



## Decision Document DD2002-42

# Determination of the Safety of BASF's Imazethapyr Tolerant PWC16 Rice

This Decision Document has been prepared to explain the regulatory decision reached under the guidelines Dir95-03 *Guidelines for the Assessment of Livestock Feed from Plants with Novel Traits* and Dir94-08 *Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits*.

The Canadian Food Inspection Agency (CFIA), specifically the Feed Section of the CFIA, with advice from the Plant Biosafety Office of the CFIA has evaluated information submitted by BASF regarding the Imazethapyr tolerant PWC16 rice line. CFIA has determined that feed derived from these plants do not present a concern to environmental or livestock feed safety, when compared to currently commercialized rice varieties in Canada.

**Livestock feed use of the rice line PWC16 is therefore authorized as of December 5, 2002. Line PWC16 and any rice 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 commercialized rice, in terms of their potential environmental impact and livestock feed safety.**

**The rice line PWC16 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:	Rice line PWC16
Applicant:	BASF
Plant Species:	Rice ( <i>Oryza sativa</i> )
Novel Traits:	Tolerance to imazethapyr, an imidazolinone herbicide
Trait Introduction Method:	Chemically induced seed mutagenesis
Proposed Use of PNT's:	Production of rice for livestock feed, human food and industrial uses. These materials will be grown outside of Canada, in the usual production areas for rice.

## II. Background Information

BASF has developed rice line PWC16 tolerant to imazethapyr, an imidazolinone herbicide. This rice line was developed to provide an alternative strategy for weed control.

The development of the PWC16 rice was accomplished using chemically induced seed mutagenesis and whole plant selection procedures. The herbicide tolerance results from a single point mutation in the major acetohydroxyacid synthase (AHAS) gene such that this enzyme, the target of imidazolinone herbicides, is no longer affected by imazethapyr.

PWC16 was field tested in the United States in 2000.

BASF has provided data on the identity of rice line PWC16, 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 rice line PWC16 such as grain yield, days to 50% head, plant height and milling were compared the parental unmodified counterpart.

Nutritional components of PWC16 such as proximates, amino acids and fatty acids were compared with unmodified rice counterparts. Anti-nutritional factors were also determined.

The Feed Section, CFIA, with input from the Plant Biosafety Office, CFIA, has reviewed the above information. The following assessment criteria as described in regulatory directives Dir95-03 and Dir94-08 were used to determine the safety and efficacy as livestock feed and the environmental safety of feed from this plant with novel trait:

- potential impact of PWC16 rice on livestock and workers/by-standers,
- potential impact of PWC16 rice on livestock nutrition,

- potential of PWC16 rice to become a weed of agriculture or be invasive of natural habitats,
- C potential for gene flow from PWC16 rice to wild relatives whose hybrid offspring may become more weedy or more invasive,
- C potential of PWC16 rice to become a plant pest,
- C potential impact of PWC16 rice or their gene products on non-target species, including humans, and
- C potential impact of PWC16 rice on biodiversity.

### 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 rice variety Cypress with ethyl methanesulfonate (EMS). Whole plant selection procedures for herbicide tolerance were used. One herbicide tolerant mutant was selected and was designated PWC16. Increases in seed was done through self pollination, or natural pollination occurring within the variety.

PWC16 is a diploid belonging to genus and species *Oryza sativa*.

#### 2. Imazethapyr 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 catalyzes 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 rice is not tolerant to imidazolinone herbicides.

A single amino acid substitution in the AHAS gene, sufficient to alter the binding site for imidazolinone herbicides such that Imazethapyr no longer inhibits the AHAS enzyme, resulted in a herbicide tolerant phenotype.

The novel Imazethapyr tolerance is under the control of the native AHAS promoter and is believed to be constitutively expressed. Sequence information from the modified AHAS gene was submitted for PWC16.

The tolerance to Imazethapyr was demonstrated by comparison of the activity of the AHAS enzyme extracted from PWC16 rice plants to that of the unmodified parental rice plants.

The levels of valine, leucine and isoleucine produced in rice are regulated by feedback inhibition. BASF provided data to support the claim that the modified 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 (~0.001% of total protein in rice seed), it is heat sensitive and susceptible to trypsin degradation. The AHAS protein from PWC16 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 5 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 rice.

The major endogenous allergens of rice are the 14-16kDa group of proteins that show homology to the alpha-amylase/trypsin inhibitor family from wheat and barley. It was demonstrated that PWC16 had comparable levels of trypsin inhibitor activity to that of unmodified comparators. Rice also contains endogenous allergens reported as 33 kDa and 56-60 kDa proteins and a lipid-transfer protein. BASF provided evidence to show that the protein components of PWC16 are not altered in comparison with the unmodified parental comparator. SDS-PAGE electrophoresis and HPLC were run on protein extracts from the unmodified parental comparator and the modified rice 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 rice containing the modified AHAS gene.

### **3. Stable Expression**

The segregation of herbicide tolerance in crosses with PWC16 is consistent with the inheritance of a single semi-dominant gene. All F1 progeny are resistant to the Imazethapyr herbicide. F2 progeny segregate in a 1:2:1 ratio (herbicide resistant: intermediate resistant: susceptible to herbicide).

