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Canadian Food Inspection Agency  
Plant Products Directorate  
Plant Biosafety Office

## Decision Document DD2004-48 Determination of the Safety of BASF Canada's Imidazolinone-Tolerant (CLEARFIELD™) Wheat Teal 11A

This Decision Document has been prepared to explain the regulatory decision reached under the regulatory directive Dir94-08 *Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits* and its companion document Dir1999-01 *The Biology of Triticum aestivum* (Wheat) and Dir95-03 *Guidelines for the Assessment of Livestock Feed from Plants with Novel Traits*.

The Canadian Food Inspection Agency (CFIA), specifically the Plant Biosafety Office and the Feed Section, have evaluated information submitted by BASF Canada regarding the Imidazolinone tolerant CLEARFIELD™ wheat line Teal 11A. The CFIA has determined that this plant with a novel trait does not present a significant risk to the environment, nor does it present livestock feed safety concerns when compared to currently commercialized wheat varieties in Canada.

**Unconfined release into the environment and livestock feed use of the CLEARFIELD™ wheat line Teal 11A is therefore authorized as of June 24, 2004. Line Teal 11A and any wheat lines derived from it may be imported and/or released, 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 to wheat currently grown in Canada, in terms of their specific use and safety for the environment and for human and animal health.**

This CLEARFIELD™ wheat line is subject to the same phytosanitary import requirements as its unmodified counterparts.

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## **I. Brief Identification of the Plant with a Novel Trait (PNT)**

<b>Designations of the PNT:</b>	CLEARFIELD™ wheat line Teal 11A
<b>Applicant:</b>	BASF Canada
<b>Plant Species:</b>	Wheat ( <i>Triticum aestivum</i> )
<b>Novel Traits:</b>	Tolerance to imazamox and imazethapyr, imidazolinone herbicides
<b>Trait Introduction</b>	Chemically induced seed mutagenesis

**Method:**

**Proposed Use of the PNT:** Production of wheat for livestock feed and human food. This material will not be grown outside the normal production area for wheat.

## II. Background Information

BASF Canada has developed a new wheat line, designated Teal 11A, which exhibits tolerance to imazamox and imazethapyr, imidazolinone herbicides. This new wheat line will be utilized in the CLEARFIELD™ wheat breeding programs.

The development of the CLEARFIELD™ wheat line Teal 11A was accomplished using chemically induced seed mutagenesis. The herbicide tolerance trait is conferred by a single point mutation modification of the acetohydroxyacid synthase (AHAS) gene such that this enzyme, the target of imidazolinone herbicides, is no longer affected by imazamox and imazethapyr.

Line Teal 11A was field tested in multiple locations in Saskatchewan, North Dakota and Minnesota from 1999 to 2001.

BASF Canada has provided data on the identity of the wheat line, a detailed description of the modification method and breeding history, information on the modified gene, the resulting protein and its mode of action and the stability of trait expression.

Agronomic characteristics of the wheat line Teal 11A such as grain yield, heading date, plant height and days to maturity were compared to those of unmodified wheat counterparts. Nutritional components of the wheat line Teal 11A such as proximates, amino acids and fatty acids were compared with unmodified wheat counterparts. Anti-nutritional factors were also determined.

BASF Canada has provided to the CFIA a method for the detection and identification of wheat containing the modified AHAS gene.

The Plant Biosafety Office, CFIA, has reviewed the above information, with respect to the assessment criteria for determining environmental safety of plants with novel traits, as described in the regulatory directive Dir94-08:

- potential of line Teal 11A to become a weed of agriculture or be invasive of natural habitats;
- potential for gene flow from line Teal 11A to wild relatives whose hybrid offspring may become more weedy or more invasive;
- potential of line Teal 11A to become a plant pest;
- potential impact of line Teal 11A or its gene products on non-target

- species, including humans; and
- potential impact of line Teal 11A 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 the regulatory directive Dir95-03:

- potential impact of line Teal 11A on livestock nutrition; and
- potential impact of line Teal 11A on livestock and workers/by-standers.

### III. Description of the Novel Trait

#### 1. Development Method

The original mutant line was isolated from a population derived by chemical-induced mutagenesis of seed of the wheat variety CDC Teal with Ethylmethane sulfonate (EMS). Whole plant selection procedures for herbicide tolerance were used. One herbicide tolerant mutant was selected and was designated Teal 11A.

