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Plant Health and Production Division,  
Plant Biosafety Office

## Decision Document DD96-09: Determination of Environmental Safety of Event 176 Bt Corn (*Zea mays* L.) Developed by Ciba Seeds and Mycogen Corporation

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](#).

Agriculture and Agri-Food Canada (AAFC), specifically the Plant Biotechnology Office (PBO) and the Feed Section of the Plant Health and Production Division, with input from the Plant Health Risk Assessment Unit, AAFC, and advice from the Pest Management Regulatory Agency of Health Canada, has evaluated information submitted by Ciba Seeds of Ciba-Geigy Canada Ltd. and cited by Mycogen Corporation regarding Event 176 *Bt* corn. This plant was transformed with genes conferring resistance to the European Corn Borer (ECB) and tolerance to glufosinate ammonium as a selectable marker. AAFC has determined that this plant with novel traits (PNT) should pose no concerns with respect to environmental safety, the safety of livestock consuming feed derived from the PNT, and is considered substantially equivalent to corn products currently approved as livestock feed.

**Unconfined release into the environment, including use as livestock feed of Event 176 *Bt* corn and other *Z. mays* lines derived from it, without the introduction of any other novel traits, is therefore considered safe.**

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

<i>Designation of the PNT:</i>	Event 176 Bt corn
<i>Applicant:</i>	Ciba Seeds of Ciba-Geigy Canada Ltd.; Mycogen Corporation.
<i>Plant Species:</i>	<i>Zea mays</i> L. (corn/maize)
<i>Novel Traits:</i>	Resistance to European Corn Borer ( <i>Ostrinia nubilalis</i> ); tolerance to glufosinate ammonium herbicide.
<i>Trait Introduction Method:</i>	Microprojectile bombardment of immature embryos
<i>Proposed Use of PNT's:</i>	For cultivation as hybrid grain corn in existing corn growing areas of Canada.

## II. Background Information

Ciba Seeds of Ciba-Geigy Canada Ltd. (Ciba Seeds) and Mycogen Corporation (Mycogen) have jointly developed a corn line that is resistant to European Corn Borer (ECB), an occasional pest of corn in Canada. This corn line, designated Event 176 Bt corn (referred to as Event 176 in this document), has been transformed using recombinant DNA technology and microprojectile bombardment of embryos, to produce an insecticidal protein, from *Bacillus thuringiensis* ssp. *kurstaki*, active against certain species of Lepidoptera, an insect order to which butterflies and moths belong, including ECB. Specifically, this protein is a truncated form of the Cry1Ab  $\delta$ -endotoxin and protects this corn against feeding damage caused by ECB larvae. In addition, Event 176 was transformed with a gene that confers tolerance to the herbicide glufosinate ammonium, used to select transformed plants at a very early development stage.

Through traditional breeding, Event 176 will be used to introduce resistance to ECB into corn hybrids adapted to Canadian Corn Heat Unit areas, mainly in Ontario and Quebec. Corn hybrids derived from Event 176 were tested in Ontario, under confined conditions, in 1993, 1994 and 1995. In August 1995, the Environmental Protection Agency of the United States conditionally approved the commercialization of field corn derived from Event 176, until the year 2000; conditions of this approval relate to the development of data in the area of resistance management research and to monitoring for the potential development of ECB populations resistant to the Cry1Ab  $\delta$ -endotoxin. Health Canada has determined that food derived from this corn is substantially equivalent to that derived from currently commercialized corn (December 19, 1995).

Ciba Seeds has submitted data and information on the identity of Event 176 and characterization of the resistance to ECB, and herbicide tolerance traits. Dietary toxicity studies on insects, birds and mammals, and *in vitro* digestibility studies were conducted using the corn-produced insecticidal protein. Data from agronomic and efficacy studies conducted in the United States, and data from proximate analysis (total ash, fat, fibre, moisture, protein and starch), fatty acid profiles, carotenoid levels (xanthophylls and  $\beta$ -carotene), and amino acid composition were also reviewed.

