



Decision Document DD2004-47

Determination of the Safety of BASF's Imazamox Tolerant (CLEARFIELD™) Wheat AP205CL

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 Imazamox tolerant CLEARFIELD™ wheat line AP205CL. 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 AP205CL is therefore authorized as of June 11, 2004. Line AP205CL 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 uses and safety for the environment and for human and animal health.

The CLEARFIELD™ wheat line AP205CL is subject to the same phytosanitary import requirements as its unmodified counterparts.

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

Designation(s) of the PNT:	CLEARFIELD™ wheat line AP205CL
Applicant:	BASF Canada
Plant Species:	Wheat (<i>Triticum aestivum</i>)
Novel Traits:	Tolerance to imazamox, an imidazolinone herbicide
Trait Introduction Method:	Chemically induced seed mutagenesis
Proposed Use of PNT:	Production of wheat for livestock feed and human food.

II. Background Information

BASF has developed a wheat line tolerant to imazamox, an imidazolinone herbicide. This wheat line was developed to provide an alternative strategy for weed control.

The development of the CLEARFIELD™ wheat line AP205CL 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.

Line AP205CL was field tested in North Dakota, Minnesota and Colorado in 2000, 2001 and 2002. These represent areas with environments contiguous with Canadian wheat production areas.

BASF 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 AP205CL such as grain yield, heading date, plant height, foliar disease and test weight were compared to those of unmodified wheat counterparts.

Nutritional components of the wheat line AP205CL such as proximates, amino acids and fatty acids were compared with unmodified wheat counterparts. Anti-nutritional factors were also determined.

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 AP205CL to become a weed of agriculture or be invasive of natural habitats;
- potential for gene flow from line AP205CL to wild relatives whose hybrid offspring may become more weedy or more invasive;
- potential of line AP205CL to become a plant pest;
- potential impact of line AP205CL or its gene products on non-target species, including humans; and
- potential impact of line AP205CL 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 AP205CL on livestock nutrition; and
- potential impact of line AP205CL on livestock and workers/by-standers.

III. Description and Assessment 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 Gunner with Ethylmethane sulfonate (EMS) and Diethyl Sulfate (DES). Whole plant selection procedures for herbicide tolerance were used. One herbicide tolerant mutant was selected and was designated AP205CL.

The AP205CL variety is a hexaploid (42 chromosomes $n=14$) belonging to genus and species *Triticum aestivum*.

2. Imazamox 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 AHAS gene, sufficient to alter the binding site for imidazolinones such that the herbicide imazamox no longer inhibits the AHAS enzyme, resulted in a herbicide tolerant phenotype.

The novel imazamox 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 AP205CL was submitted.

The tolerance to imazamox was demonstrated by comparison of the activity of the AHAS enzyme extracted from AP205CL wheat plants to that of unmodified wheat plants.

The levels of valine, leucine and isoleucine produced in wheat are regulated by feedback inhibition of AHAS. BASF 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 is susceptible to trypsin. The modification does not change AHAS expression. The AHAS protein from AP205CL was shown to be heat sensitive, with no detectable activity of AHAS after 1 min of heating at 100 °C. AHAS 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 provided evidence to show that the protein components of AP205CL 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 protein or increased protein expression occurred as a result of the mutagenic event.

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

3. Stable Expression

The segregation of herbicide tolerance in crosses with AP205CL is consistent with the inheritance of a single semi- or co-dominant allele. AP205CL segregates in a 3:1 ratio (herbicide resistant: susceptible to herbicide).

IV. Criteria for the Environmental Assessment

Note: Wheat line AP205CL is not intended for cultivation in Canada

1. Potential of line AP205CL 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.

The CFIA evaluated data submitted by BASF on the biology of wheat line AP205CL, and determined that vegetative vigour, time to maturity, seed production, as well as resistance to disease, were within the normal range of expression of these traits currently displayed by the parent line.

