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Canadian Food Inspection Agency

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Decision Document DD2005-55

Determination of the Safety of Dow AgroSciences Canada Inc. and Pioneer Hi-Bred Production Inc.'s Insect Resistant and Glufosinate-Ammonium Herbicide Tolerant Corn (*Zea mays* L.) Line 59122

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 Dir94-11 *The Biology of Zea mays L. (Corn/Maize)* and Dir95-03 *Guidelines for the Assessment of Novel Feeds: Plants Sources*.

The Canadian Food Inspection Agency (CFIA), specifically the Plant Biosafety Office and the Feed Section, have evaluated information submitted by Dow AgroSciences Canada Inc. and Pioneer Hi-Bred Production Inc. This information is in regard to the insect resistant and glufosinate-ammonium herbicide tolerant corn line 59122. The CFIA has determined that this plant with a novel trait (PNT) does not present altered environmental risk nor, as a novel feed, does it 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 line 59122 is therefore authorized as of November 18, 2005. The authorization is limited to one year. Renewal of the one year authorization is conditional upon the submission of additional research results related to rootworm resistance management. 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 line 59122 is subject to the same phytosanitary import requirements as its unmodified counterparts.

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.

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

Designation(s) of the PNT:	*B.t.* Cry34/35Ab1 Insect Resistant Glufosinate-Ammonium Herbicide Tolerant Maize Line 59122, OECD identifier DAS-59122-7
Applicants:	Dow AgroSciences Canada Inc. and Pioneer Hi-Bred Production Inc.
Plant Species:	Corn (*Zea mays* L.)
Novel Traits:	Resistance to Western and Northern Corn Rootworms. (*Diabrotica virgifera virgifera and Diabrotica barberi)* Tolerance to glufosinate-ammonium herbicide.
Trait Introduction Method:	*Agrobacterium*-mediated transformation.
Proposed Use of PNT:	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. and Pioneer Hi-Bred Production Inc. have developed a corn line resistant to rootworms and tolerant to the glufosinate-ammonium herbicide. The corn line, designated as corn line 59122, was developed to provide a method to control yield losses from insect feeding damage caused by rootworm larvae, as well as a method to control weeds in corn production.

Corn line 59122 was developed using recombinant DNA technology, resulting in the introduction of bacterial genes conferring resistance to rootworms and tolerance to glufosinate-ammonium herbicide.

Dow AgroSciences Canada Inc. and Pioneer Hi-Bred Production Inc. have provided data on the identity of corn line 59122, 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 line 59122 conducted in 2003 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 line 59122 such as stand establishment, vegetative vigour, time to maturity, flowering period, susceptibilities to various corn pests and pathogens and seed production, were compared to those of corn counterparts. Nutritional components of corn line 59122 such as proximates, amino acids and fatty acids were compared with unmodified corn counterparts. 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 Dir94-08:

- potential of corn line 59122 to become a weed of agriculture or be invasive of natural habitats,
- potential for gene flow from corn line 59122 to wild relatives whose hybrid offspring may become more weedy or more invasive,
- potential for corn line 59122 to become a plant pest,
- potential impact of corn line 59122 or it's gene products on non-target species, including humans, and
- potential impact of corn line 59122 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 Dir95-03. The following have been considered:

- potential impact of corn line 59122 on livestock nutrition and,
- potential impact of corn line 59122 on livestock and workers/bystanders.

Dow AgroSciences Canada Inc. and Pioneer Hi-Bred Production Inc. have provided to the CFIA a method for detection and identification of corn specifically containing the Cry34Ab1 protein.

III. Description of the Novel Traits

1. Development Method

Corn line 59122 was produced via *Agrobacterium*-mediated transformation of the corn line Hi-II, derived from a cross of A188 x B73. The synthetic *cry*34Ab1 gene, the synthetic *cry*35Ab1 gene and the herbicide tolerance *pat* 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. Corn line 59122 was identified as a successful transformant and was chosen for further development.

2. Resistance to Corn Rootworms

Bacillus thuringiensis is a common gram-positive soil-borne bacterium. In its spore forming stage, it produces several insecticidal protein crystals, including the δ-endotoxin Cry proteins that are active against certain insect groups. 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.