Information pertaining to the efficacy of resistance to ECB and to the management of potential Cry1Ab resistant ECB strains were submitted for review by the Pest Management Regulatory Agency (PMRA) of Health Canada, which acts in an advisory capacity to the Plant Biotechnology Office, AAFC, regarding plants with novel pesticidal properties. The potential for development of ECB resistance to the insecticidal protein was discussed, and an insect resistance management plan will be implemented by Ciba Seeds and Mycogen.

The Plant Biotechnology Office of the Plant Health and Production Division, with input from the Plant Health Risk Assessment Unit on behalf of the Plant Protection Division, AAFC, has reviewed the information submitted by Ciba Seeds and cited by Mycogen, for the determination of environmental safety, in light of the assessment criteria described in the regulatory directive [Dir94-08](#) *Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits*:

- 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 Plant Health and Production Division, AAFC, has also reviewed the information submitted by Ciba Seeds and cited by Mycogen in light of the assessment criteria for determining safety and efficacy of livestock feed, as described in the regulatory directive [Dir95-03](#) *Guidelines for the Assessment of Livestock Feed from Plants with Novel Traits*:

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

### III. Description of the Novel Traits

#### 1. Resistance to European Corn Borer (ECB)

- *Bacillus thuringiensis subsp. kurstaki* (B.t.k.) is an endospore-forming, gram-positive, soil-borne bacterium. In its spore forming stage, it produces several insecticidal protein crystals, including the  $\delta$ -endotoxin Cry1Ab which is active against certain lepidopteran insects such as ECB, Spruce Budworm, Tent caterpillar, Gypsy moth, Diamondback moth, Cabbage looper, Tobacco budworm, and Cabbage worm. The protein has been repeatedly shown to be non-toxic to humans, other vertebrates and beneficial insects. B.t.k.-based foliar insecticides have been registered for over 30 years in Canada and have a long history of safe use.
- A synthetic *cry1Ab* gene, derived from B.t.k. strain HD-1, coding for a truncated form of the Cry1Ab  $\delta$ -endotoxin, and modified to enhance its expression in corn was developed. The synthetic gene has approximately 65% homology at the nucleotide level with the native gene. The truncated Cry1Ab protein contains the insecticidal region of the native Cry1Ab. The insecticidal protein binds to receptor sites in the epithelium of the susceptible insect's mid-gut, resulting in the formation of pores,

disruption of the osmotic potential of the cells, cessation of feeding and the eventual death of the insect.

- Event 176 was transformed with two of these synthetic *cry1Ab* genes. One gene is linked to a specific promoter which confers expression in green tissue, while the other is linked to a pollen-specific promoter, resulting in expression in pollen. Expression of the Cry1Ab protein in green tissues is intended to render the plant resistant to first generation ECB larvae feeding on leaves. Expression in pollen is intended to target second generation ECB larvae, which are known to feed on pollen.
- Cry1Ab production was quantified in leaves and pollen among three genotypes. Expression in leaves, across genotypes, ranged from 0.596 to 1.159 µg/g (fresh weight) in seedlings, 0.530 to 3.029 µg/g (f.w.) at anthesis, 0.442 to 0.471 µg/g (f.w.) at physiological maturity, and 0.066 to 0.225 µg/g (f.w.) at senescence. Maximum expression in leaves was detected at either the vegetative or anthesis stages, depending on the genotype tested, and expression in all genotypes declined as plants senesced. Expression in pollen, across genotypes, ranged from 1.137 to 2.348 µg/g (f.w.). Expression of Cry1Ab in roots (0.008 µg/g f.w.), pith (<0.008 µg/g f.w.), and kernels (<0.005 µg/g f.w.) was below levels of quantification. Whole plant expression at anthesis, as a percentage of total protein ranged from 0.00025 to 0.0014 % (f.w.).
- Biological activity of the truncated Cry1Ab protein produced in Event 176 leaves was compared to that of the native B.t.k. Cry1Ab on ECB, and other lepidopteran species. While quantitative comparisons were not possible, a similar rank order of sensitivity was observed to the Event 176 and native B.t.k. proteins among the tested lepidopteran species, other than Southwestern Corn Borer which did not feed on the Event 176 leaf extract. Non-susceptible species were unaffected by either the Event 176-expressed or native Cry1Ab proteins.
- Western blot analyses of Event 176-expressed Cry1Ab, and of the native protein, displayed similar bands at approximately 65 kDa. Three additional immunoreactive proteins weighing approximately 60, 40 and 36 kDa were also detected in the leaves, but not in the pollen. It was suggested that these may represent breakdown products resulting from intrinsic proteolysis in Event 176 leaves. Toxicological studies were conducted using corn-expressed leaf and pollen Cry1Ab protein, thus the potential toxicity of the additional peptides detected in the leaves was assessed.
- No evidence was found of post-translational modifications such as acetylation, glycosylation or phosphorylation in Event 176-expressed *cry1Ab* gene.
- Cry1Ab protein from Event 176 leaves was subjected to *in vitro* digestibility studies under simulated mammalian gastric conditions and was shown to be digested as conventional dietary protein.

