Government of Canada
Canadian Food Inspection Agency
Plant Health and Production Division

Gouvemement du Canada
Agence Canadienne d'inspection des aliments
Division de la production et de protection des végétaux

# Decision Document DD2002-39

## Determination of the Safety of Aventis CropScience Canada Inc's Glufosinate Ammonium Tolerant Sugar Beet (*Beta vulgaris*) Lines Derived from Event T120-7

This Decision Document has been prepared to explain the regulatory decision reached under the regulatory directive Dir94-08 *Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits* and its companion document Bio2002-01 *The Biology of Beta vulgaris L.* (Sugar beet) and Dir95-03 *Guidelines for the Assessment of Livestock Feed from Plants with Novel Traits.*

The Canadian Food Inspection Agency (CFIA), specifically the Plant Biosafety 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 Aventis CropScience Canada Inc. This information is in regard to glufosinate ammonium tolerant sugar beet lines derived from Event T120-7. The CFIA has determined that these plants with novel traits do not present significant altered environmental interactions or pose concerns for the safety of livestock consuming feed derived from lines derived from Event T120-7, when compared to currently commercialized sugar beet varieties in Canada.

**Unconfined release into the environment and use as livestock feed of the sugar beet lines derived from Event T120-7 are therefore authorized as of January 9, 2001. Any other *Beta vulgaris* 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 nutritionally equivalent to currently grown sugar beet, in terms of their potential environmental impact and livestock feed safety.**

**The sugar beet lines derived from Event T120-7 are subject to the same phytosanitary import requirements as their unmodified counterparts.**

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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 the Plants with Novel Traits (PNTs)

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| **Designation of the PNTs:** | Glufosinate ammonium tolerant lines derived from Event T120-7 |
| **Applicant:** | Aventis Crop Science Canada Inc. |
| **Species:** | *Beta vulgaris* L. |
| **Novel Traits:** | Herbicide tolerance (glufosinate ammonium)<br>Antibiotic resistance (kanamycin) |
| **Trait Introduction Method:** | *Agrobacterium* mediated transformation |
| **Intended Use of the PNT:** | Production of *B. vulgaris* for processing into sugar for human consumption and by-product for livestock feed. The PNT will not be grown outside the normal production area for sugar beet in Canada |

# II. Background Information

Aventis CropScience Canada Inc. has developed sugar beet lines derived from Event T120-7 containing a gene that codes for a phosphinothricin acetyltransferase (PAT) enzyme, which imparts novel tolerance to the herbicide glufosinate ammonium. This herbicide tolerance trait allows for the control or suppression of weeds in sugar beet production.

The development of the glufosinate ammonium tolerant sugar beet lines derived from Event T120-7 was accomplished with recombinant DNA technology through the introduction of a synthetic version of the *pat* gene isolated from soil bacterium *Streptomyces viridochromogenes*. The PAT enzyme detoxifies glufosinate ammonium, thereby providing reduced sensitivity to plants exposed to this herbicide. The sugar beet lines derived from Event T120-7 contain the same bacterial enzyme of PNTs that have already received approval for unconfined environmental release in Canada in crops such as canola, corn and soybeans. In addition to the *pat* gene, lines derived from Event T120-7 also contain a gene coding for the *Escherichia coli* derived neomycin phosphotransferase II enzyme which imparts resistance to aminoglycoside antibiotics.

Aventis CropScience Canada Inc. has provided data on the identity of lines derived from Event T120-7, 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 genes and regulatory sequences in donor organisms and the full nucleotide sequences. The novel proteins were identified, characterized and compared to the original plant protein, including an evaluation of their potential toxicity to livestock and non-target organisms. References to relevant scientific publications were included in the submission.

The material has been field tested in Canada under confined conditions in Canada from 1997 to 2000. Field trials have also been conducted in the USA from 1994 to 1998 as well as in Germany, U.K., France and the former USSR.

Agronomic characteristics of the sugar beet lines derived from Event T120-7 such as seed germination, plant vigour, flowering period, time to maturity, overwintering capacity, and susceptibilities to various sugar beet pests and pathogens were compared to those of unmodified sugar beet counterparts.

