Decision Document DD2006-59

Determination of the Safety of Dow AgroSciences Canada Inc.'s Insect Resistant and Glufosinate - Ammonium Tolerant Corn (*Zea mays* L.) Event DAS-06275-8

This Decision Document has been prepared to explain the regulatory decision reached under the directive Dir94-08 *Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits* and its companion document BIO1994-11 *The Biology of Zea mays L. (Corn/Maize)* and the directive Dir95-03 *Guidelines for the Assessment of Novel Feeds: Plant Sources.*

The Canadian Food Inspection Agency (CFIA), specifically the Plant Biosafety Office and the Feed Section of the CFIA, have evaluated information submitted by Dow AgroSciences Canada Inc. This information is in regard to the glufosinate-ammonium tolerant, lepidopteran insect resistant corn event DAS-06275-8. The CFIA has determined that these plants with a novel trait (PNT) do not present altered environmental risk nor, as a novel feed, do they present livestock feed safety concerns when compared to currently commercialized corn varieties in Canada.

Unconfined release into the environment and livestock feed use of corn event DAS-06275-8 is authorized as of June 19, 2006. All its progeny and sister lines which have been derived from the original transformation event and their respective progenies, are also authorized for unconfined release and livestock feed, provided that: (i) no inter-specific crosses are performed, (ii) the intended uses are similar, (iii) based on characterization, these plants do not display any additional novel traits and are substantially equivalent, in terms of their specific use and safety for the environment and for human and animal health, to plants currently being cultivated, (iv) the novel genes are expressed at a level similar to that of the authorized line and (v) that insect resistance management requirements described in the present document are applied.

The corn event DAS-06275-8 is subject to the same phytosanitary import requirements as its unmodified counterpart.

Please note, that the livestock feed and environmental safety of PNTs and novel feeds are critical steps in the potential commercialization of these plant types. Other requirements, such as the evaluation of food safety by Health Canada, have been addressed separately from this review.

(*publié aussi en français*)

June 19, 2006

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

Plant Biosafety Office
Plant Products Directorate
59 Camelot Drive, Ottawa
Ontario K1A 0Y9
613-225-2342

Feed Section
Animal Health and Production Division
Animal Products Directorate
59 Camelot Drive, Ottawa
Ontario K1A 0Y9
613-225-2342

Canada

Table of Contents

I. Brief Identification of the Modified Plant

Designation(s) of the modified plant:	Bt Cry1F maize event DAS-06275-8, OECD identifier DAS-06275-8
Applicant:	Dow AgroSciences Canada Inc.
Plant Species:	Corn (*Zea mays* L.)
Novel Traits:	Resistance to lepidopteran pests of corn, including European Corn Borer (*Ostrinia nubilalis*.), corn earworm (*Helicoverpa zea*), fall army worm (*Spodopera frugiperda*) and black cutworm (*Agrotis ipsilon*). Tolerance to glufosinate-ammonium herbicide.
Trait Introduction Method:	*Agrobacterium*-mediated transformation.
Proposed Use of the modified Plant:	Production of corn for human consumption (wet mill products, dry mill products and seed oil) and oil, meal, grain, silage and other by-products for livestock feed. These materials are not intended to be grown outside the normal production area for corn in Canada.

II. Background Information

Dow AgroSciences Canada Inc. developed corn lines from event DAS-06275-8 resistant to certain lepidopteran pests and tolerant to the herbicide glufosinate-ammonium. This line, designated as corn event DAS-06275-8, was developed to provide a method to control yield losses from insect feeding damage caused by certain lepidopteran pests, as well as a method to control weeds in corn production.

Corn event DAS-06275-8 was developed using recombinant DNA technology, resulting in the introduction of bacterial genes conferring resistance to lepidopteran pests and tolerance to glufosinate-ammonium herbicide.

Dow AgroSciences Canada Inc. has provided data on the identity of corn event DAS-06275-8, a detailed description of the transformation method, the inserted genes and regulatory sequences, information on the gene insertion site, gene copy number and levels of gene expression in the plant, and the full amino acid sequences of the novel proteins. Each novel protein was identified, characterized and compared to the original donor bacterial proteins. An evaluation of their potential toxicity to livestock and non-target organisms and potential allergenicity to humans and to livestock was provided. Relevant scientific publications were also supplied.