Bacillus thuringiensis strain PS149B1 produces a binary insecticidal crystal protein (BICP or ICP) which consists of two proteins, Cry34Ab1 and Cry35Ab1, approximately 14 kDa and 44 kDa in molecular weight respectively. This binary Cry34/35Ab1 ICP has been shown to be selectively toxic to certain coleopteran species, including the western and northern corn rootworms.

Synthetic *cry*34Ab1 and *cry*35Ab1 genes were developed to maximize their expression in corn, and introduced into the Hi-II line. The amino-acid sequences of the Cry34Ab1 and Cry35Ab1 proteins expressed in corn line 59122 are identical to the native protein sequences. Similar to other *B.t.* delta-endotoxins, insecticidal activity of the Cry34/35Ab1 ICP is believed to depend on the binding of the ICP 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. The Cry34Ab1 protein is active alone, but it is substantially synergised by Cry35Ab1.

The *cry*34Ab1 and *cry*35Ab1 genes of corn line 59122 are linked to constitutive promoters. The amino-sequences of the Cry34Ab1 and Cry35Ab1 proteins expressed in 59122 corn are identical to the native Cry34Ab1 and Cry35Ab1 protein sequences. Cry34Ab1 and Cry35Ab1 protein expression was determined from plants grown in Canada and in the USA. The expression levels were measured at various stages of plant growth by enzyme-linked immunosorbent assay. Average Cry34Ab1 protein expression in nanograms protein per milligram dry weight tissue (ng/mg dwt) was as follows: 54.9 to 266.4 across growth stages in leaf, 35.4 to 43.7 across growth stages in root, 49 in stalk, 36.4 in grain, 64.7 in pollen and 97.7 in forage. Average Cry35Ab1 protein expression in nanograms protein per milligram dry weight tissue (ng/mg dwt) was as follows: 52.3 to 97.1 across growth stages in leaf, 5.3 to 15.5 across growth stages in root, 19.3 in stalk, 2.0 in grain, 0.06 in pollen and 28.1 in forage.

The levels of Cry34Ab1 and Cry35Ab1 tend to decrease in senescent corn tissues and the Cry34Ab1 and Cry35Ab1 protein was shown to degrade readily in soil.

Dow AgroSciences Canada Inc. and Pioneer Hi-Bred Production Inc. submitted data showing that, unlike many allergens, the Cry34Ab1 and Cry35Ab1 proteins degrade readily in simulated gastric fluid (SGF). More than 97% of the Cry35Ab1 protein was digested after 5 min exposure to SGF, and 90% of Cry34Ab1 protein was digested after 6.5 min exposure to SGF. Known protein allergens also tend to be stable to heat and processing, unlike the Cry34Ab1 and Cry35Ab1 proteins. The Cry34Ab1 and Cry35Ab1 proteins are completely inactivated by exposure for 30 min to 90ºC and 60ºC respectively, indicating that both proteins are heat labile. A search for amino-acid sequence similarity between the Cry34Ab1 and Cry35Ab1 proteins and known allergens revealed no significant amino-acid homologies based on sequence identity of 8 or more contiguous amino acids. The Cry34Ab1 and Cry35Ab1 proteins expressed in corn line 59122 were demonstrated not to be glycosylated, providing additional evidence that Cry34Ab1 and Cry35Ab1 proteins do not have the properties of known allergens.

To obtain sufficient quantities of Cry34Ab1 and Cry35Ab1 proteins for evaluation of environmental and feed safety, it was necessary to express the cry34Ab1 and cry35Ab1 genes in a bacterial production system. The cry34Ab1 and cry35Ab1 sequences expressed in the bacterial production system are identical to the cry34Ab1 and cry35Ab1 sequences introduced into corn, except for a four amino acid difference found in the C-terminal region of the Cry35Ab1 protein**.** In order to assess the equivalence between the plant produced proteins and the bacterial produced proteins, various studies were submitted. The bacterium-derived and transgenic corn-produced Cry34Ab1 and Cry35Ab1 proteins were assessed to be equivalent in regards to molecular weight, immunorecognition, N-terminal sequence, tryptic peptide mass fingerprinting by matrix-assisted laser adsorption ionization/time-of-flight mass spectometry (MALDI-TOF MS), the lack of glycosylation, and biological activity.