The Plant Biosafety 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 for lines derived from Event T120-7 to become weeds of agriculture or be invasive of natural habitats,
- potential for gene flow from lines derived from Event T120-7 to wild relatives whose hybrid offspring may become more weedy or more invasive,
- potential for lines derived from Event T120-7 to become plant pests,
- potential impact of lines derived from Event T120-7 or their gene products on non-target species, including humans, and
- potential impact of lines derived from Event T120-7 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 of lines derived from Event T120-7 to livestock and
- potential impact of lines derived from Event T120-7 on livestock nutrition.

## III. Description of the Novel Traits

### 1. Glufosinate Ammonium Tolerance

Phosphinothricin, the active ingredient of glufosinate ammonium, inhibits glutamine synthetase, which results in the accumulation of lethal levels of ammonia in susceptible plants within hours of application. Plants produce ammonia as a result of normal metabolic processes.

The gene engineered into sugar beet lines derived from Event T120-7 codes for PAT an enzyme which detoxifies phosphinothricin by acetylation into an inactive compound. PAT has extremely high substrate specificity and data included in the submission indicates that it does not acetylate other enzymes or proteins.

The introduced *pat* gene was originally isolated from *Streptomyces viridochromogenes*, an aerobic soil actinomycete bacterium. The PAT enzyme is therefore naturally occurring in the soil. More generally, acetyltransferases are ubiquitous in nature.

The *pat* gene is linked to a constitutive promoter. The expression of PAT was evaluated in the tops and roots of field grown plants, using ELISA assays. Composite samples of up to 30 plants were analysed. Higher expression levels were detected in tops than in roots. Overall expression of the PAT protein in mature plants ranged from 74-208 ng/g tissue in roots with an average of 137 ng/g and a range of 732-1176 ng/g in tops with an average of 966 ng/g.

Studies showed that the enzyme was inactivated within one minute when subjected to typical mammalian stomach conditions.

The gene nucleotide sequence and the enzyme amino acid sequence were provided. The nucleotide sequence showed no significant homology to the toxins or allergens entered into GENEBANK DNA database.

The *pat* gene was expressed in a bacterial expression system and the resulting enzyme compared to the plant expressed PAT protein by western blot analysis. The enzymes expressed from the two sources were shown to be functionally and structurally similar. Molecular weights were similar, indicating that the protein had not been glycosylated, nor had it undergone post-transcriptional modifications. The microbial expressed PAT enzyme was used to evaluate it's enzyme kinetics, to perform toxicology studies and as a standard in the determination of protein expression from the modified plant.

**2. Kanamycin Resistance**

The neomycin phosphotransferase II (*nptII*) gene isolated from the common enteric bacterium *E. coli* was also inserted into the plant's genome. This gene codes for an enzyme which imparts resistance to aminoglycoside antibiotics, such as kanamycin.

The gene is linked to a constitutive promoter. Expression levels for the NPTII enzyme were found to range from 11-30 ng/g tissue in roots with an average of 20 ng/g and to range from 28-76 ng/g tissue in tops with an average of 44 ng/g.

This protein occurs naturally in bacteria and was shown to degrade rapidly in simulated mammalian gastric and intestinal fluids.

The expressed enzyme was compared to the bacterial expressed NPTII enzyme and this study demonstrated that molecular weights were similar, indicating that the protein had not been glycosylated nor had it undergone post-transcriptional modifications.

The nucleotide sequence showed no significant homology with the toxins or allergens entered in the GENEBANK DNA database.

### 3. Development Method

Sugarbeet line RO1 was transformed with a plasmid vector containing the *pat* and *nptII* genes to confer the glufosinate ammonium tolerance and aminoglycoside antibiotic resistance traits. The DNA sequences were introduced by transformation with *Agrobacterium tumefaciens*. Multiple plants from a single transformation event designated T-120-7 were subsequently crossed to other elite lines, followed by appropriate backcrosses to introgress the novel traits into additional sugar beet lines derived from Event T120-7.

### 4. Stable Integration Into the Plant's Genome

Southern blot analysis of the sugar beet lines derived from Event T120-7 indicate the presence of a single insertion of the *pat* and *nptII* genes. Field trials demonstrated that the genes were inherited in a Mendelian manner. The line continued to display tolerance to glufosinate ammonium during breeding.