Data obtained from confined field trials of corn event DAS-06275-8 conducted in 2002 was submitted to CFIA. Data generated from field trials in the United States and Chile were also used to support the application.

Agronomic characteristics of corn hybrids derived from corn event DAS-06275-8 such as early stand establishment, vegetative vigour, time to maturity, flowering period, susceptibility to various corn pests and pathogens, and seed production, were compared to those of unmodified corn counterparts and modified corn counterparts containing the authorized transformation line 1507. These comparisons contributed to the safety assessment.

The Plant Biosafety Office, 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 Directive 94-08 (Dir94-08), entitled "*Assessment Criteria for Determining Environmental Safety of Plants With Novel Traits*". The PBO has considered:

- potential of corn event DAS-06275-8 to become a weed of agriculture or be invasive of natural habitats,
- potential for gene flow from corn event DAS-06275-8 to wild relatives whose hybrid offspring may become more weedy or more invasive,
- potential for corn event DAS-06275-8 to become a plant pest,
- potential impact of corn event DAS-06275-8 or it's gene products on non-target species, including humans, and
- potential impact of corn event DAS-06275-8 on biodiversity.

The Feed Section, 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 Directive 95-03 (Dir95-03), entitled "*Guidelines for the Assessment of Novel Feeds: Plant Sources*". The Feed Section has considered:

- potential impact of corn event DAS-06275-8 on livestock nutrition, and
- potential impact of corn event DAS-06275-8 on livestock and workers/by-standers.

III. Description of the Novel Traits

1. Development Method

Corn event DAS-06275-8 was produced via *Agrobacterium*-mediated transformation of the corn line Hi-II, derived from a cross of A188 x B73. The synthetic *cry1F* gene and the *bar* gene carried by a binary plasmid vector were introduced by a disarmed *Agrobacterium tumefaciens* strain into immature embryos. Transformants were selected based on tolerance to the herbicide glufosinate-ammonium in the culture

medium. Event DAS-06275-8 was identified as a successful transformant and was chosen for further development.

2. Resistance to Lepidoperan pests of Corn

Bacillus thuringiensis var. *aizawai* is a common gram-positive soil-borne bacterium. In its spore forming stage, it produces several insecticidal protein crystals, including the δ-endotoxin Cry1F protein that is active against certain Lepidopteran insect pests, such as European Corn Borer. This protein has been shown to be non-toxic to humans, other vertebrates and non-lepidopteran invertebrates. Foliar insecticides based on Cry endotoxins (generally known as B.t.) have been registered for over 30 years in Canada and have a long history of safe use.

A synthetic *cry1F* gene was developed to maximize it's expression in corn, and introduced into the Hi-II hybrid. The gene codes for a truncated, core insecticidal protein with a very high degree of similarity to the *B. thuringiensis* var. *aizawai* native protein. The Cry1F protein expressed in event DAS-06275-8 is identical to the Cry1F protein expressed in corn line 1507, that is authorized for unconfined release and livestock feed in Canada (Decision Document DD2002-41). The protein expressed by *B. thuringiensis* var. *aizawai* is insecticidal to some lepidopteran species after cleavage in the insect's gut to a bio-active, trypsin resistant core. Insecticidal activity is believed to depend on the binding of the active fragment to specific receptors present in susceptible insects on midgut epithelial cells, forming pores which disrupt osmotic balance and eventually results in cell lysis and insect death.

Samples of corn event DAS-06275-8 tissues were collected at various growth stages from six representative field trial sites in the US and Canada. The expression levels were measured at various stages of plant growth by enzyme-linked immunosorbent assay. Average Cry1F protein expression in nanograms protein per milligram dry weight tissue (ng/mg dwt) was as follows: 15 to 30.9 across growth stages in leaf, 6.75 to 8.82 across growth stages in root, 7.61 in stalk, 1.04 in grain, 4.58 in pollen and 13.9 in forage. As expected, the levels of Cry1F decreased in senescent corn tissues and the Cry1F protein was previously shown to degrade readily in soil.