Acute oral toxicity studies using mice were conducted to determine the toxicological effects of the bacterially expressed Cry34Ab1 and Cry 35Ab1 proteins. The proteins were tested individually (2700 mg and 1850 mg of protein per kg body weight for Cry34Ab1 and Cry35Ab1, respectively) and as a mixture (482 mg/kg Cry34Ab1 and 1520 mg/kg Cry35Ab1), administered by gavage, followed by a two-week observation period. No mortality or adverse effects were observed in these studies at the doses tested. Based on the calculated maximum exposure levels for livestock to Cry34Ab1 and Cry35Ab1 proteins from their diet, no adverse effects would be expected.

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 (*pat* gene) engineered into corn line 59122 codes for the enzyme phosphinothricin N-acetyltransferase (PAT). This enzyme detoxifies phosphinothricin by acetylation into an inactive compound.

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

The *pat* gene of corn line 59122 is liked to a constitutive promoter. The PAT protein expression was determined from plants grown in Canada and the USA. The expression levels were measured at various stages of plant growth by enzyme-linked immunosorbent assay. Average PAT protein expression in nanograms protein per milligram dry weight tissue (ng/mg dwt) was as follows: 0.25 to 11.4 across growth stages in leaf, 0.18 to 0.42 across growth stages in root, 0.38 in stalk, 0.1 in grain, and 2.4 in forage. The PAT level in pollen was below the detection limit (0.30 ng/mg dwt).

Due to the low levels of PAT protein expressed in the corn plant it was necessary to produce PAT protein by bacterial fermentation to obtain sufficient quantities to conduct some of the safety studies (e.g. acute oral toxicity study in mice, simulated gastric fluid digestion studies). The bacterial produced protein was compared to the plant produced protein and shown to be of similar molecular weight and immunological reactivity. Neither the plant nor bacterially expressed PAT is glycosylated.

The PAT protein has been extensively studied and was previously shown to lack allergen traits. The PAT protein did not exhibit any acute oral toxicity when administered to mice at 5000 mg/kg body weight.

4. Stable Integration into the Plant's Genome

Southern blot analysis of different generations derived from corn line 59122 indicated a single insertion of one intact copy of the T-DNA region from the transformation plasmid at one locus in the corn genome. The absence of transformation plasmid sequences outside the T-DNA borders was confirmed.

The genetic stability of the insert across four breeding generations was demonstrated.

The inheritance and stability of the introduced traits was determined within a segregating generation using a combination of Southern blot analyses, immunoassays for expressed Cry34Ab1 protein and herbicide tolerance bioassay for expressed PAT protein. The linkage between the genotype and phenotypic trait expression was

demonstrated.

The Mendelian inheritance of the *cry*34Ab1 and *pat* genes and the corresponding traits across 8 generations was also confirmed.

The segregation and stability data were consistent with the insertion of at a single locus behaving in a Mendelian hereditary fashion. Mendelian segregation data provides evidence of the stable inheritance of the genetic elements introduced into corn line 59122.

IV. Criteria for the Environmental Assessment

1. Potential of Corn line 59122 to Become a Weed of Agriculture or be Invasive of Natural Habitats

The biology of corn, 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 nature of corn cobs, and poor competitive ability of seedlings. According to the information provided by Dow AgroSciences Canada Inc. and Pioneer Hi-Bred Production Inc., corn line 59122 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. and Pioneer Hi-Bred Production Inc. on the reproductive and survival biology of corn hybrids derived from corn line 59122, 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 line 59122 has no altered weed or invasiveness potential compared to currently commercialized corn.

2. Potential for Gene Flow from Corn line 59122 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.
CFIA therefore concludes that gene flow from corn line 59122 to wild corn relatives

is not possible in Canada.

3. Altered Plant Pest Potential of Corn line 59122

The intended effects of both novel traits are unrelated to plant pest potential, and corn itself 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. Field observations did not indicate modifications to disease and pest susceptibilities, other than to rootworm which is not known to be a limiting factor in the establishment and spread of corn in Canada.

Some of the genetic elements introduced into corn line 59122 were derived from known plant pathogens, but in all cases the genes responsible for the pathogenic qualities of the pathogen were not introduced. Therefore, the introduction of this genetic material would not be expected to result in corn line 59122 expressing novel pathogenic characteristics.