## IV. Assessment Criteria for Environmental Safety

### 1. Potential for the Lines Derived from Event T120-7 to Become Weeds of Agriculture or be Invasive of Natural Habitats

The biology of sugar beet (*Beta vulgaris*), described in Bio2002-01, shows that unmodified plants of this species are not invasive of unmanaged habitats in Canada. According to the information provided by Aventis CropScience Canada Inc., the sugar beet lines derived from Event T120-7 were determined not to be different from their counterparts in this respect.

The CFIA evaluated data submitted by Aventis CropScience Canada Inc. on the biology of sugar beet lines derived from Event T120-7, and determined that seed germination, plant vigour, flowering period, time to maturity, and overwintering capacity was comparable to unmodified sugar beet counterparts.

No competitive advantage was conferred to these plants, other than that conferred by tolerance to glufosinate ammonium herbicide. Tolerance to glufosinate ammonium will not, in itself, render sugar beet 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 sugar beet lines derived from Event T120-7 have no altered weed or invasiveness potential compared to currently commercialized sugar beets.

**2. Potential for Gene Flow from Lines Derived from Event T120-7 to Wild Relatives Whose Hybrid Offspring may Become more Weedy or More Invasive**

The biology of sugar beet, as described in Bio2002-01, indicates that there are no wild relatives in Canada that can hybridize with sugar beet.

The CFIA therefore concludes that gene flow from sugar beet lines derived from Event T120-7, to wild 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 sugar beet is not a plant pest in Canada (Bio2002-01). In addition, agronomic characteristics of the modified sugar beet were shown to be comparable to those of the unmodified counterpart, and indicate that the growth characteristics of sugar beet was not inadvertently altered. Glufosinate ammonium is commonly used for chemical fallow production and tolerant sugar beet volunteer plants will not be controlled by the herbicide. Despite the tolerance to glufosinate ammonium, volunteers can still be managed by growers using alternative herbicides with different modes of action, or by control methods that do not involve the use of herbicides. Field observations did not indicate modifications of disease and pest susceptibilities.

The CFIA has therefore determined that the sugar beet lines derived from Event T120-7 do not display any altered plant pest potential.

**4. Potential Impact on Non-Target Organisms**

The detailed characterization of the novel genes and resulting enzymes, as briefly summarized in Part III of the present document, has led to the conclusion that the expression of the novel proteins do not result in altered toxic or allergenic properties.

The enzymes are rapidly inactivated in mammalian stomach and intestinal fluids and do not confer resistance to agricultural pests. Searches of the amino-acid sequence databases revealed no significant homology of the novel proteins with known toxins or allergens.

Based on the above, the CFIA has determined that the unconfined release of the sugar beet lines derived from Event T120-7 will not result in altered impacts on interacting organisms, including humans, compared to current varieties.

## 5. Potential Impact on Biodiversity

The sugar beet lines derived from Event T120-7 have no novel phenotypic characteristics which would extend its use beyond the current geographic range of sugar beet production in Canada. Since sugar beet has no wild relatives in Canada that it can outcross to, there will be no transfer of novel traits to unmanaged environments. In addition the novel traits were determined to be safe to non-target organisms.

The use of broad spectrum herbicides has the intended effect of reducing local weed populations within agricultural fields and this may reduce local weed species biodiversity, and possibly other trophic levels which utilize these weed species. It must be noted however that reduction in weed biodiversity in agricultural fields is not unique to the use of PNTs, and is a common factor in virtually all modern agricultural systems.

The CFIA has therefore concluded that the potential impact on biodiversity of the sugar beet lines derived from Event T120-7 does not differ from conventional sugar beets.

## 6. Potential for the Development of Multiple Herbicide Tolerant Volunteers and Herbicide Tolerant Weeds

If there is general adoption of several different crop and specific herbicide weed management systems, then the potential exists for the development of crop volunteers with a combination of novel tolerances to different herbicides. As a result, this technology should be managed as part of an integrated approach which may include currently available weed control products with alternate modes of action, or alternative methods of weed control. Of additional note is the use several crop species in rotation which all rely on tolerance to the same herbicide. The continued use of a specific herbicide may provide significant selective pressure for the potential development of herbicide resistant weeds. 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 herbicide tolerant crop volunteers as well as tolerant weed populations.

The CFIA is currently working with the Pest Management Regulatory Agency of Health Canada to develop strategies which address the issues of multiple herbicide resistant volunteers and herbicide tolerant weeds.