The Cry1F protein expressed in corn event DAS-06275-8 is identical to the Cry1F protein expressed in corn line 1507. Studies submitted in the corn line 1507 application have shown that, unlike many allergens, the Cry1F protein degrades readily in simulated gastric fluid. The Cry1F protein is not biologically active following exposure to elevated temperature (> 75ºC). A search for amino-acid sequence similarity between the Cry1F protein and known allergens revealed no significant amino-acid homologies based on sequence identity of 8 or more contiguous amino acids.

Additionally, the Cry1F protein expressed in event DAS-06275-8 was demonstrated not to be glycosylated, providing additional evidence that Cry1F does not have the properties of known allergens.

Tests were done to demonstrate the equivalency of the Cry1F protein expressed in corn event DAS-06275-8 tissues with the bacterially-produced Cry1F protein used in the non-target organism tests and biochemical assays. The Cry1F protein extracted from corn tissues or from the bacteria was found to be biochemically equivalent. The Cry1F protein from both expression sources was sensitive to protease cleavage, resulting in a core truncated form of about 65 kDa. Both proteins were shown to be of similar immunological reactivity and lacked detectable glycosylation. Matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and N-terminal sequencing were used to determine the sequence equivalency of the two proteins.

3. Glufosinate-Ammonium Herbicide Tolerance

Phosphinothricin, the active ingredient of glufosinate-ammonium herbicide, inhibits the plant enzyme glutamine synthetase, resulting in the accumulation of lethal levels of ammonia in susceptible plants within hours of application. Ammonia is produced by plants as a result of normal metabolic processes.

The glufosinate-ammonium tolerance gene (*bar* gene) engineered into corn event DAS-06275-8 codes for the enzyme phosphinothricin N-acetyltransferase (BAR). This enzyme detoxifies phosphinothricin by acetylation into an inactive compound.

The *bar* gene was originally isolated from *Streptomyces hygroscopicus*, an aerobic soil bacterium. The BAR enzyme is therefore naturally occurring in the soil. More generally, acetyltransferase enzymes are ubiquitous in nature.

The *bar* gene expressed in corn event DAS-06275-8 is linked to a constitutive promoter. Samples of corn event DAS-06275-8 tissues were collected at various growth stages from six representative field trial sites in the US and Canada. The expression levels were measured at various stages of plant growth by enzyme-linked immunosorbent assay. BAR protein expression in nanograms protein per milligram dry weight tissue (ng/mg dwt) was as follows: 129.2 to 224.2 across growth stages in leaf, 61.1 to 70.0 across growth stages in root, 103.3 in stalk, 5.94 in grain, 0.73 in pollen and 106.9 in forage. As expected, the BAR protein levels decreased in senescent corn tissues.

Protein allergens are normally resistant to digestion unlike the BAR protein which was shown to be rapidly digested under simulated gastric conditions.

The amino acid sequence of the BAR protein shows no significant homology to toxins in the Genbank database or allergens (based on sequence identity of 8 or more contiguous amino acids) from standard protein sequence databases.

The *bar* gene was expressed in a bacterial expression system and the resulting enzyme was used to perform toxicology studies and as a standard in the determination of protein expression from the modified plant. The BAR protein expressed in corn event DAS-06275-8 was compared to the bacterial-produced protein. The BAR protein extracted from corn tissues or from the bacteria was found to be biochemically equivalent. Both proteins were shown to be of similar molecular weight and immunological reactivity and lacked detectable glycosylation. Matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and N-terminal sequencing were used to determine the sequence equivalency of the two proteins.

4. Stable Integration into the Plant's Genome

Southern blot analysis of corn event DAS-06275-8 genome and sequencing of the insert and flanking regions indicate that a single intact copy of the *bar* gene expression cassette and a 5'-truncated copy of the *cry1F* gene expression cassette are integrated at a single locus in corn event DAS-06275-8 genome. Southern blot analysis and sequencing confirmed the absence of plasmid backbone corn event DAS-06275-8 genome.

