



Decision Document

DD2002-40

Determination of the Safety of BASF's Imazethapyr Tolerant (CLEARFIELD™) 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 CLEARFIELD™ rice lines CL121, CL141 and CFX51. CFIA has determined that feed derived from these plant with novel traits does not present a concern to environmental or livestock feed safety, when compared to currently commercialized rice varieties in Canada.

Livestock feed use of the CLEARFIELD™ rice lines CL121, CL141 and CFX51 is therefore authorized as of February 5, 2002. Lines CL121, CL141 and CFX51 and any rice lines derived from them 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 CLEARFIELD™ rice lines are subject to the same phytosanitary import requirements as their unmodified counterparts.

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I. Brief Identification of the Plants with Novel Traits (PNTs)

Designation of the PNTs:	CLEARFIELD™ rice lines CL121, CL141 and CFX51
Applicant:	BASF
Species:	Rice (<i>Oryza sativa</i>)
Novel Traits:	Tolerance to imazethapyr, an imidazolinone herbicide
Trait Introduction Method:	Chemically induced seed mutagenesis
Intended Use of the PNT:	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 three rice lines tolerant to imazethapyr, an imidazolinone herbicides. These rice lines, referred to as CLEARFIELD™ rice (lines CL121, CL141 and CFX51 all of which were derived from crosses with the 93AS3510 mutant), were developed to provide an alternative strategy for weed control.

The development of the CLEARFIELD™ rice 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 imazethapyr

93AS3510 was field tested in the United States in 2000.

BASF has provided data on the identity of CLEARFIELD™ rice lines, 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. Relevant scientific publications were also supplied.

Agronomic characteristics of rice lines CL121, CL141 and CFX51 derived from 93AS3510 such as grain yield, days to 50% head, plant height and milling were compared to those of unmodified rice counterparts.

Nutritional components of rice lines CL121, CL141 and CFX51 derived from 93AS3510 such as proximates, amino acids and fatty acids were compared with unmodified rice counterparts. Anti-nutritional factors were also determined.

The Feed Section of the Animal Health and Production Division, CFIA, with input from the Plant Biosafety Office of the Plant and Health Production Division, 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 suitability as livestock feed and the environmental safety of feed from these plant with novel traits:

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

III. Description and Assessment of the Novel Trait

1. 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 catalyses the first step in the biosynthesis of the essential branched chain amino acids isoleucine 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 imidazolinones such that the herbicide 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 93AS3510.
- The tolerance to imidazolinone herbicides was demonstrated by comparison of the activity of the AHAS enzyme extracted from 93AS3510 rice plants to that of wild type 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 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 protease susceptible. The AHAS protein from 93AS3510 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 93AS3510 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 93AS3510 are not altered in comparison with an unmodified comparator. SDS-PAGE electrophoresis and HPLC were run on protein extracts from unmodified and modified rice to indicate that no new major proteins or increased protein expression occurred as a result of the mutagenic event.

2. Development Method

- The original mutant line was isolated from a population derived by chemical-induced mutagenesis of seed of rice line AS3510 with ethyl methanesulfonate (EMS). Whole plant selection procedures for herbicide tolerance were used. One herbicide tolerant mutant was selected and was designated 93AS3510. 93AS3510 was crossed with commercial varieties using conventional plant breeding techniques to create the imazethapyr tolerant CLEARFIELD™ rice lines CL121, CL141 and CFX51.
- CL121 was derived from an initial cross of 93AS3510 to the rice variety Cocodrie. Selections in the F3, F4, F5 and F6 progeny rows were made to isolate CL121.
- CL141 was derived from an initial cross of 93AS3510 to the rice variety Maybelle. Two additional backcrosses were made to Maybelle. Selections in the F4, F5 and F6 progeny rows were made to isolate CL141.
- CFX51 was derived from an initial cross of 93AS3510 to the rice variety Cypress. Selections in the F3, F4, F5 and F6 progeny rows were made to isolate CFX51.
- The four lines (93AS3510, CL121, CL141 and CFX51) are diploids belonging to genus and species *Oryza sativa*.

