



Decision Document DD2003-44

Determination of the Safety of BASF's Imazamox Tolerant (CLEARFIELD™) Wheat AP602CL

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 AP602CL. 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 AP602CL is therefore authorized as of March 20, 2003. Line AP602CL and any wheat lines derived from it may be imported and/or released, provided (i) no inter-specific crosses are performed, (ii) the intended use is similar, (iii) it is known following thorough characterization that these plants do not display any additional novel traits and are substantially equivalent to currently commercialized wheat, in terms of their potential environmental impact and livestock feed safety.

This CLEARFIELD™ wheat line 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 AP602CL
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 herbicides. This wheat line was developed to provide an alternative strategy for weed control.

The development of the CLEARFIELD™ wheat was accomplished using chemically induced seed mutagenesis. The herbicide tolerance results from 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 AP602CL 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 AP602CL 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 AP602CL 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 AP602CL to become weeds of agriculture or be invasive of natural habitats;

- potential for gene flow from AP602CL to wild relatives whose hybrid offspring may become more weedy or more invasive;
- potential of AP602CL to become a plant pest;
- potential impact of AP602CL or their gene products on non-target species, including humans; and
- potential impact of AP602CL 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 AP602CL on livestock nutrition; and
- potential impact of AP602CL 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 AP602CL.

The AP602CL 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 AP602CL was submitted.

The tolerance to Imazamox was demonstrated by comparison of the activity of the AHAS enzyme extracted from AP602CL wheat plants to that of wild type 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 trypsin susceptible. The AHAS protein from AP602CL 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 AP602CL 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.

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 AP602CL is consistent with the inheritance of a single semi- or co-dominant allele. AP602CL segregates in a 3:1 ratio (herbicide resistant: susceptible to herbicide).

IV. Criteria for the Environmental Assessment

Note: Wheat line AP602CL is not intended for cultivation in Canada

1. Potential of line AP602CL 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 AP602CL, 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 AP602CL 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 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.

Wheat line AP602CL 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 AP602CL 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 AP602CL to wild or weedy species in Canada is very unlikely.

3. Altered Plant Pest Potential of line AP602CL

The intended effect of the novel trait is unrelated to plant pest potential. *T. aestivum* is not a plant pest in Canada. Additionally, the agronomic characteristics of wheat line AP602CL were shown to be within the normal range of conventional wheat varieties.

The CFIA has therefore determined that wheat line AP602CL does not present a plant pest concern.

4. Potential Impact on Non-Target Organisms of line AP602CL

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 AP602CL. BASF has submitted data indicating that the modified AHAS is substantially equivalent to its unmodified counterparts. 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. The mutant AHAS in AP602CL has not significantly affected the biosynthesis of the branched-chain amino acids, valine, leucine and isoleucine. The detailed compositional analysis has led to the conclusion that the wheat line AP602CL are substantially equivalent to the parent line.

Based on the above, the CFIA has determined that wheat line AP602CL will not result in altered impacts on non-target organisms, including humans, compared to current wheat varieties.

5. Potential Impact on Biodiversity of line AP602CL

Wheat line AP602CL 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 and AP602CL is equivalent to that of currently commercialized wheat lines.

Over the longer term, 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 the wheat line AP602CL to minimize the development of imidazolinone-tolerant weed populations.

V. Criteria for the Livestock Feed Assessment

1. Potential Impact on Livestock Nutrition

Nutrient and anti-nutrient composition of wheat line AP602CL was compared with its control (Gunner), grown in replicated trials in North Dakota and Minnesota.

Anti-nutritional Factors

Phytic acid content of AP602CL averaged 1.3% and was not different from Gunner. Trypsin inhibitors in both AP602CL and Gunner were below the limit of detection of 2000 TIU/ml.

Nutritional Composition

Composition of AP602CL 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.

There were small but statistically significant differences observed, including lower fat content, lower oleic acid content, and higher threonine content in AP602CL compared

with Gunner. There were no differences in branched chain amino acids, or any of the other components analysed. The differences observed were very small in biological terms and would not impact 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. AHAS from AP602CL 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 AP602CL with unmodified wheat counterparts, the Plant Biosafety Office, CFIA, has concluded that the novel genes and their corresponding traits do not confer to wheat line AP602CL 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 AP602CL with unmodified wheat 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 wheat line AP602CL. 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 AP602CL has been assessed and found to be as safe as nutritious as traditional wheat varieties. AP602CL 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 AP602CL is therefore authorized as of March 20, 2003. Any other wheat lines derived from it may be imported and/or released, provided no inter-specific crosses are performed, provided the intended use is similar, and provided it is known, following thorough characterization that these plants do not display any additional novel traits and are substantially equivalent to currently grown wheat, in terms of their potential environmental impact and livestock feed safety.

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:

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

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