The truncated *cry1F* gene expression cassette contains the complete Cry1F coding region with a truncated promoter region and intact terminator region. Sequencing of endogenous corn DNA 521 base pairs upstream and 2059 base pairs downstream of the truncated insert showed that an endogenous maize gene has not been interrupted by the insert. Upstream sequencing did not reveal any known promoter sequences. A study by Salgueiro *et al.* (2000) suggest that transcription of the *cry1F* gene could be directed by the truncated ubi intron present in the genetic construct.

The inheritance and stability of each introduced trait was determined using a combination of Southern blot analysis, lateral-flow immunoassays for expressed Cry1F protein and herbicide tolerance bioassay for expressed BAR protein.

The genetic stability of the insert within one stage in the breeding process, designated as the BC4S1 generation, and the linkage between the genotype and phenotypic trait expression was demonstrated. The stability of the insert across 2 additional generations was also confirmed. Segregation analyses across 4 additional generations were performed to determine the inheritance of the insect resistance and herbicide tolerance traits.

The results of the analysis are consistent with the finding of a single active site of insertion that segregates according to the Mendelian laws of genetics.

Dow AgroSciences Canada Inc. has provided the CFIA with a method for detection and identification of corn containing the 6275 transformation line.

IV. Criteria for the Environmental Assessment

1. Potential of Corn Line 6275 to Become a Weed of Agriculture or be Invasive of Natural Habitats

The biology of corn, described in BIO1994-11, 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 nature of corn cobs, and poor competitive ability of seedlings. According to the information provided by Dow AgroSciences canada Inc., corn event DAS-06275-8 and derived hybrids were determined not to be significantly different from their counterparts in this respect.

CFIA evaluated data submitted by Dow AgroSciences Canada Inc. on the reproductive and survival biology of corn hybrids derived from event DAS-06275-8 and determined that 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 resistance to the target pests and tolerance to glufosinate-ammonium herbicide. These traits were demonstrated not to 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 demonstrable effects on weediness or invasiveness, led the CFIA to conclude that corn event DAS-06275-8 has no altered weed or invasiveness potential compared to currently commercialized corn.

2. Potential for Gene Flow from Corn Event DAS-06275-8 to Wild Relatives Whose Hybrid Offspring May Become More Weedy or More Invasive

The biology of corn, as described in BIO1994-11, indicates that there are no wild relatives in Canada that can hybridize with corn. CFIA therefore concludes that gene flow from corn event DAS-06275-8 to wild corn relatives is not possible in Canada.

3. Altered Plant Pest Potential of Corn Event DAS-06275-8

The intended effects of both novel traits are unrelated to plant pest potential, and corn itself is not a plant pest in Canada (BIO1994-11). 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. Field observations did not indicate modifications to disease and pest susceptibilities, other than to ECB and other lepidopteran pests of corn which are not known to be limiting factors in the establishment and spread of corn in Canada.

Some of the genetic elements introduced into corn event DAS-06275-8 were derived from *Agrobacterium tumefaciens*, but in all cases genes responsible for pathogenic qualities of the bacteria were not introduced. Therefore, the introduction of this genetic material would not be expected to result in corn event DAS-06275-8 expressing novel pathogenic characteristics.

Based on these points, the CFIA has determined that corn event DAS-06275-8 does not display any altered plant pest potential.

4. Potential Impact of Corn Event 6275 on Non-Target Organisms

The history of use and available literature indicate that bacterial *B.t.* δ-endotoxins are active against only specific insect groups and are not toxic to other organisms including humans and other vertebrates.

The Cry1F *B.t.* protein produced in corn event DAS-06275-8 is active only against specific lepidopteran insects; no lepidopteran species which are listed as threatened or endangered species in Canada will have significant exposure to the Cry1F protein produced by widespread cultivation of corn plants derived from event DAS-06275-8.

The Cry1F protein expressed in event DAS-06275-8 is identical to the Cry1F protein expressed in a previously authorized corn line, line 1507. Pollen and grain are likely sources of non-target exposure to Cry1F protein expressed in corn. There is a 3 to 6-fold reduction Cry1F expression in pollen compared to line 1507. Cry1F expression in grain of both lines is similar. Cry1F protein was previously shown to degrade readily in the soil.

