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Agence Canadienne d'inspection des aliments

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Decision Document DD2002-35
Determination of the Safety of Monsanto Canada Inc.'s Roundup Ready™ Corn (*Zea mays* L.) Line 603

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 Biosafety Office (PBO) of the Plant Health and Production Division and the Feed Section of the Animal Health and Production Division have evaluated information submitted by Monsanto Canada Inc. This information is in regard to the glyphosate herbicide tolerant corn line 603. 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 603 is therefore authorized as of March 7, 2001. Any other corn 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 603 corn line is subject to the same phytosanitary import requirements as its unmodified counterparts.

Please note that the Health Canada evaluation of food safety has been addressed separately from this review.

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

Designation(s) of the PNT: Line 603

Applicant: Monsanto Canada Inc.

Plant Species: *Zea mays* L.

Novel Trait(s): Herbicide Tolerance (glyphosate).

Trait Introduction Method: Microprojectile bombardment of corn cells.

Proposed Use of PNT's: Production of *Z. mays* for seed, meal and oil for human consumption and grain, cobs, oil, meal, and other by-products 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 AW x CW, containing an *epsps* gene, coding for 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS), 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. The *epsps* coding sequence from *Agrobacterium sp.* strain CP4 (i.e., CP4 EPSPS), was inserted into cells of a corn cell culture line. Cells which produced the CP4 EPSPS protein were identified by tolerance to exposure to the herbicide glyphosate. The CP4 EPSPS protein imparts reduced sensitivity to glyphosate herbicide at the site of action of the herbicide in the chloroplast. The CP4 *epsps* coding sequence is fused to chloroplast transit peptide sequence which directs the translated protein to the chloroplast, the site of amino acid biosynthesis.

Monsanto Canada Inc. has provided data on the identity of line 603, 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 amino acid sequence. The CP4 EPSPS protein from corn was shown to be equivalent to the protein produced in an *Escherichia coli* expression system developed to produce the protein. The *E. coli* produced CP4 EPSPS was used to generate sufficient quantities of pure protein for safety studies. References to relevant scientific publications were included in the submission.

Plants of corn line 603 were field tested in the USA in 1997, 1998 and 1999 and in Canada, USA, Argentina, France and Italy in 2000.

Agronomic characteristics of corn hybrids derived from line 603 such as seed germination, vegetative vigour (plant height), 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 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 of the PNT 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 PNT to become plant pests,
- potential impact of the PNT or their gene products on non-target species, including humans, and
- potential impact of the PNT 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 the regulatory directive; Dir95-03. The Feed Section has considered:

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

III. Description of the Novel Trait

1. Development Method

The corn line 603 was generated using microprojectile bombardment and a gel-isolated DNA fragment, designated PV-ZMGT32L, which contained the *epsps* gene from *Agrobacterium*

sp. strain CP4 (CP4 EPSPS). The plasmid vector was constructed to contain two adjacent plant gene expression cassettes, each containing a single copy of the *epsps* gene. In the first gene cassette, the CP4 *epsps* coding sequence was under the control of a rice actin promoter and intron while the second cassette utilized an enhanced CaMV 35S promoter (e35S) and intron from the maize heat-shock protein. No antibiotic marker genes are present in corn line 603.Glyphosate tolerant transformed cells were selected, then cultured in tissue culture medium for plant regeneration.

2. Glyphosate Tolerance

- A gene derived from an *Agrobacterium* sp. strain CP4 (CP4 *epsps*) which imparts field level tolerance to glyphosate, the active ingredient in Roundup® herbicide, was introduced into corn line 603.

- A plant-derived coding sequence expressing an optimized chloroplast transit peptide was fused to the CP4 *epsps* coding sequence. This peptide facilitates the import of the newly translated EPSPS enzyme into the chloroplast, the site of amino acid biosynthesis.

- EPSPS is an enzyme involved in the shikimic acid metabolic pathway which is essential for the production of the aromatic amino acids. The native corn EPSPS enzyme is sensitive to glyphosate. The herbicide disrupts the shikimic acid pathway, leading to growth suppression or death of the plant. The CP4 EPSPS version of this enzyme is expressed in corn line 603, and confers glyphosate tolerance since it continues to catalyze the production of aromatic amino acids in the presence of glyphosate due to a reduction in the binding of glyphosate to the CP4 EPSPS in comparison to the native corn EPSPS.

- A gene coding for CP4 *epsps* was introduced into *E. coli* and the resulting enzyme compared to the CP4 EPSPS produced by corn line 603, and the EPSPS expressed in Monsanto company's Roundup-Ready™ soybeans by western blot. Roundup-Ready™ soybeans were previously approved by the CFIA for unconfined environmental release The enzymes expressed from all sources were shown to be identical immunologically and electrophoretically. The *E. coli* CP4 EPSPS derived enzyme was used to evaluate enzyme kinetics, to perform toxicology studies and as a standard in the determination of protein expression within the modified plant.

- The expression of the novel enzyme in the plant was quantified by enzyme-linked immunosorbent assay (ELISA). The CP4 EPSPS protein was expressed at low levels as

based on evaluation of levels present in forage and in grain from corn line 603 plants sampled from both replicated and non-replicated sites in 1998. ELISA assay demonstrated that the mean CP4 EPSPS protein levels were 25.6 ug/g fresh weight in forage and 10.9 ug/g fresh weight in grain.

