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Français	Contact Us	Help	Search	Canada Site
Table of Contents	What's New	Acts and Regulations	Site Map	
Food Safety	Animal Health	Plant Protection	Corporate Affairs	

Quick Pick By Commodity / Key Topic

- [Plant Products](#)
- [Plant Biosafety](#)
- [Acts and Regulations](#)
- [Biotechnology Notices of Submission Project](#)
- [Decision Documents](#)
- [Regulation of Plants with Novel Traits](#)
 - [Confined or Unconfined Release of Plants with Novel Traits](#)
 - [Assessment Criteria for Determining Environmental Safety](#)
- [Overview of Regulation of Plants With Novel Traits in Canada](#)
- [Contacts](#)

**Canadian Food Inspection Agency
Plant Products Directorate
Plant Biosafety Office**

Decision Document DD2004-46 Determination of the Safety of the BASF Canada Imidazolinone-Tolerant Lentil Line RH44

This Decision Document has been prepared to explain the regulatory decision reached under the regulatory directive 94-08 *Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits* and the companion document BIO2003-12 *The Biology of Lens Culinaris (Lentil)* and the regulatory directive 95-03 *Guidelines for the Assessment of Plants with Novel Traits as Livestock Feed*.

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 lentil line RH44, which will be known commercially as CLEARFIELD™ lentil. 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 lentil varieties in Canada.

Unconfined release into the environment and livestock feed use of line RH44, is therefore authorized as of June 24, 2004. Line RH44 and any other lentil 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 lentils, in terms of their potential environmental impact and livestock feed safety.

Line RH44 is subject to the same phytosanitary import requirements as its unmodified counterparts.

Table of Contents

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

II. Background Information

III. Description of the Novel Traits

1. Development Method
2. Imidazolinone Tolerance
3. Stable Expression

IV. Criteria for the Environmental Safety Assessment

1. Potential of Line RH44 to become a Weed of Agriculture or to be Invasive of Natural Habitats
2. Potential for Gene Flow from Line RH44 to Relatives Whose Hybrid Offspring May Become More Weedy or More Invasive
3. Altered Plant Pest Potential of Line RH44
4. Potential Impact of Line RH44 on Non-Target Organisms
5. Potential Impact of Line RH44 on Biodiversity

V. Criteria for the Livestock Feed Assessment

1. Potential Impact of Line RH44 on Livestock Nutrition
2. Potential Impact of Line RH44 on Livestock and Workers/By-standers

VI. New Information Requirements

VII. Regulatory Decisions

Appendices

1. [CLEARFIELD Lentil Herbicide Tolerance Stewardship](#)

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

Designations of the PNT:	Line RH44
Applicant:	BASF Canada
Plant Species:	Lentil (<i>Lens culinaris</i>)
Novel Traits:	Tolerance to imidazolinone herbicides

Trait

Introduction	Chemically induced seed mutagenesis
Method:	
Proposed Use of the PNT:	Production of <i>L. culinaris</i> for human food and livestock feed. This material will not be grown outside the normal production area for lentil.

II. Background Information

BASF Canada has developed a lentil line tolerant to imidazolinone herbicides. This *L. culinaris* line, designated line RH44, exhibited no significant injury when treated with imidazolinone herbicides at normal field application rates. This will allow the use of imidazolinones in post-emergence on lentil crops, thus providing an alternative means of weed control in lentil production.

The development of line RH44 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 imidazolinones.

Line RH44 has been field tested in Canada from 1999 to 2002 under confined conditions in the provinces of Alberta, Manitoba, and Saskatchewan.

BASF Canada has provided data on the identity of line RH44, 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. References to scientific publications were also included.

Agronomic characteristics such as grain yield, days to maturity, plant height, and disease susceptibilities were compared with those of unmodified *L. culinaris* counterparts.

Nutritional components of line RH44 such as proximates, amino acids and fatty acids were compared with unmodified lentil counterparts. Levels of anti-nutritional factors were also compared between the line RH44 and the unmodified counterparts.

The Plant Biosafety Office (PBO) Canadian Food Inspection Agency (CFIA) reviewed the above information, in accordance with the following assessment criteria for determining environmental safety of plants with novel traits (PNTs), as described in regulatory directive Dir94-08:

- potential of line RH44 to become weeds of agriculture or to be invasive of natural habitats,
- potential for gene-flow from line RH44 to wild relatives whose hybrid

- offspring may become more weedy or more invasive,
- potential for line RH44 to become plant pests,
- potential impact of line RH44 or its gene products on non-target species, including humans,
- potential impact of line RH44 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 novel livestock feed, as described in the regulatory directive Dir95-03:

- potential impact of line RH44 on livestock nutrition; and
- potential impact of line RH44 on livestock and workers/bystanders.

Additionally, CFIA has reviewed a method submitted by BASF Canada for the detection and identification of lentils containing this modified AHAS gene.