Data from dietary toxicity studies on non-target organisms submitted in support of line 1507 application have shown that Cry1F protein has no effect on honeybees, green lacewing larvae, ladybird beetles, daphnia, collembola, parasitic hymenoptera, earthworm, bobwhite quail and mice, when ingested at doses greatly exceeding the levels of exposure to Cry1F protein from line 1507 tissues. It was also demonstrated that the Cry1F protein exhibits virtually no toxicity to larvae of the monarch butterfly at levels as high as 10 mg toxin/ml diet, a level which greatly exceeds anticipated environmental exposure, based on field trial data. Since pollen expression for line

6575 is substantially lower than for line 1507, there are extra margins of safety for event DAS-06275-8 over the previous line.

In addition, a new dietary toxicity study with the rainbow trout has been provided by Dow AgroSciences Canada Inc. No mortality or sublethal effects were observed over 8 days when the fish were fed daily with a standard fish diet containing 100 mg of Cry1F protein per kg of diet, supporting the lack of toxicity of event DAS-06275-8 to fish.

The herbicide tolerance trait of event DAS-06275-8 is conferred by expression of the phosphinothricin N-acetyltransferase (BAR). An acute oral gavage study was used to test the toxicity of BAR protein to mice. No mortality or abnormal clinical observations were noted over 2 weeks after the mice received a dose of 3250 mg of BAR protein per kg body weight. Phosphinothricin N-acetyltransferase enzymes, including the BAR protein expressed in event DAS-06275-8, have been studied extensively and have been found to be safe for food and feed uses.

Corn is not known for the production of significant levels of endogenous toxins and the transformation line that produced event DAS-06275-8 would not be expected to induce their synthesis. Corn is however, known to produce low levels of trypsin inhibitor and phytic acid. The levels of these compounds in event DAS-06275-8 were found to be equivalent to levels found in the control lines. The genetic modification, therefore did not alter the expression of endogenous toxins.

Based on the above, the CFIA has determined that the unconfined release of corn event DAS-06275-8, when compared with currently commercialized corn, will not result in altered impacts on non-target organisms.

5. Potential Impact of Corn Event DAS-06275-8 on Biodiversity

Corn event DAS-06275-8 has no novel phenotypic characteristics that would extend its use beyond the current geographic range of corn production in Canada. Since there are no wild relatives of corn in Canada, there will be no transfer of novel traits to unmanaged environments.

Corn event DAS-06275-8 targets certain lepidopteran pest species, but it has been demonstrated to be safe for non-target organisms. The control of agricultural pest species is a common practice in Canada that is not restricted to the environmental release of PNTs, therefore the reduction in local pest species as a result of the release of corn hybrids containing event DAS-06275-8 does not present a significant change from existing agricultural practices.

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 herbicide tolerant crops, and is a common practice in virtually all modern agricultural systems.

The CFIA has therefore concluded that the potential impact on biodiversity of corn event DAS-06275-8 does not present a significantly altered impact in comparison to corn varieties currently being grown in Canada.

6. Potential for Development of Target Pest Resistance to Corn Event DAS-06275-8

In order to significantly minimize the likelihood of the development of insect pest resistance to PNTs expressing novel insect resistance, the CFIA requires that an insect resistance management (IRM) plan be implemented for these products. Lepidopteran insects have a significant ability to develop resistance to conventional chemical insecticides, therefore it is reasonable to expect that resistance to the insecticidal properties of corn event DAS-06275-8 may develop. *B. thuringiensis* var *aizawai* preparations are commercially available for control of various lepidopteran pests and the development of resistance to Cry1F due to the environmental release of corn event DAS-06275-8 may result in the reduction or loss of efficacy of the foliar spray Bt products. Corn event DAS-06275-8 produces Cry1F throughout the growing season therefore target insects will be exposed to significantly higher levels of Cry1F than through the current foliar spray treatments, leading to high selection pressures for resistant ECB individuals.

The following IRM design is intended to reduce or delay European corn borer resistance to the Cry1F protein. A component of the IRM strategy that will be used with corn event DAS-06275-8, is the establishment of a refuge of ECB-susceptible corn within or near the Bt corn field. Should resistant insects occur, they would then be able to mate with susceptible insects to keep the frequency of resistance genes diluted in the insect population.