No competitive advantage was conferred to these plants, other than that conferred by tolerance to imidazolinone herbicide. Tolerance to imidazolinone herbicide will not, in itself, render wheat weedy or invasive of natural habitats since none of the reproductive or growth characteristics were modified. Imidazolinone tolerance will not cause AP205CL to become more weedy or invasive in managed habitats than unmodified *T. aestivum*. Imidazolinone-tolerant wheat volunteers will not be controlled in subsequent crops if imidazolinone 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.

If cultivation of wheat line AP205CL is intended in Canada, the CLEARFIELD™ Wheat Herbicide Tolerance Stewardship Plan will be implemented (please refer to Decision Document DD2004-48).

Wheat line AP205CL is not intended for cultivation in Canada and the novel trait has no intended or observed effects on weediness or invasiveness. The CFIA has therefore concluded that this wheat line has no altered weed or invasiveness potential in Canada when compared to conventional wheat varieties.

2. Potential for Gene Flow from line AP205CL 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 line AP205CL to wild or weedy species in Canada is very unlikely. Moreover, gene flow from line AP205CL 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 AP205CL

The novel trait is not expected to affect the plant pest potential of wheat. *T. aestivum* is not a plant pest in Canada. Additionally, the agronomic characteristics of wheat line AP205CL were shown to be within the normal range of conventional wheat varieties.

The CFIA has therefore determined that wheat line AP205CL does not present a plant pest concern.

4. Potential Impact on Non-Target Organisms of line AP205CL

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 AP205CL. BASF has submitted data indicating that the modified AHAS is substantially equivalent to the native AHAS enzyme. The mutation in the AHAS gene in line AP205CL 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 and pathogen interactions of line AP205CL were shown 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 line AP205CL that could have adverse impacts on non-target organisms.

5. Potential Impact on Biodiversity of line AP205CL

Wheat line AP205CL is safe to non-target organisms, does not present altered weediness or plant pest potential and is not intended to be grown in Canada. In addition the novel trait has not altered the ability of this line to persist in the Canadian environment.

The CFIA has therefore concluded that the potential impact on biodiversity of wheat line AP205CL is equivalent to that of currently commercialized wheat lines.

V. Criteria for the Livestock Feed Assessment

1. Potential Impact on Livestock Nutrition

Nutrient and anti-nutrient composition of wheat line AP205CL was compared with its control (Gunner), grown in replicated trials in North Dakota and Minnesota.

Anti-nutritional Factors

Phytic acid content of line AP205CL averaged 1.2% and was not different from Gunner. Trypsin inhibitor in both line AP205CL and Gunner was below the limit of detection of 2000 TIU/ml.

Nutritional Composition

Composition of line AP205CL and Gunner were compared in terms of proximate analysis, branched chain and essential amino acids, fatty acids, the minerals, P, Mg, Zn, Fe, and thiamine, niacin, pantothenic acid, pyridoxine and vitamin E were analysed. One statistically significant difference was observed; thiamine concentration was lower in line AP205CL (2.5%) than Gunner (2.8%). There were no other differences in composition observed. The slight difference in thiamine levels would not impact on livestock nutrition or feed safety, and is likely not related to the novel trait.

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. AHAS from line AP205CL 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, 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 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 will immediately provide such information to CFIA. On the basis of such new information, CFIA will re-evaluate the potential impact of the proposed use 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, and through comparisons of line AP205CL with unmodified wheat counterparts, the Plant Biosafety Office, CFIA, has concluded that the modified gene and its corresponding novel trait do

not confer to wheat line AP205CL any characteristic that would result in intended or unintended significant environmental effects following unconfined release.

Based on the review of data and information submitted by BASF, including comparisons of line AP205CL with unmodified wheat counterparts, the Feed Section, CFIA, has concluded that the modified gene and its corresponding novel trait do not confer to these plants any characteristic that would raise any concerns regarding the safety or nutritional composition of wheat line AP205CL. 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 AP205CL has been assessed and found to be as safe as nutritious as traditional wheat varieties. Line AP205CL 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 AP205CL is therefore authorized as of June 11, 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 uses and safety for the environment and for human and animal health.

The CLEARFIELD™ wheat line AP205CL 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:

www.hc-sc.gc.ca/food-aliment/mh-dm/ofb-bba/nfi-ani/e_novel_foods_and_ingredient.html