## 2. Glufosinate Ammonium Herbicide Tolerance

- The glufosinate ammonium tolerance gene (*bar* gene), derived from the common soil bacterium *Streptomyces hygroscopicus*, codes for phosphinothricin acetyltransferase (PAT), and is linked to a constitutive promoter active in all plant tissues except pollen. Phosphinothricin, a glutamine synthetase inhibitor, is the active moiety of glufosinate ammonium. The herbicidal activity of phosphinothricin is characterized by the inhibition of glutamine synthetase resulting in the accumulation of lethal amounts of ammonia in the plant. PAT catalyses the acetylation of phosphinothricin, thus eliminating its herbicidal activity.
- PAT was shown to act specifically on phosphinothricin, since no other activity was observed on other common acetyltransferase substrates, including pyruvate, choline or serine.
- A sequence similarity search for the PAT protein revealed similarity only to other phosphinothricin resistance gene products and no similarity was found to known sequenced toxins.
- Southern blot analysis revealed that the *bar* gene is present in transformed tissue, however, PAT protein levels were below the limit of quantification in tested tissues (<0.2 ppm in leaf, root, pith and whole plant samples).
- *In vitro* digestibility studies, under simulated mammalian gastric conditions, were conducted with *E. coli* expressed PAT and revealed that this protein is digested as

conventional dietary protein.

- This trait was introduced to permit the identification of transformed embryos on selective medium and is also used to track genes during plant breeding.

### 3. Development Method

- Two synthetic *cry1Ab* genes were cloned into a single plasmid vector. A second vector contained the herbicide tolerance gene (*bar*). These genes were introduced into the corn by microprojectile bombardment of immature embryos. Southern blot analysis indicated that there may be two or more copies of each plasmid integrated into the genome of Event 176. An ampicillin resistance gene, regulated by a bacterial promoter, also present in the vectors used to transform Event 176, was not expressed in either leaf tissue or pollen as confirmed by assays and Northern blot analysis. Other regulatory DNA elements inserted to enhance expression levels of *cry1Ab* did not code for any other protein.

### 4. Stability of Insertion of the Traits

- The production of Cry1Ab and PAT proteins, in leaves and pollen of greenhouse-grown plants was determined to be stable over four successive backcross generations. Segregation analyses indicated that the resistance to ECB and herbicide tolerance traits co-segregate as linked Mendelian traits. A study of 3240 plants indicated that only five plants (0.15%) were identified as being tolerant to glufosinate ammonium but susceptible to damage by ECB larvae.

## IV. Assessment Criteria for Environmental Safety

### 1. Potential of the PNT to Become a Weed of Agriculture or Become 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.