3. Stable Expression

- The segregation of herbicide tolerance in crosses with 93AS3510 is consistent with the inheritance of a single semi- or co-dominant allele. Backcrossed F2 progeny of 93AS3510

consistently showed imazethapyr tolerance.

IV. Criteria for Safety Assessment

Note: Rice lines CL121, CL141 and CFX51 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 lines CL121, CL141 and CFX51 would be capable of persisting in the Canadian environment.

1. Potential of lines CL121, CL141 and CFX51 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 lines CL121, CL141 and CFX51, 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 lines CL121, CL141 and CFX51 are not intended for cultivation in Canada and the novel traits have 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 lines CL121, CL141 and CFX51 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 lines CL121, CL141 and CFX51 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 lines CL121, CL141 and CFX51

- *Oryza sativa* is not a plant pest in Canada, additionally the agronomic characteristics of rice lines CL121, CL141 and CFX51 were shown to be within the normal range of conventional rice varieties.

The CFIA has therefore determined that rice lines CL121, CL141 and CFX51 do not present a plant pest concern.

4. Potential Impact on Non-Target Organisms of lines CL121, CL141 and CFX51

- 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 lines CL121, CL141 and CFX51 will not result in altered impacts on non-target organisms, including humans, compared to current rice varieties.

5. Potential Impact on Biodiversity of lines CL121, CL141 and CFX51

- Rice lines CL121, CL141 and CFX51, are safe to non-target organisms, do 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 lines CL121, CL141 and CFX51 does not present any adverse impacts on biodiversity in Canada.

V. Nutritional Assessment Criteria as Livestock Feed

The nutritional assessment was based on nutrient and anti-nutrient composition of rice grain from the three lines derived from 93AS3510, compared with five representative commercial lines (Cocodrie, Cypress, Drew, Wells, Bengal) grown in three locations in the rice growing area of the United States.

1. Anti-Nutritional Factors

- Phytic acid, trypsin inhibitor and lectin concentrations were determined in rice grain samples from each of CL121, CL141 and CFX51 and five commercial rice varieties. Trypsin inhibitor and lectin concentrations were both below the limit of detection in all of the varieties. Phytic acid levels in CL121, CL141 and CFX51 were equivalent to the levels

observed in the five commercial varieties.

2. Nutritional Composition

- Nutritional components were measured in grain from rice lines CL121, CL141 and CFX51, and from five commercial control varieties grown in three US locations. Components included crude fat, crude protein, crude fibre, amino acid content and fatty acid content. The concentration of these components in the three lines was within the range observed in the five control unmodified lines.

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 these rice lines.

VII. Regulatory Decision

Based on the review of data and information submitted by BASF, including comparisons of rice hybrids derived from 93AS3510 with unmodified rice counterparts, the Feed Section of the Animal Health and Production Division, 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 CLEARFIELD™ rice lines CL121, CL141 and CFX51. 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 lines CL121, CL141 and CFX51 derived from 93AS3510 have been assessed and found to be substantially equivalent to traditional rice varieties, with respect to safety and nutritional quality. CL121, CL141 and CFX51 and their products are considered to meet the present ingredient definitions and are approved for use as livestock feed ingredients in Canada. None of the rice lines will be grown in Canada nor can the seed overwinter, therefore the release of the feed into the environment would result in no intended or unintended environmental effects.

Livestock feed use of the CLEARFIELD™ rice lines CL121, CL141 and CFX51 derived from 93AS3510 is therefore authorized as of February 5, 2002. CL121, CL141 and CFX51 and any other rice lines derived from them 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.

The CLEARFIELD™ rice lines are subject to the same phytosanitary import requirements as their unmodified counterparts.

Please refer to Health Canada's Decisions on Novel Foods for a description of the food safety assessment of CLEARFIELD™ rice 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