The line Teal 11A is a hexaploid ( $2n=6x=42$ ) belonging to genus and species *Triticum aestivum*.

#### 2. Imidazolinone Tolerance

Imidazolinone herbicides are active against the enzyme acetohydroxyacid synthase (AHAS), also known as acetolactate synthase (ALS).

AHAS is an enzyme found in bacteria, certain other micro-organisms and plants. This enzyme catalyses the first step in the biosynthesis of the essential branched chain amino acids isoleucine, leucine and valine. Herbicide induced AHAS inhibition results in a lethal decrease in protein synthesis. Unmodified wheat is not tolerant to imidazolinone herbicides.

A single amino acid substitution in the *A/s2* AHAS gene, sufficient to alter the binding site for imidazolinones such that the herbicide imazamox and imazethapyr no longer inhibits the AHAS enzyme, resulted in a herbicide tolerant phenotype. This is the same mutation in the *A/s2* AHAS gene that was seen in the previously approved wheat line AP602CL. The modified amino acid sequences of Teal 11A and AP602CL wheat lines were aligned and were confirmed to be 100% identical.

The novel imazamox and imazethapyr tolerance is under the control of the native AHAS promoter and is believed to be constitutively expressed. Sequence information for the modified AHAS gene in Teal 11A was submitted.

The tolerance to imazamox and imazethapyr was demonstrated by

comparison of the activity of the AHAS enzyme extracted from Teal 11A wheat plants to that of wild type wheat plants, CDC Teal.

The levels of valine, leucine and isoleucine produced in wheat are regulated by feedback inhibition of AHAS. BASF Canada provided data to demonstrate that the modified AHAS shows similar feedback inhibition by valine and leucine as compared to unmodified AHAS. The modification of the AHAS does not affect feedback inhibition and hence, the regulation and levels of these amino acids.

Unlike known food allergens, AHAS is a minor protein in plant tissue, it is heat sensitive and trypsin susceptible. The AHAS protein from the *Als2* mutation was shown to be heat sensitive, with no detectable activity of AHAS after 1 min of heating at 100°C. The AHAS protein from the *Als2* mutation was completely degraded within 30 minutes of trypsin treatment. The unmodified form of the AHAS protein shows no amino acid similarity to known allergens. The amino acid sequence of mutated AHAS differs by one amino acid from that of unmodified wheat.

BASF Canada provided evidence to show that the protein components of Teal 11A are not altered in comparison with an unmodified comparator. HPLC was run on protein extracts from unmodified and modified wheat to indicate that no new major proteins or increased protein expression occurred as a result of the mutagenic event.

### **3. Stable Expression**

The segregation of herbicide tolerance in crosses with Teal 11A is consistent with the inheritance of a single semidominant allele.

## **IV. Criteria for the Environmental Assessment**

### **1. Potential of line Teal 11A to Become a Weed of Agriculture or Invasive of Natural Habitats**

The centre of origin of wheat is considered to be in the Middle East. Modern wheat does not have high potential for weediness and its survival outside cultivation is limited to short periods. Wheat plants can grow as volunteers in a cultivated field following a wheat crop and are usually eliminated via cultivation or the use of herbicides. After hundreds of years of cultivation in North America and throughout the world, there have been no reports of wheat becoming an invasive pest.

According to the information provided by BASF Canada, no competitive advantage was conferred to wheat line Teal 11A, other than that conferred by tolerance to imidazolinone herbicides. The mutation of the AHAS gene in Teal 11A has not affected the physiology of the plant, as supported by agronomic and compositional data. It is therefore not expected that wheat line Teal 11A would possess traits that would render

it invasive of natural habitats since none of the reproductive or growth characteristics were modified.

Imidazolinone tolerance in itself will not cause Teal 11A to become more weedy or invasive in managed habitats than non-transformed *T. aestivum*. Imidazolinone-tolerant wheat volunteers will not be controlled in subsequent crops if an imidazolinone herbicide is used as the sole weed control tool. However, control of imidazolinone tolerant wheat as a volunteer weed in other crops or in fallow ground, can readily be achieved by the use of classes of herbicides other than imidazolinones, or by mechanical means.

The above considerations have led the CFIA to conclude that wheat line Teal 11A has no short-term ecological advantages when compared with currently commercialized wheat varieties.