AAFC evaluated information submitted by Ciba Seeds and cited by Mycogen, on the reproductive and survival biology of hybrids derived from Event 176, and determined that growth habit, vegetative vigour, overwintering capacity, flowering period, plant height, insect and disease susceptibility, other than resistance to ECB, and grain yield and quality, were substantially equivalent to non-transformed isogenic lines. No genes for cold tolerance or winter hibernation were inserted in Event 176.

No competitive advantage was conferred to Event 176, other than resistance to ECB and tolerance to glufosinate ammonium herbicide. Resistance to ECB, in itself, will not render corn weedy or invasive of natural habitats since none of the reproductive or growth characteristics were modified. Tolerance to glufosinate ammonium will not render corn weedy, since this herbicide is not presently used in crop rotation cycles. Any glufosinate tolerant corn volunteer plants can be easily managed by either mechanical means or by the use of other available herbicides.

**NOTE:** A longer term concern, if there is general adoption of several different crop and specific herbicide weed management systems, is the development of crop volunteers with novel tolerances to specific herbicides. This could result in the loss of the use of these herbicides in some crop rotation cycles. Agricultural extension personnel, in both the private and public sectors, should therefore promote careful management practices for growers who use any of these herbicide tolerant crops.

The above considerations, together with the fact that the novel traits have no intended effects on weediness or invasiveness, led AAFC to conclude that Event 176 does not possess altered weed or invasiveness potential compared to currently commercialized corn.

## **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 freely hybridize with *Zea mays*. AAFC therefore concludes that gene flow from Event 176 to corn relatives is not possible in Canada.

## **3. Altered Plant Pest Potential**

The novel traits in this plant do not inherently confer to Event 176, and hybrids derived from it, any potential to become a plant pest and *Zea mays* is not a plant pest in Canada ([Dir94-11](#)). Results from proximate analysis, and analyses of fatty acid profiles, carotenoids, and amino acid profiles showed that Event 176 was substantially equivalent to non-transformed isogenic lines, and indicated that there were no unintended effects on metabolic pathways. Trials were conducted comparing disease and pest susceptibility of Event 176 with non-transformed isogenic lines. Visual observations showed no differences in resistance or susceptibility to diseases such as Eyespot (*Kabatiella zeae*), Stewart's wilt (caused by various bacterial spp.), common rust (*Puccinia sp.*) and Gray leaf spot (*Cercospora sp.*). Non-Bt corn exhibited a higher incidence of Anthracnose stalk rot (*Colletotrichum sp.*) and Fusarium stalk rot (*Fusarium sp.*) when compared to Event 176. This may be due to less ECB-caused stalk damage in Event 176 resulting in reduced pathogenic infection.

AAFC has therefore determined, based on the above considerations, that Event 176, and hybrids derived from it, do not display any altered plant pest potential.

## **4. Potential Impact on Non-target Organisms**

European Corn Borer (ECB), the target insect, is controlled in Event 176 via the insects' consumption of green leaves and pollen, and only during specific growth stages when the Cry1Ab protein is present at sufficiently high levels. The relatively higher expression of Cry1Ab in green leaf tissue, at vegetative and anthesis stages, is intended to target first generation ECB larvae, while expression in pollen targets second generation larvae.

Ciba Seeds submitted data on the effect of Event 176 on populations of nontarget insects, specifically insect predators and parasites (of the orders Diptera, Hymenoptera and the family Coccinellidae), and insects (homopterans) that are parasitized and predated upon by these. Results indicated that Event 176 had no effect on the presence of these insects when compared to conventional non-Bt corn.

Dietary toxicity studies using Event 176-produced Cry1Ab protein were conducted on beneficial insects (honeybee larvae and ladybird beetles) and other non-target species (*Daphnia*, earthworms, and *Collembola*). None of these species were negatively affected by Event 176 in the dietary toxicity tests when compared to non-Bt corn. The original study on honeybee larvae did not yield conclusive data. Ciba Seeds and Mycogen submitted a revised honeybee dietary toxicity study in the document "Evaluation of the Dietary Effect(s) of Transgenic Bt Maize (Corn) Pollen on Honeybee Development" dated December 20, 1996. This study demonstrated that Event 176 has no insecticidal activity against honeybee larvae in comparison to non-Bt corn.