Based on these points, the CFIA has determined that corn line 59122 does not display any altered plant pest potential.

4. Potential Impact of Corn line 59122 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 binary Cry34/35Ab1 protein is active only against specific coleopteran insects. There are no coleopteran species currently listed by the Committee on the Status of Endangered Wildlife in Canada as being a threatened or endangered species.

Dow AgroSciences Canada Inc. and Pioneer Hi-Bred Production Inc. have submitted data from dietary toxicity studies on the effect of the Cry34 and Cry35Ab1 proteins on non-target invertebrates, including honeybee larvae, green lacewing larvae, ladybird beetles (*Hippodamia convergens* and *Coleomegilla maculata*), parasitic wasp, ground beetle, daphnia, collembola, and earthworm. Data were also submitted on non-target vertebrates including mice and the rainbow trout. Cry34 and Cry35Ab1 proteins were demonstrated to be safe to these indicator species when ingested alone or in combination, at doses exceeding the levels of direct or indirect exposure to Cry34 and Cry35Ab1 proteins from corn 59122 tissues. Although growth inhibition was reported when *Coleomegilla maculata* larvae were fed Cry34Ab1 protein at about 10 times the field pollen value, no delay in development or weigh reduction were observed when larvae were fed a diet composed of 50% corn line 59122 pollen, which represents a conservative estimate of the potential exposure in the field. Therefore, no sub-lethal effects on *C. maculata* ladybird beetle are expected from exposure to corn

line 59122 pollen in field conditions.

A study on broiler chicken using corn line 59122 grain showed corn line 59122 grain is safe and as nutritious to birds as non-modified corn grain.

The Cry34 and Cry35Ab1 protein was shown to degrade readily in soil, indicating that it is unlikely to accumulate and persist in soil.

Dow AgroSciences Canada Inc. and Pioneer Hi-Bred Production Inc. submitted field based observational studies on the stability and abundance of non-target arthropods communities and individual species where Cry34 and Cry35Ab1 expressing corn is grown. The species studied were representatives of various functional groups (predators, parasitoids, herbivores and detritivores) and were sampled using visual observations of corn plants, sticky traps, pitfall traps or litterbag traps. The studies did not reveal any negative impact on the abundance of these non-target organisms relative to the non-*B.t.* control hybrid.

The herbicide tolerance trait of corn line 59122 is conferred by expression of the phosphinothricin N-acetyltransferase (PAT). The PAT protein has been studied extensively and has been found to be safe for non-target organisms, including human beings.

Corn is not known for the production of significant levels of endogenous toxins and the transformation event that produced corn line 59122 would not be expected to induce their synthesis. Corn is however, known to produce low levels of trypsin inhibitor and phytic acid and the levels of these compounds in corn line 59122 were found to be within the range of conventional corn lines. The genetic modification, therefore did not alter the expression of endogenous anti-nutrients.

Based on the above, the CFIA has determined that the unconfined release of corn line 59122, when compared with currently commercialized corn, will not result in altered impacts on non-target organisms.

5. Potential Impact of Corn line 59122 on Biodiversity

Corn line 59122 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 line 59122 targets certain coleopteran 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 line 59122 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 factor in virtually all modern agricultural systems.

The CFIA has therefore concluded that the potential impact on biodiversity of corn line 59122 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 Line 59122

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. Coleopteran 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 line 59122 may develop. The following IRM design is intended to reduce or delay corn root worm (CRW) resistance to the Cry34/35Ab1 binary protein. A component of the IRM strategy that will be used with corn line 59122, is the establishment of a refuge of CRW-susceptible corn within or adjacent to the *B.t.* 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 Cry34/35Ab1 binary protein resistant CRW populations, however the CRW populations must be monitored for the development of resistance in a regular and consistent manner.

CFIA understands that Dow AgroSciences Canada Inc. and Pioneer Hi-Bred Production Inc. have 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 CRW that have not been exposed to the Cry34/35Ab1 binary proteins and are available to reproduce with potentially resistant insects that may emerge from the *B.t.* crop.

(ii) The early detection of CRW populations resistant to the corn-expressed insecticidal protein is extremely important. Close monitoring for the presence of such populations, in CRW-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 Pioneer Hi-Bred Production Inc., and strategies to be followed if unexpected levels of CRW damage occur.