CFIA believes that sound management practices and IRM strategies can significantly reduce and delay the development of Cry1F resistant ECB populations, however the ECB populations must be monitored for the development of resistance in a regular and consistent manner. CFIA understands that Dow AgroSciences Canada Inc. has developed and will implement an insect resistance management plan that includes the following key components:

(i) The use of structured refuge to provide a population of insects that have not been exposed to the Cry1F protein and are available to reproduce with potentially resistant insects that may emerge from the Bt crop.

(ii) The early detection of ECB populations resistant to the corn-expressed insecticidal protein is extremely important. Close monitoring for the presence of such populations, in ECB-resistant corn fields and surrounding areas, is therefore

warranted. Monitoring includes the development of appropriate detection tools such as visual field observations and laboratory bioassays, education of growers, reporting schedules, and enforcement procedures in case of resistance development.

(iii) Education tools will be developed and provided to all growers, district managers and field managers. These will include information on product performance, resistance management, monitoring procedures and timetables, detection protocols for resistant individuals, instructions to contact Dow AgroSciences Canada Inc., and strategies to be followed if unexpected levels of lepidopteran pest damage occur.

(iv) Dow AgroSciences Canada Inc. will have procedures in place for responding to these reported instances of unexpected target pest damage. These procedures will include, where warranted, the collection of plant tissue and pest insects and use of appropriate bioassays to evaluate suspected Cry1F resistant individuals, and a protocol for immediate action to control resistant individuals.

(v) Detection of confirmed resistant lepidopteran pest populations and subsequent action plan will immediately be reported to CFIA.

(vi) Integrated Pest Management practices will be promoted, such as prediction of infestation problems from previous years and crop rotation.

Notes: Corn event DAS-06275-8 also targets corn earworm, black cutworm and fall armyworm, however no modification is required to the present IRM design to address resistance in these pests since there are no significant over wintering populations of these insects in Canada.

The Plant Biosafety Office periodically audits compliance with the IRM requirements.

7. Potential for the development of multiple herbicide tolerant volunteers and herbicide tolerant weeds

If there is general adoption of several different crop species with novel herbicide tolerances, then the potential exists for the development of crop volunteers with a combination of tolerances to different herbicides. Therefore, 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. Another potential concern is that the continued use of a specific herbicide may provide significant selective pressure for the potential development of herbicide tolerant 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 understands that Dow AgroSciences Canada Inc. has developed and will implement a Herbicide Management Plan for glufosinate-tolerant corn that adequately addresses these issues.

V. Criteria for the Livestock Feed Assessment

1. Potential Impact of Corn Event DAS-06275-8 on Livestock Nutrition

Nutritional Composition of corn event DAS-06275-8

Composition of grain and whole plant from corn line TC6275 was compared with a control line with the same genetic background. Whole plant analysis included proximates, ADF, NDF, Ca and P, while grain analysis included proximates, major fatty acids, amino acids, vitamin A, folic acid, tocopherols, B vitamins, minerals, secondary metabolites (inositol, raffinose, furfural, P-coumaric acid, ferulic acid) and anti-nutrients (phytic acid and trypsin inhibitor). In grain, there was significantly lower P in line TC6275 than control, but all of the values were within literature values. Measurements for several of the analytes, although similar across treatments, were outside of the range of literature values. These included Ca, vitamin A, and total tocopherols.

Secondary Metabolites and Anti-Nutritional Factors

Concentration of inositol, raffinose, furfural, P-coumaric acid, ferulic acid, phytic acid and trypsin inhibitor was shown to be equivalent in control vs event DAS-06275-8 corn grain.

The applicant has demonstrated that the nutritional composition of corn event DAS-06275-8 is equivalent to control corn lines.