Over the longer term, the continued use of imidazolinones on herbicide tolerant crops may provide additional significant selection pressure for the development of Group 2-herbicide tolerant weeds. BASF Canada provided the CFIA with a stewardship plan that describes appropriate strategies that will allow the deployment of line Teal 11A, as well as other approved wheat lines expressing imidazolinone tolerance, while managing these concerns (see appendix 1). The stewardship plan submitted by BASF Canada is based the biology of the wheat plant and on associated agronomic practices.

The CLEARFIELD™ Wheat Herbicide Tolerance Stewardship Plan comprises the *Best Management Practice Program for the CLEARFIELD™ Wheat Production System*.

As part of its stewardship plan, BASF Canada is responsible for communicating to Canadian wheat producers the general recommendations of the CLEARFIELD™ Wheat Stewardship Guide. A number of vehicles have been developed by BASF to communicate the best management strategies to growers adopting the technology and allow them to report any problems. In addition, BASF is required to monitor grower compliance to determine the effectiveness of the stewardship plan and make any changes to the plan as appropriate.

## **2. Potential for Gene Flow from line Teal 11A to Wild Relatives Whose Offspring May Become More Weedy or More Invasive**

No known wild *Triticum* species exist in North America. The weedy relative *Aegilops cylindrica*, jointed goat grass, is present in winter wheat crops in the United States, but is not reported in Canada and does not readily produce fertile progeny when hybridized with wheat. *A. cylindrica* is included in the provincial Noxious Weed List in British Columbia to deter the spread of this weed into Canada.

The only weedy species related to wheat in Canada is *Agropyron repens*, quack grass. *A. repens* is a troublesome weedy grass of agricultural areas throughout Canada. However, no known naturally-occurring hybrids between wheat and *Agropyron* species have been reported.

The CFIA has therefore determined that gene flow from Teal 11A to wild or weedy species in Canada is very unlikely. Moreover, gene flow from line Teal 11A to wild or weedy species in Canada would not be expected to result in increased invasiveness of the offspring.

### **3. Altered Plant Pest Potential of line Teal 11A**

*T. aestivum* is not a plant pest in Canada and the novel trait in wheat line Teal 11A is not expected to affect its plant pest potential. The mutation of the AHAS gene function in Teal 11A, which is analogous to the approved wheat line AP602CL, is not associated with disease or insect resistance and, therefore, has very unlikely altered plant pest potential. The agronomic characteristics of wheat line Teal 11A were shown to be within the normal range of conventional wheat varieties.

The CFIA has therefore determined that wheat line Teal 11A does not present a plant pest concern.

### **4. Potential Impact on Non-Target Organisms of line Teal 11A**

Single amino acid modification of the AHAS enzyme, which alters the herbicide binding site on the enzyme, is the molecular basis for imidazolinone tolerance in wheat line Teal 11A. BASF Canada has submitted data and information indicating that the modified AHAS is substantially equivalent to the native AHAS enzyme. The mutation in the AHAS gene in line Teal 11A has not significantly affected the biosynthesis of the branched-chain amino acids, valine, leucine and isoleucine, or the nutritional composition. The CFIA has therefore determined that the modified AHAS enzyme will not have altered impacts on interacting organisms, including humans, compared with the unmodified counterpart.

The AHAS enzyme is not a known toxin, does not confer resistance to agricultural pests and is commonly found in a wide variety of plants and micro-organisms with a history of safe use. No novel toxins were introduced into this variety. Therefore, no negative interactions with non-target symbiotic or consumer organisms are anticipated.

In addition, agronomic characteristics, pathogen interactions, and insect pest susceptibility of line Teal 11A are expected to be within the range of values displayed by currently commercialized wheat varieties. The CFIA concluded that there were not likely to be significant unintended changes to wheat line Teal 11A that could have adverse impacts on non target organisms.

## 5. Potential Impact on Biodiversity of line Teal 11A

Wheat line Teal 11A has no novel phenotypic characteristics which would extend its use beyond the current geographic range of spring wheat production in Canada. In addition, Teal 11A was shown to be safe to non-target organisms and does not present altered weediness or plant pest potential. Since wheat does not outcross under natural conditions to wild relatives in Canada, the transfer of novel traits to other plant species in unmanaged environments is highly unlikely. The novel trait has not altered the ability of this line to persist in the Canadian environment. The CFIA has therefore concluded that the impact on biodiversity of wheat line Teal 11A is equivalent to that of currently commercialized wheat lines.