Negative effects were observed in *Collembola* fed the Event 176 leaf protein (5 % mortality at 0.088 mg Cry1Ab /kg soil) while *Collembolans* fed with non-Bt corn protein were not adversely affected. This level of Cry1Ab is approximately 10 times greater than the maximum

soil concentration that would occur if Event 176 plants were to be incorporated into the soil at anthesis. Corn is normally plowed under in the fall when plants have senesced, and the Cry1Ab protein in Event 176 is present at very low levels at this time. Furthermore, the ever-increasing practice of not tilling corn residues into the soil after harvest (commonly referred to as "no-till" or "conservation-till"), recommended for the conservation of soil, will minimize this exposure.

Acute oral toxicity studies were conducted with northern bobwhite quail and mice. These animals were fed Event 176-produced protein, and in the case of mice, were also fed bacterial-expressed protein. No mortality was observed and was not expected since Event 176-produced protein was shown to degrade very rapidly under simulated mammalian gastric conditions (enzymatic proteolysis at low pH).

The issue of pulmonary toxicity was taken into consideration by AAFC due to the presence of the Cry1Ab protein in Event 176 pollen. However, corn pollen is large (diameter: 73.4 to 92.6  $\mu\text{m}$ ), and is too heavy to be aspirated. Also, pulmonary toxicity studies using B.t.k. based foliar sprays showed that there were no effects attributable to the  $\delta$ -endotoxins. AAFC has therefore concluded that, based on the large size and weight of corn pollen, and, the history of safe use of B.t.k. based foliar sprays, there should be no concerns for human or mammalian pulmonary exposure to Event 176 pollen.

Based on the above, AAFC has determined that the unconfined release of Event 176, when compared to currently commercialized corn, will not result in significantly altered impacts on interacting organisms, including humans, with the exception of ECB and, possibly, Collembola. The impact on honeybees will be reported in a future addendum to this document.

## 5. Potential Impact on Biodiversity

Event 176 does not possess novel phenotypic characteristics which would extend the use of its hybrid derivatives 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.

At present, the use of chemical insecticides to control ECB is not recommended for grain corn produced in Canada, but is recommended in sweet corn given the higher cash value of the crop, and less consumer tolerance for insect-damaged cobs. Insecticides for the control of ECB in corn are only effective when the larvae first emerge and before these begin to move to the whorl of the plant and into the stalk. It is therefore not usually cost-effective to use insecticides to control ECB in grain corn. Current recommendations by provincial extension services for the control of ECB in grain corn are: to grow corn hybrids which have been traditionally bred for natural resistance to ECB, to avoid planting at densities that are too high for the hybrid chosen, to plant early to reduce infestations by the second generation and to harvest early in order to decrease losses from dropped ears and broken stalks. Despite the promotion of no-till practices in corn for soil conservation, it is still recommended to plow-down corn residues in the fall, in order to kill over-wintering larvae.

The cultivation of Event 176, as hybrid grain corn, will not result in any overall change in the use of chemical insecticides<sup>1</sup>, thus AAFC has concluded that the potential impact on biodiversity will be neutral.

**Note:** <sup>1</sup> At present there is very little chemical insecticide use in hybrid grain corn production in Canada. A current recommendation for the control of insect pests in hybrid grain corn is to practice crop rotation and this is presently being done with soybeans and other crops. Crop rotations help to break both disease and insect cycles. For example, the Ontario Ministry of Agriculture and Food and Rural Affairs (OMAFRA) recommends the use of crop rotation in order to control Corn Rootworm and advises the use of insecticides only if crop rotation is not practical and only in the second year of corn production. For the control of outbreaks of

Cutworm, OMAFRA recommends treatment with insecticides only in areas of the field that show evidence of cutworm feeding.