- The EPSPS protein was labile to digestion. The enzyme was completely digested within 15 seconds in simulated gastric fluid (pepsin) and within 10 minutes in simulated intestinal fluid (pancreatin), as demonstrated by western blots.

- An acute mouse gavage study demonstrated that the CP4 EPSPS is not toxic. No treatment related adverse effects were observed in animals administered CP4 EPSPS protein by oral gavage at doses up to 572 mg/kg.

- The amino acid sequence of the CP4 EPSPS protein shares no structurally significant sequence similarity to known toxins, allergens or gliadins (allergins known to be present in wheat and other grains excluding corn). A database of 4,677 protein toxin sequences was assembled from public databases and compared to the amino acid sequence of the EPSPS. Using the FASTA alignment tool, no biologically relevant sequence similarities were detected between EPSPS and known toxins. A database of 567 allergen and gliadin sequences was constructed from public domain databases. No significant sequence similarities between CP4 EPSPS and known allergens and gliadins were identified. EPSPS is an enzyme present in many foods with a long history of safe use in Canada, and therefore would not be expected to be toxic or allergenic.

3. Stable Integration into the Plant's Genome

Southern blot analysis of the transformed corn line indicate the presence of a single insertion site, with one complete copy of the CP4 insert containing the two *CP4 epsps* gene cassettes.

Southern blot data was presented from two generations of corn line 603. The Southern blots showed identical banding patterns demonstrating the stable insertion of the genetic elements into the genome of corn line 603. Data was also presented which demonstrated several generations of corn line 603 continue to display tolerance to glyphosate herbicide.

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*) as 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., line 603 and derived corn hybrids were determined not to be significantly 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 line 603, 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 competitive advantage was conferred to these plants, other than that conferred by tolerance to glyphosate herbicide. Tolerance to glyphosate 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 corn weediness or invasiveness, led the CFIA to conclude that the corn line 603 has no altered weed or invasiveness potential compared to currently commercialized corn.

A longer term consideration, 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 tolerances to different herbicides. This necessitates the management of this technology as a part of an integrated approach which may include currently available weed control products with alternate modes of action. 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.

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 corn.

The CFIA therefore concludes that gene flow from corn line 603 to corn relatives is not possible in Canada.

3. Altered Plant Pest Potential

The intended effect 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 in such a way as to impact on the plant pest potential of corn. Despite glyphosate tolerance, line 603 volunteers can still be managed by growers using alternative herbicides with different modes of action, or cultivation practices which do not involve the use of herbicides. Field observations did not indicate modifications of disease or pest susceptibilities.

The CFIA has therefore determined that the 603 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 CP4 EPSPS protein is not a known toxin, does not confer resistance to agricultural pests and is commonly found in a wide variety of plants and micro-organisms with a history of safe use. Additionally, mouse acute gavage tests demonstrated the lack of acute oral toxicity of the CP4 EPSPS protein.

Based on the above, the CFIA has determined that the unconfined release of corn line 603 will not result in altered impacts on non-target organisms, including humans, compared to current corn varieties.

5. Potential Impact on Biodiversity

Line 603 expresses no novel phenotypic characteristics which could extend its use beyond the current geographic range of corn production in Canada. Since corn does not out cross to wild relatives in Canada, there will be no transfer of novel traits to species in unmanaged environments. In addition the novel trait was determined to pose minimal risks 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 corn line 603 is equivalent to that of currently commercialized corn varieties.

V. Nutritional Assessment Criteria for Use as Livestock Feed

1. Nutritional Composition of the PNT

Comparisons of protein, fat, ash, moisture, fibre, carbohydrates, amino acids, fatty acids, vitamin E, and minerals (Ca, Cu, Fe, Mg, Mn, P, K, Na and Zn) of corn grain and protein, fat, ash, moisture, fibre and carbohydrates in forage from the PNT line vs. its parent line and commercial non-PNT varieties were made. In total, 51 different compositional components were evaluated. In both the grain and the whole plant, there were occasional significant differences in individual trials between the PNT and the non-PNT in various compositional parameters. Only one nutrient showed statistically significant differences across all replicates and locations; stearic acid in grain. Stearic acid levels in corn line 603 were found to differ by 3.7% to 5.1% in comparison with the unmodified counterpart. However, stearic acid levels in corn line 603 are still within the normal range for corn.

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 603 is substantially equivalent to traditional corn varieties.

2. Anti-Nutritional Factors

Corn is not known for the production of significant levels of anti-nutritional factors and the introduction of genetic elements which confer tolerance to the herbicide glyphosate would not be expected to induce their synthesis. Trypsin inhibitor and phytic acid are known to be produced by corn at low levels, and the levels of these compounds in line 603 were found to be equivalent to levels found in the control lines. The genetic modification, therefore did not alter the expression of endogenous anti-nutritional factors.

VI. Regulatory Decision

Based on the review of data and information submitted by Monsanto Canada Inc., and through comparisons of corn hybrids derived from line 603 with unmodified corn counterparts, the Plant Biosafety 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 significant 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 does not in itself raise any concerns regarding the safety or nutritional composition of line 603. 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. Line 603 and corn hybrids derived from it have been assessed and found to be substantially equivalent to traditional corn varieties. Line 603 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 603 is therefore authorized as of March 7, 2001. Any other corn 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 and efficacy.

The corn line 603 is subject to the same phytosanitary import requirements as its unmodified counterparts.

Please refer to Health Canada's Decisions on Novel Foods for a description of the food safety assessment of corn line 603. 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