2. Potential Impact of Corn Event DAS-06275-8 on Livestock and Workers/Bystanders

- The history of use and literature suggest that the bacterial B.t. Cry proteins are not toxic to humans and other vertebrates. The low mammalian toxicity of B.t. microbial insecticide mixtures containing Cry proteins has been demonstrated over the last 40 years. The amino acid sequence of the Cry 1F protein found in corn event DAS-06275-8 is identical to a Cry 1F protein in crops previously approved in Canada. The Cry 1F protein shares no biologically relevant significant homology with known toxins or allergens, it is present in small amounts in the feed, it is heat labile and it is rapidly degraded under the conditions present in the gastrointestinal tract. A mouse acute oral

toxicity study using bacterially expressed Cry 1F protein indicated there were no adverse effects at 576 mg/kg body weight. A study looking at the dietary toxicity of Cry1F protein in bobwhite quail was also conducted. No adverse effects were demonstrated.

- PAT is a highly substrate specific enzyme that has been well defined. Exposure to PAT protein is not new. The *bar* gene is isolated from *Streptomyces hygroscopicus* a common soil bacterium. The pat gene is present in the environment with no known adverse effects on humans and animals. In addition, PAT from the *bar* gene has been expressed in various crops authorized in Canada. The PAT protein from the bar gene also does not share any biologically relevant significant homology with known toxins or allergens. Studies indicate that the protein was heat and pH labile, and was inactivated within one minute when subjected to typical mammalian stomach and intestinal conditions. A mouse acute oral toxicity study using the bacterially expressed PAT protein indicated that there were no adverse effects at 3250 mg/kg body weight.

  From the information provided by Dow AgroSciences Canada Inc., the Cry1F and the PAT proteins are unlikely to be novel toxins or allergens. Based on the predicted exposure levels and the results of the above tests, no significant risk to livestock and workers/by-standers is expected from exposure to the Cry1F and PAT proteins.

VI. New Information Requirements

Where, at any time after providing notification of the proposed unconfined release or receiving authorization for the unconfined release of corn event DAS-06275-8, Dow AgroSciences Canada Inc. becomes aware of any new information regarding the environmental safety or animal or human health safety of corn event DAS-06275-8 that could result from the release, Dow AgroSciences Canada Inc. must immediately provide the CFIA with the new information. On the basis of such new information, the CFIA will re-evaluate the potential risk to environmental, animal or human health that could result from release of corn event DAS-06275-8 and will re-evaluate its decision with respect to the livestock feed use and environmental release authorizations of corn event DAS-06275-8. The CFIA may maintain, change, or remove existing conditions respecting the release; impose additional conditions; or refuse or cancel the authorization and require the applicant to stop the release and take any appropriate action necessary to eliminate from, or minimize the risk to, the environment.

VII. Regulatory Decision

Based on the review of data and information submitted by Dow AgroSciences Canada Inc. and through comparisons of corn event DAS-06275-8 with corn counterparts, the Plant Biosafety Office, CFIA, has concluded that the novel genes and their corresponding traits do not confer to these plants any characteristic that would result in intended or unintended significant environmental effects following unconfined release.

Dow AgroSciences Canada Inc. has developed and will implement an insect resistance management plan.

Based on the review of submitted data and information by Dow AgroSciences Canada Inc., including comparisons of corn event DAS-06275-8 with unmodified corn counterparts, the Feed Section, CFIA, has concluded that the modified gene and its corresponding novel trait will not confer to these plants any characteristic that would raise any concerns regarding the safety or nutritional composition of corn event DAS-06275-8 for livestock animals. 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. Corn event DAS-06275-8 has been assessed and found to be substantially equivalent to traditional corn varieties, with respect to safety and nutritional quality. Corn event DAS-06275-8 and its products are considered to meet present ingredient definitions and are approved for use as livestock feed ingredients in Canada.

Unconfined release into the environment and livestock feed use of the corn event DAS-06275-8 is therefore authorized as of June 19, 2006. All its progeny and sister lines which have been derived from the original transformation line and their respective progenies, are also authorized for unconfined release and livestock feed, provided that no inter-specific crosses are performed, provided the intended uses are similar, provided that based on characterization, these plants do not display any additional novel traits and are substantially equivalent, in terms of their specific use and safety for the environment and for human and animal health, to plants currently being cultivated, provided the novel genes are expressed at a level similar to that of the authorized line and provided that insect resistance management requirements described in the present document are applied.

The corn event DAS-06275-8 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 event DAS-06275-8. The food safety decisions are available at the following Health Canada web site:

http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/index_e.html