# V. Nutritional Assessment Criteria for Use as Livestock Feed

## 1. Nutritional Composition

Beet pulp and molasses are the major feed byproducts of sugar beets used in livestock feeding. Beet pulp could be used at up to 20%, while molasses could be included in feeds at up to10% in ruminant rations. Both beet pulp and molasses could be used in horse rations at 10% of the diet. Beet tops (leaves) could be grazed by ruminants after harvest of sugar beets. Whole root is not commonly used as livestock feed. Sucrose could be used in all livestock rations at a maximum of about 5%. Sugar beet byproducts including condensed Steffen filtrate, Condensed separator byproduct, and molasses yeast solubles could be used in all livestock rations.

The applicant submitted two studies on nutrient composition. In the first study, proximate, amino acid, mineral and sugar analyses of compound leaf and root samples from each of 32 lines of conventional sugar beets and two hybrids derived from Event T120-7 grown in Europe were conducted. This study showed that for the leaves, there was no difference in nutrient composition between the control and the hybrid lines. In the tap root samples, there were no differences in proximate analysis between the control and the T120-7 hybrids. Of a total of 63 variables measured, three of them (Ca, Mn, Arg) were observed to be different in the T120-7 hybrids compared with the control lines.

The second study was conducted using US grown sugar beets. Sugar beet roots and tops from T120-7 vs. a control non-transgenic variety from seven sites in three locations (North Dakota, California, Idaho) were analysed. In this study, there were overall no differences between the T120-7 lines and the control. Analysis by location showed small but statistically significant differences in Ca, fibre in the roots, and Na in the leaves in one location, but these differences were not consistent with other locations or with the differences shown in the first study. Further analysis of processed fractions i.e., pulp, molasses and refined sugar from the California location were also conducted, and confirmed that there were no nutritionally significant differences between the T120-7 lines and the control line.

Beet pulp, beet molasses and other sugar beet byproducts are approved for use in livestock feeds, and these ingredients are listed in Schedule IV, Part 1 of the Feeds Regulations. As lines derived from Event T120-7 have been demonstrated to be nutritionally equivalent to traditional varieties of sugar beets, this line can also be used in livestock feeds.

**2. Anti-Nutritional Factors**

Sugar beet is not known for the production of significant levels of anti-nutritional or other endogenous toxins, and the introduction of genetic elements related to herbicide tolerance would not be expected to induce their synthesis.

The CFIA has therefore concluded that the nutritional composition of sugar beet lines derived from Event T120-7 do not differ from that of conventional sugar beets.

## VI. Regulatory Decision

Based on the review of data and information submitted by Aventis CropScience Canada Inc., and through comparisons of beet lines derived from Event T120-7 with unmodified sugar beet counterparts, the Plant Biosafety Office of the Plant Health and Production Division, CFIA has concluded that the novel genes and their corresponding traits do not confer to these plants any characteristic that would result in unintended environmental effects as a result of 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 does not in itself raise any concerns regarding the safety or nutritional composition of lines lines derived from Event T120-7. Sugar beet lines derived from Event T120-7 have been assessed and found to be equivalent to traditional sugar beet varieties in terms of safety and nutritional composition. Sugar beets lines derived from Event T120-7 and their byproducts are therefore considered to meet present ingredient definitions, as per Schedule IV, Part 1 of the Feeds Regulations, and are approved for use as livestock feed ingredients in Canada.

If at any time, Aventis CropScience Canada Inc. becomes aware of any information regarding risk to the environment, including the development of ECB resistance or risk to human or animal health that could result from release of these materials in Canada, or elsewhere, Aventis CropScience Canada Inc. will immediately provide such information to CFIA. On the basis of such new information, CFIA will re-evaluate the potential impact of the proposed feed use and environmental release and will re-evaluate its decision with respect to the livestock feed and environmental release authorizations of these sugar beet lines.

**Unconfined release into the environment and use as livestock feed of the sugar beet lines derived from Event T120-7 are therefore authorized as of January 9, 2001. Any**

**other sugar beet 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 sugar beets, in terms of their potential environmental impact and livestock feed safety and efficacy.**

Please refer to Health Canada's Decisions on Novel Foods for a description of the food safety assessment of sugar beet lines lines derived from Event T120-7. The food safety decisions are available at the following Health Canada web site:

http://www.hc-sc.gc.ca/food-aliment/english/subjects/novel_foods_and_ingredient/novel_foods_and_ingredient.html