiversity](#)
- V. [Nutritional Assessment Criteria for Use as Livestock Feed](#)

1. Nutritional Composition of the PNT's
 2. Anti-Nutritional Factors
- VI. Regulatory Decision

I. Brief Identification of the Plants with Novel Traits (PNT's)

<i>Designation(s) of the PNT:</i>	Liberty Link™ corn lines T14 and T25
<i>Applicant:</i>	AgrEvo Canada Inc.
<i>Plant Species:</i>	Corn (<i>Zea mays</i>)
<i>Novel Traits:</i>	Tolerance to the glufosinate ammonium herbicide Liberty™
<i>Trait Introduction Method:</i>	<i>Agrobacterium tumefaciens</i> -mediated transformation
<i>Proposed Use of PNT's:</i>	Production of corn of the yellow dent type for grain and silage. These materials will not be grown outside the normal production area for corn

II. Background Information

AgrEvo Canada Inc. has developed corn lines T14 and T25, that are tolerant to Liberty™, a broad spectrum, non-residual, glufosinate ammonium herbicide. These corn lines will permit the use of Liberty™ as a post-emergence herbicide, thus providing an additional means of weed control in corn production, which may reduce reliance on soil-incorporated herbicides.

Lines T14 and T25 were produced using recombinant DNA technology which permitted the introduction of a bacterial based synthetic gene into a line of *Z. mays*. This inserted gene codes for phosphinothricin acetyltransferase (PAT), an enzyme that inactivates glufosinate ammonium through acetylation. A second gene, conferring resistance to ampicillin antibiotic, was included in the original genetic sequence, but no intact copies were detected in either T14 or T25. This gene of no agronomic interest but was used in the development of the genetic sequence carrying the herbicide tolerance gene. No gene product from the ampicillin resistance gene was detected.

Lines T14 and T25 have been field tested in Ontario, Canada under confined conditions since 1993.

AgrEvo has provided data on the identity of events T14 and T25, their molecular characterization, a detailed description of the modification method, data and information on the stability of the gene insertion, the role of the inserted gene and regulatory sequences in donor organisms, and full nucleotide sequences of the inserted elements. The novel protein was identified and characterized and information on its expression in the transformed plants was provided.

Agronomic characteristics such as yield, plant height, overwintering capacity, flowering period, a disease susceptibility were compared to those of unmodified *Z. mays* counterparts. Effects of residues from T14 and T25, were addressed and AgrEvo also provided data on seed dormancy and the emergence in subsequent years of volunteer plants. Stress adaptation was evaluated, including susceptibilities to various *Z. mays* pests and pathogens and to herbicides, other than glufosinate ammonium, that are normally used to control corn or weeds in corn crops. Invasiveness studies were performed under disturbed, undisturbed, and agronomic conditions.

The Plant Biotechnology Office of the Plant Health and Production Division, CFIA with input from the Plant Health Risk Assessment Unit, 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 assessment included the following:

- potential of the PNT to become a weed 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 PNT to become a plant pest
- potential impact of the PNT or its gene products on non-target species, including humans