(iv) Dow AgroSciences Canada Inc. and Pioneer Hi-Bred Production 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 Cry34/35Ab1 resistant individuals, and a protocol for immediate action to control resistant individuals.

(v) Detection of confirmed resistant CRW 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.

Research related to the proposed IRM plan is ongoing, and as research progresses, the new information will be used to determine if the present IRM plan should be maintained in its present form, or if it will be modified. Therefore, the renewal of the present one year authorization will be contingent upon Dow AgroSciences Canada Inc. and Pioneer Hi-Bred Production Inc. demonstrating significant progress in research related to rootworm resistance management.

Note: 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. and Pioneer Hi-Bred Production Inc. have 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 on Livestock Nutrition

Nutritional Composition of corn line 59122

Composition of corn grain and forage from corn line 59122 was compared with the control line (05F/581) in a field study conducted in the United States and Canada with 5 separate field locations. Forage analysis included crude protein, crude fat, ash, crude fibre, ADF, NDF, calcium and phosphorous. Grain analysis included, in addition, amino acids, minerals (Cu, Fe, Mg, Mn, K, Na, and Zn), vitamins (beta-carotene, B1, B2, folic acid, E) secondary metabolites (inositol, raffinose, furfural, p-coumaric acid and ferulic acid) and phytic acid. Composition was determined for line 59122, both treated and untreated with glufosinate, and the untreated control.

A second study conducted in Chile at 6 separate field locations included the same compositional analysis of forage and grain from corn line 59122 (treated and untreated with glufosinate) and untreated control.

Consistent differences that were observed in some locations in both studies, including higher crude protein, calcium and phosphorous in corn line 59122 than the control. In the North American study, Vitamin B1 levels were also significantly higher, but this difference was not observed in the Chile study. In all cases, other than for Vitamin B1, nutrient and antinutrient levels were shown to be similar to literature values. In the case of Vitamin B1, the level was within the reference range provided by the applicant, for hybrids grown and analyzed in a similar manner to the studies submitted. The company noted that the variation in composition observed in these studies is similar to that observed among commercial maize lines. The applicant has demonstrated that corn line 59122 is equivalent to conventional corn in terms of livestock nutrition.

2. Potential Impact on Livestock and Workers/By-standers

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. Cry34Ab1 and Cry 35Ab1 proteins 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 conditions in the gastrointestinal tract.

Based on the calculated maximum exposure levels for livestock to Cry34Ab1 and Cry35Ab1 proteins from their diet, no adverse effects would be expected.

PAT is a highly substrate specific enzyme that has been well defined. Exposure to PAT protein is not new. The *pat* gene was originally isolated from *Streptomyces viridochromogenes*, an aerobic soil bacterium. The *pat* gene is present in the environment with no known adverse effects on humans and animals. In addition, PAT from the *pat* gene has been expressed in various crops authorized in Canada. The PAT 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 conditions in the gastrointestinal tract. Based on the calculated maximum exposure levels for livestock to the PAT protein from their diet, no adverse effects would be expected.

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 Cry34Ab1, Cry 35Ab1 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 line 59122, Dow AgroSciences Canada Inc. and Pioneer Hi-Bred Production Inc. become aware of any new information regarding the environmental safety or animal or human health safety of corn line 59122 that could result from the release, Dow AgroSciences Canada Inc. and Pioneer Hi-Bred Production 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 line 59122 and will re-evaluate its decision with respect to the livestock feed use and environmental release authorizations of corn line 59122. 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 Pioneer Hi-Bred Production Inc. and through comparisons of corn line 59122 with unmodified 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. and Pioneer Hi-

Bred Production Inc. have developed and will implement a insect resistance management plan.

Based on the review of submitted data and information by Dow AgroSciences Canada Inc. and Pioneer Hi-Bred Production Inc., including comparisons of corn line 59122 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 line 59122 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 line 59122 has been assessed and found to be substantially equivalent to traditional corn varieties, with respect to safety and nutritional quality. Corn line 59122 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 line 59122 is therefore authorized as of November 18, 2005. The authorization is limited to one year. Renewal of the one year authorization is conditional upon the submission of additional research results related to rootworm resistance management. 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 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 line 59122 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 59122. 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