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

**Note:** Rice line PWC16 will not be grown in Canada and will only be imported as human food or livestock feed. The majority of rice imported to Canada does not have an intact hull, which results in the seed being incapable of germination and growth. Due to the unfavorable climatic conditions for rice in Canada, it is unlikely that grain from rice line PWC16 would be capable of persisting in the Canadian environment.

### **1. Potential of Line PWC16 to Become Weeds of Agriculture or Invasive of Natural Habitats**

The centre of origin of rice is considered to be in the subtropics of Southeast Asia. Rice is not grown in Canada and is not adapted to the environmental conditions encountered in Canadian agricultural environments.

The CFIA evaluated data submitted by BASF on the biology of rice line PWC16 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 rice varieties.

No competitive advantage was conferred to these plants, other than that conferred by tolerance to an imidazolinone herbicide. Tolerance to an imidazolinone herbicide will not, in itself, render rice weedy or invasive of natural habitats since none of the reproductive or growth characteristics were modified.

Rice line PWC16 is not intended for cultivation in Canada and the novel trait has no intended effects on weediness or invasiveness. The CFIA has therefore concluded that these rice lines have no altered weed or invasiveness potential in Canada when compared to conventional rice varieties.

## 2. Potential for Gene Flow from Line PWC16 to Wild Relatives Whose Offspring May Become More Weedy or More Invasive

Species sexually compatible with rice do not occur in Canada. The wild “rice” which occurs in Canada (*Zizania aquatica*) belongs to a species that is not sexually compatible with domesticated rice (*Oryza sativa*). Rice line PWC16 will not be cultivated in the Canada and if released, would not persist.

The CFIA has therefore determined that gene flow to sexually compatible species in Canada is not possible.

## 3. Altered Plant Pest Potential of Line PWC16

*Oryza sativa* is not a plant pest in Canada, additionally the agronomic characteristics of rice line PWC16 were shown to be within the normal range of conventional rice varieties.

The CFIA has therefore determined that rice line PWC16 does not present a plant pest concern.

## 4. Potential Impact on Non-Target Organisms of Line PWC16

The detailed characterization of the modified gene containing a single base pair change and the resulting enzyme, has led to the conclusion that the expression of the novel protein does not result in altered toxic or allergenic properties. 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.

Based on the above, the CFIA has determined that rice line PWC16 will not result in altered impacts on non-target organisms, including humans, compared to current rice varieties.

## 5. Potential Impact on Biodiversity of Line PWC16

Rice line PWC16 is safe to non-target organisms, does not present altered weediness or plant pest potential and will not be grown in Canada. In addition the novel trait has not altered the ability of these lines to persist in the Canadian environment.

The CFIA has therefore concluded that the potential impact on biodiversity of rice line PWC16 does not present any adverse impacts on biodiversity in Canada.

## V. Criteria for the Livestock Feed Assessment

### 1. Potential Impact on Livestock Nutrition

Nutrient and anti-nutrient composition of rice grain from PWC16 was compared with its control (Cypress), grown in replicated trials in three locations in the southern United States.

#### Anti-nutritional Factors

Phytic acid and trypsin inhibitor concentrations were determined in rice grain samples from PWC16 and Cypress. There were no differences between the lines in concentration of either phytic acid or trypsin inhibitor.

#### Nutritional Composition

Nutritional components were measured in grain from rice line PWC16 and Cypress. Components included crude fat, crude protein, crude fibre, amino acids, fatty acids, B vitamins, vitamin E, and the minerals phosphorous, magnesium, iron, zinc. There were no differences between PWC16 and Cypress in nutrient composition, and the composition was typical for rice grain. The CFIA has therefore concluded that rice line PWC16 has nutritional composition equivalent to conventional rice.

### 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 PWC16 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. 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 protein profiles of the modified plant compared to the unmodified comparators) it is unlikely that secondary mutations causing unintended effects have occurred in the rice 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 authorization of this rice line.

## VII. Regulatory Decision

Based on the review of data and information submitted by BASF, including comparisons of PWC16 with the unmodified parental 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 rice line PWC16. Rice grain, groats, hulls, bran and rice bran oil are currently listed in Schedule IV of the *Feeds Regulations* and are, therefore approved for use in livestock feeds in Canada. Rice line PWC16 has been assessed and found to be substantially equivalent to the unmodified parental rice variety, with respect to safety and nutritional quality. PWC16 and its products are considered to meet the present ingredient definitions and are approved for use as livestock feed ingredients in Canada. This rice line will not be grown in Canada nor can the seed overwinter, therefore the release of the feed into the environment would result in neither intended nor unintended environmental effects.

**Livestock feed use of rice line PWC16 is therefore authorized as of December 5, 2002. PWC16 and any other rice 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 rice, in terms of their livestock feed safety and environmental impact.**

**PWC16 is subject to the same phytosanitary import requirements as it's unmodified counterpart.**

Please refer to Health Canada's Decisions on Novel Foods for a description of the food safety assessment of PWC16 rice lines. The food safety decisions are available at the following Health Canada web site:

[http://www.hc-sc.gc.ca/food-aliment/english/subjects/novel\\_foods\\_and\\_ingredient/novel\\_foods\\_and\\_ingredient.html](http://www.hc-sc.gc.ca/food-aliment/english/subjects/novel_foods_and_ingredient/novel_foods_and_ingredient.html)