- potential impact on biodiversity

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

- potential impact to livestock
- potential impact to livestock nutrition

III. Description of the Novel Traits

1. Glufosinate Ammonium Tolerance

- Phosphinothricin (PPT), the active ingredient of glufosinate ammonium, inhibits glutamine synthetase, which results in the accumulation of lethal levels of ammonium in susceptible plants within hours of application.
- The phosphinothricin tolerance gene engineered into AgrEvo's corn lines T14 and T25 codes for PPT-acetyltransferase (PAT). This enzyme detoxifies phosphinothricin by acetylation into an inactive compound. PAT has extremely high substrate specificity for L-PPT and dimethylphosphinothricin (DMPT), and experimental data clearly showed that neither L-PPT's analog L-glutamic acid, D-PPT, nor any other amino acid can be acetylated by the PAT enzyme.
- The synthetic PAT gene was derived from a PAT gene isolated from *Streptomyces viridochromogenes*, an aerobic soil bacteria. The PAT enzyme occurs naturally in the soil and acetyltransferases are ubiquitous in nature.
- The gene is linked to a constitutive promoter, and protein expression was detected in plant tissues, grain and silage. The PAT enzyme was not detected in protein extracts from the pollen.
- The PAT enzyme from T14 and T25 was compared to PAT expressed from the transform canola line HCN-92 (see Decision Document DD95-01, "*Determination of Environmental Safety of AgrEvo Canada Inc's Glufosinate Ammonium-Tolerant Canola*." The molecular weight and immunoreactivity of the enzyme from the plant derived sources was similar. When the plant derived PAT enzyme was compared to PAT enzyme from a bacterial expression system, the molecular weights were similar, indicating that the protein was unlikely to have been glycosylated or undergone other post translational modifications. Studies showed that the enzyme was inactivated within one minute when subjected to typical mammalian stomach conditions.
- The gene nucleotide sequence was provided. The nucleotide sequence showed no significant homology with any known toxins or allergens.

2. The Ampicillin Resistance Gene

- An ampicillin resistance gene was used during the development of the genetic sequence inserted into the T14 and T25 corn lines. This gene was not intended to have an agronomical purpose.
- Analysis of lines T14 and T25 demonstrated that neither event possessed a complete sequence of the ampicillin resistance gene. The ampicillin resistance gene introduced into T14 and T25 is not functional in the modified corn lines, as it does not have the necessary regulatory sequences for expression in plants.

3. Development Method

- A tissue cultured cell line of corn was used as a source of protoplasts. DNA containing the PAT gene was combined with the protoplasts. Cell colonies derived from this protoplast/DNA mixture were then regenerated and transformants were selected on a medium containing glufosinate ammonium.
- Two primary transformants, T14 and T25, were then backcrossed with both commercial public inbred lines and proprietary inbred lines of the yellow dent corn type.

4. Stable Integration into the Plant's Genome

- The data provided demonstrated the integration of one and three copies of the genetic sequence in transformation events T25 and T14, respectively.
- Liberty Link™ corn lines are several generations removed from the two original transformation events.

IV. Assessment Criteria for Environmental Safety

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

The CFIA has evaluated data submitted by AgrEvo Canada Inc. on the reproductive and survival biology of T14 and T25, and determined that vegetative vigour, overwintering capacity, flowering period, seed production, and dormancy were within the normal range of expression of characteristics in unmodified *Z. mays* counterparts. The genetic sequence introduced in transformation events T14 and T25 had no added genes for cold tolerance or winter hibernation. The number of volunteers in the year following a field trial were comparable between plots of Liberty Link™ corn lines and traditionally developed counterpart *Z. mays* plants.

Based on the submitted data, the CFIA have determined that corn lines derived from transformation events T14 and T25 did not show any stress adaptation other than its resistance to glufosinate ammonium herbicide. Resistance or susceptibility to major *Z. mays* pests and pathogens fall within the ranges currently displayed by commercial varieties.

The biology of *Z. mays*, described in Dir 94-11, 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 AgrEvo Canada Inc. T14 and T25 were determined not to be different from their traditionally developed counterparts in this respect. Invasiveness was studied in disturbed and undisturbed habitats. Data showed that T14 and T25 corn lines were neither more invasive nor more persistent than commercially available counterparts. No competitive advantage was conferred to glufosinate ammonium tolerant plants, other than that conferred by tolerance to glufosinate ammonium herbicide. Tolerance to glufosinate ammonium will not render corn weedy, since this herbicide is not presently used in crop rotation cycles involving corn. Glufosinate ammonium tolerant corn volunteer plants can easily be managed by mechanical means or by the use of other available herbicides.

The above considerations, together with the fact that the novel trait has no intended effect on weediness or invasiveness, led the CFIA to conclude that T14 and T25 have no altered weed or invasiveness potential compared to currently commercialized corn varieties.

Note: A longer term concern, if there is general adoption of several different crop/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 Invasive

The biology of corn, as described in Dir 94-11, indicates that there are no wild relatives in Canada that can hybridize with cultivated *Z. mays*.

The CFIA has therefore concluded that gene flow from T14 and T25 to wild corn relatives will not occur in Canada.