## 6. Potential for Development of ECB Resistance to the PNT

Hybrid corn derived from Event 176 produces insecticidal levels of Cry1Ab protein in leaves up until anthesis, after which levels declines to very low levels at senescence. Insecticidal levels are also produced in pollen. ECB may thus be steadily exposed to levels of Cry1Ab protein and this could lead to high selection pressure for resistant ECB individuals.

It is currently accepted that ECB has one or more generations a year in Canada. The number of ECB generations that will develop in any one season will be influenced by the environmental conditions in a given area, particularly temperature and daylength. The development of resistant ECB populations may also increase in areas with multi-generations.

A component of the Ciba Seeds/Mycogen resistance management strategy for Event 176 hybrids is the presence of unstructured refugia, i.e., nearby fields of non-*Bt* corn where susceptible insect populations will be maintained. Should resistant insects be selected in Event 176, they would be able to mate with susceptible insects from non-*Bt* corn resulting in Event 176-susceptible heterozygous offspring. The behaviour of ECB during mating is such that individuals migrate to grassy areas adjacent to corn fields to mate, hence increasing the likelihood that any resistant ECB individuals mate with susceptible ones. Initially there will exist sufficient unstructured non-*Bt* corn refugia and this may delay the development of resistance. Should the acreage of Event 176 hybrids become greater than the non-*Bt* hybrids, careful management resulting in the maintenance of non-*Bt* corn (structured refugia) may be necessary to provide the required non-*Bt* refugia. However, there does not exist consensus in the scientific community that the use of refugia will be effective; it is difficult to predict the extent and rapidity of resistance development without field validation of the proposed strategy. Ciba and Mycogen will establish baseline susceptibilities of ECB to Event 176 in the United States. This may provide a basis for evaluating any future reports of ECB resistance.

The development of resistance to the Cry1Ab protein in Event 176 in non-target insect pests, that may then cause further problems in other crops, warrants consideration. Two lepidopteran insects that could be exposed to Event 176 and that could develop resistance are Armyworm (*Pseudaletia unipuncta*) and Corn Earworm (*Helicoverpa zea*). Armyworm, a sporadic pest in Canada, feeds on corn and other crops such as forage grasses (e.g. timothy), wheat, oats and barley. Dipel®, a B.t.k. foliar spray, is currently registered for the control of a lepidopteran, European Skipper, but not Armyworm, in timothy. Should resistance to Cry1Ab protein occur in Armyworm as a result of exposure to Event 176, this will not, at present, impact on the use of this insecticidal protein. Corn Earworm (*Helicoverpa zea*) feeds on the silks and developing ears (pre-dough stage of kernels) of corn; their exposure to Cry1Ab protein would be at extremely low doses given the expression in these tissues in Event 176. Thus the risk of Corn Earworm developing resistance to Cry1Ab in Event 176 in Canada is low.

Cry1Ab is one of five  $\delta$ -endotoxins found in B.t.k. (strain HD-1) foliar sprays registered for the control of various lepidopterous pests (but not ECB) on crops such as peppers, timothy and tomatoes in Canada. A B.t.k. foliar spray (Bactospeine®) is currently registered for control of ECB in hybrid seed corn production. Thus, any ECB individuals having developed resistance to Cry1Ab through continued exposure to Event 176 may not be controlled in hybrid seed corn treated with Bactospeine®. ECB also attacks green peppers, however, Dipel® is currently registered only for the control of Cabbage Looper in this crop.

Evidence exists that selection for resistance to one or more  $\delta$ -endotoxins may also cause resistance to others - this phenomenon, known as cross-resistance, typically occurs when mechanisms of toxicity are similar (i.e. sharing same binding sites). The development of resistance to Cry1Ab in ECB could result in cross-resistance to other Cry1 (e.g. Cry1Ac)

proteins. This could result in the loss of other B.t.k. protein types that may be used for the control of ECB.