## V. Criteria for the Livestock Feed Assessment

### 1. Potential Impact on Livestock Nutrition

The nutritional assessment was based on the comparison of nutrient and antinutrient composition of wheat line CDC Teal and Teal 11A grown in two locations in Saskatchewan in 1999 and two locations in the North Dakota and Minnesota in 2001.

#### *Anti-nutritional Factors*

Two antinutrients, phytic acid and trypsin inhibitor in whole grain samples of Teal 11A and CDC Teal from the four locations were compared. Trypsin inhibitor levels in both lines were below the detection limit of 2000 TIU/ml and phytic acid levels were similar in the two lines.

#### *Nutritional Composition*

Nutritional components were measured in whole grain wheat from CDC Teal and Teal 11A. Analyses included, for all locations, crude protein, crude fat, crude fibre, and the amino acids valine, isoleucine, leucine, threonine, cystine, lysine, and methionine. In addition, in the two United States locations, fatty acids, B vitamins (Thiamine HCl, Niacin, Pantothenic acid, Vitamin B6), phosphorous, zinc, magnesium, and iron were measured. There were no differences between Teal and Teal 11A in crude protein, crude fat, branched chain and essential amino acids, B vitamins and minerals. Two minor but statistically significant differences between the two lines were noted; crude fibre and oleic acid were slightly lower in Teal 11A than CDC Teal. There would be no impact of these slight differences on livestock nutrition.

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

The AHAS enzyme is found in a wide variety of plants and micro-organisms. AHAS is not a known toxin or allergen and a single base pair change would not be expected to change this. The AHAS from Teal 11A

is feedback inhibited as is unmodified AHAS, it is present in small amounts in the feed, it is heat labile and it is rapidly degraded under conditions in the gastrointestinal tract. The expression of AHAS is not changed by the modification. Based on the information provided by BASF Canada, the modified AHAS is unlikely to be a novel toxin or allergen.

Based on the detailed characterization provided (nutritional composition, agronomic data and HPLC protein profiles of the modified plant compared to the unmodified comparator) it is unlikely that secondary mutations causing unintended effects have occurred in the wheat genome.

## VI. New Information Requirements

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

## VII. Regulatory Decision

Based on the review of data and information submitted by BASF Canada, and through comparisons of Teal 11A with unmodified wheat counterparts, the Plant Biosafety Office, CFIA, has concluded that the modified gene and its corresponding novel trait will not confer any ecological advantage to Teal 11A following unconfined release. Wheat line Teal 11A was also determined not to pose any additional plant pest risk compared to its unmodified counterparts.

Based on the review of data and information submitted by BASF Canada, including comparisons of line Teal 11A with the unmodified parental counterpart, the Feed Section, CFIA, has concluded that the modified gene and its corresponding novel trait will not confer to these plants any characteristics that would raise any concerns regarding the safety or nutritional composition of wheat line Teal 11A. Wheat grain, its byproducts and wheat germ oil, are currently listed in Schedule IV of the *Feeds Regulations* and are, therefore approved for use in livestock feeds in Canada. Wheat line Teal 11A has been assessed and found to be as safe as nutritious as traditional wheat varieties. Teal 11A and its products are considered to meet the 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 CLEARFIELD™ wheat line Teal 11A is therefore authorized as of June 24, 2004. Any other wheat lines derived from it may be imported and/or released, provided no inter-specific crosses are**

**performed, provided the intended uses are similar, and provided it is known, based on characterization, that these plants do not display any additional novel traits and are substantially equivalent to currently grown wheat in Canada, in terms of their specific use and safety for the environment and for human and animal health.**

**The CLEARFIELD™ wheat line 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 CLEARFIELD™ wheat lines. The food safety decisions are available at the following Health Canada web site: [http://www.hc-sc.gc.ca/food-aliment/mh-dm/ofb-bba/nfi-ani/e\\_novel\\_foods\\_and\\_ingredient.html](http://www.hc-sc.gc.ca/food-aliment/mh-dm/ofb-bba/nfi-ani/e_novel_foods_and_ingredient.html)

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