3. Altered Plant Pest Potential

The intended effect of the novel trait is unrelated to plant pest potential, and *Z. mays* is not a plant pest in Canada (Dir 94-11). In addition, agronomic characteristics, stress adaptation, and qualitative and quantitative composition of corn lines derived from transformation events T14 and T25 were shown to be within the range of values displayed by currently commercialized *Z. mays* varieties, leading to the conclusion that plant pest potential was not inadvertently altered.

The CFIA have therefore determined that Liberty Link™ corn does not display any altered pest potential.

4. Potential Impact on Non-Target Organisms

The PAT enzyme responsible for glufosinate ammonium tolerance has very specific enzymatic activity, does not possess proteolytic or heat stability and does not affect the metabolism of the

plant. Other crops such as wheat, barley, lentils, peas, flax and alfalfa, have been modified by recombinant DNA techniques to express the PAT enzyme with no apparent effect on the agronomic performance of succeeding crops. Expression levels of PAT in T14 and T25 are comparable to other transformed species and therefore the CFIA concludes that no significant residual effects from T14 and T25 are expected. PAT is rapidly inactivated in mammalian stomach and intestinal fluids by enzymatic degradation and pH-mediated proteolysis. Furthermore, PAT does not contain potential glycosylation sites nor does it possess proteolytic or heat stability, indicating that PAT is not a likely allergen. A search of the GENE BANK DNA sequence database revealed no significant homology with any known toxins or allergens.

Based on the above, the CFIA has determined that the unconfined release of T14 and T25 corn lines will not result in altered impacts on interacting organisms, including humans, when compared with currently commercialized counterparts.

5. Potential Impact on Biodiversity

T14 and T25 have no novel phenotypic characteristics which would extend their use beyond the current geographic range of corn production in Canada. The overall relative impact on plant biodiversity is neutral. Impact on animal and microbe biodiversity is also neutral since the introduced PAT enzyme would not be expected to alter the plant's metabolism such that novel compounds would be produced.

The CFIA has therefore concluded that the potential impact on biodiversity of T14 and T25 corn lines is equivalent to that of currently commercialized corn lines.

V. Nutritional Assessment Criteria as Livestock Feed

1. Nutritional Composition of the PNT

Comparisons of protein fat and fibre of corn grain and whole plant material from the glufosinate-ammonium tolerant corn lines and related lines were made. Overall protein content in the grain and whole plant was significantly higher in the PNT lines compared to the unmodified controls. The statistical differences were explained by the fact that the transformed plant lines were not complete genetic isolines of the control plants and thus the variation arose from genetic differences unrelated to the transformation event. In all cases protein content was within the normal published range for corn.

Small, less significant differences were apparent in some lines for fibre and fat content. Depending on the multiple comparison test used, results may or may not be significant. This suggests that differences between the transformed lines and the controls likely arose from experimental variation rather than as a result of the gene insertion. As was the case with protein, all values are within the normal published range for corn.

2. Anti-Nutritional Factors

The parent plant *Z. mays* is not known for the production of anti-nutritional factors and the transformation event which produced T14 and T25 would not be expected to induce their synthesis.

VI. Regulatory Decision

Based on the review of data and information submitted by AgrEvo Canada Inc., and through comparisons of T14 and T25 lines with unmodified *Z. mays* counterparts, the CFIA has concluded that neither the novel gene, nor its resulting gene product and associated novel trait, confers any intended or unintended ecological advantage to T14 and T25 corn lines.

Unconfined release into the environment and use as livestock feed of AgrEvo's corn lines T14 and T25 is therefore authorized. Any other *Z. mays* lines and intra-specific hybrids resulting from the same transformation events and all their descendants are also approved 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 provided that the resulting lines are substantially equivalent to currently grown corn in terms of their potential environmental impact and livestock feed safety.

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

Canadian Food Inspection Agency
59 Camelot Drive
Nepean, Ontario K1A 0Y9
Telephone: (613) 225-2342
Facsimile: (613) 228-6629

March 27, 1998


Top of Page

Important Notices