AAFC believes that these corn plants should therefore be responsibly managed and ECB populations monitored for the development of resistant populations in a regular and consistent manner.

AAFC also believes that sound management practices can reduce and delay the development of resistant ECB populations. Ciba Seeds and Mycogen Corporation have developed and will implement pest resistance management plans that include the following key components:

- The early detection of ECB populations resistant to the corn-expressed insecticidal protein is extremely important, thus:
  - Systematic monitoring for the development of such populations at selected sites will be conducted. These sites will be chosen based on their higher potential for resistance development, and will include sites with more than one ECB generation per growing season, areas of routinely heavier ECB infestations, and areas of highest product distribution. Detection tools will include visual field observations and, where needed, laboratory bioassays of plants and suspected resistant ECB.
  - Education tools will be provided to all growers and field managers. These will include information on product performance, integrated pest management practices, resistance management, the importance of establishing sound monitoring protocols, and instructions to contact Ciba Seeds/Mycogen if unexpected levels of ECB damage occur. Ciba Seeds/Mycogen will have procedures in place for responding to these reported instances of unexpected ECB damage. These procedures will include, where warranted, the collection of plant tissue and/or ECB, and use of appropriate, developed, bioassays to assess ECB susceptibility status of suspected Cry1Ab resistant individuals.
- Detection of any confirmed resistant ECB populations shall immediately be reported to AAFC and a procedure for control of resistant individuals must be available for immediate action.
- The strategy for ECB control using plants that produce a B.t.k. type insecticidal protein in green tissue and in pollen, and the use of insect refugia, has not been previously tested in the field on a large scale. Continued research in this area using sound science will be conducted.

All of the above information and data are to be made available to AAFC upon request. If at any time either Ciba Seeds or Mycogen become aware of any new information regarding risk to the environment, including risk to agriculture, such as the development of ECB resistance, or risk to animal or human health, that could result from the release of these materials in Canada, or elsewhere, Ciba Seeds and Mycogen will immediately provide such information to AAFC. On the basis of such new information, AAFC may re-evaluate the potential impact of the proposed release, and re-evaluate its decision.

## **V. Nutritional Assessment Criteria for Use as Livestock Feed**

### **1. Nutritional Composition of PNT**

Comparisons of protein, fat, fibre and ash concentration of corn grain and whole plant material from each PNT line and its respective parent line were made. In both the grain and the whole plant, there were occasional significant differences in fat and protein content between a PNT line and its respective nonmodified control, but these differences were inconsistent (ie., the PNT line was sometimes higher, and sometimes lower in fat or protein than its control). The grain analysis showed no differences between any PNT vs control line for either fibre or ash concentration. In the whole plant analysis, there were no differences in fibre content for any PNT vs. control line. Whole plant ash content of one PNT (564x526-176)

line was significantly higher than its control. The ash content of the grain of these two lines was not different.

Protein, fat, fibre and ash concentration of all lines were within the published range for corn. The observed variation described above are judged to be normal variation rather than due to the inserted novel trait. Agriculture and Agri-Food Canada has determined that Event 176 lines are 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 Event 176, would not be expected to induce their synthesis.

## VI. Regulatory Decision

Based on the review of data and information submitted by Ciba Seeds and cited by Mycogen, and through comparisons of Event 176 with unmodified corn counterparts, the Plant Biotechnology Office of the Plant Health and Production Division, AAFC, has concluded that the novel genes and their corresponding traits do not confer to Event 176 any characteristics 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 Plant Products Division has concluded that the novel traits do not in themselves raise any concerns regarding the safety or nutritional composition of Event 176. Grain corn and its various fractions, and corn oil are currently listed in Schedule IV of the *Feeds Regulations* and are, therefore, approved for use in livestock feeds in Canada. As Event 176 has been assessed and found to be substantially equivalent to traditional corn, Event 176 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, including feed use, of Event 176, and corn hybrids derived from it, but without the introduction of any other novel traits, is therefore authorized.**

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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 or the Feed Section at:

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