III. Description of the Novel Trait

1. Development Method

The RH44 mutant was derived by chemical-induced mutagenesis. Seeds from many lentil cultivars were treated as a batch with ethyl-methane-sulphonate (EMS) then planted. Whole plant selection procedures for herbicide tolerance were used. One herbicide tolerant mutant was selected and was designated line RH44.

Line RH44 is a diploid ($2n=14$ chromosomes) belonging to genus and species *Lens culinaris*.

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 lentils are not tolerant to imidazolinone herbicides.

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

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

The tolerance to imidazolinone herbicides was demonstrated by comparison of the activity of the AHAS enzyme extracted from line RH44 lentil plants to that of wild type lentil plants.

The levels of valine, leucine and isoleucine produced in lentil 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 line RH44 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 lentil. Data presented demonstrated that protein banding patterns and IgE activity from sera obtained from lentil allergic individuals are similar between the modified and unmodified lentils.

3. Stable Expression

The imidazolinone tolerance trait from line RH44 was shown to segregate according to the manner expected for a single dominant allele. Line RH44, which is several generations removed from the original mutation event, consistently shows imidazolinone tolerance.

IV. Assessment Criteria for Environmental Safety

1. Potential of Line RH44 to become a Weed of Agriculture or be Invasive of Natural Habitats

The biology of *L. culinaris*, described in BIO2003-12, shows that unmodified plants of this species are not invasive of unmanaged habitats in Canada. According to the information provided by BASF Canada, line RH44 was determined not to be different from its counterparts in this respect. The mutation of the AHAS gene in line RH44 has not significantly affected the physiology of the plant, as supported by agronomic and compositional data. No significant differences between RH44 and comparator lines were detected in the yield, seed germination, days to maturity, or nutritional data including amino acid composition, and percentage fat, fiber and protein. No data was submitted that indicated line RH44 would possess traits that would render it invasive of unmanaged habitats, or that a competitive advantage was conferred to line RH44, other than tolerance to imidazolinones.

Imidazolinone tolerance in itself will not cause line RH44 to become more

weedy or invasive in managed habitats than conventional lines of *L. culinaris*. Imidazolinone-tolerant lentil volunteers will not be controlled in subsequent crops if imidazolinone herbicide is used as the sole weed control tool. However, control of imidazolinone tolerant weeds, or imidazolinone tolerant lentil 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 line RH44 has no short-term ecological advantages when compared with currently commercialized lentil varieties.

In the longer term, the continued use of imidazolinones on herbicide tolerant crops may provide additional selection pressure for the development of group 2 herbicide tolerant weeds. BASF provided the CFIA with a stewardship plan that describes appropriate strategies that will allow the deployment of line RH44 while managing these concerns. The stewardship plan submitted by BASF is based on the biology of the lentil plant and on associated agronomic practices. It also received endorsement from the Saskatchewan Pulse Growers and the Saskatchewan Crop Development Centre. The stewardship plan is included in [Appendix 1 of this document](#).

CFIA acknowledges that BASF implementation of this stewardship plan may be affected by future scientific developments, significant future changes to crop management practices used by lentil growers, and/or grower compliance with the plan.

In order to monitor the implementation of the *Clearfield Lentil Herbicide Tolerance Stewardship Plan*, CFIA will require BASF to submit a report to the PBO on a triennial basis. This report will describe BASF's monitoring of grower compliance with the stewardship plan, as well as any changes that may be made to the plan itself. As outlined in Section VI of this document, reporting may occur more frequently if BASF becomes aware of any new information about the environmental safety of line RH44 or lentil lines derived from RH44.

2. Potential for Gene Flow from Line RH44 to Relatives Whose Hybrid Offspring May Become More Weedy or More Invasive

The biology of lentil, as described in BIO2003-12, indicates that a) lentil plants have less than one per cent outcrossing rates and b) there are no wild or weedy relatives in Canada that can freely hybridize with *L. culinaris*.

The CFIA therefore concludes that gene flow from line RH44 to wild or weedy relatives is extremely unlikely.

3. Altered Plant Pest Potential of Line RH44

The intended effect of the novel trait is not expected to affect plant pest potential, and *L. culinaris* is not a plant pest in Canada.

Line RH44 and an appropriate non-mutagenized line of similar genetic background were tested for responses to several common insect pests of lentil and several important fungal pathogens. There were no indications in the data supplied by BASF that line RH44 has an altered plant pest potential. In all cases, responses of line RH44 were within the normal range of typical commercial lentil cultivars.

In addition, agronomic characteristics of line RH44 were shown to be within the range of values displayed by currently commercialized *L. culinaris* varieties, leading to the conclusion that plant pest potential has not been inadvertently altered.

No gene sequences from plant pathogens were used in its development and it is therefore highly unlikely that line RH44 expresses any novel plant pathogen characteristics.

The CFIA has therefore determined that line RH44 is unlikely to display any altered plant pest potential.

4. Potential Impact of Line RH44 on Non-Target Organisms

Single amino acid modifications of the AHAS enzyme, which alters the herbicide binding site on the enzyme, is the molecular basis for imidazolinone tolerance in several plants. 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 RH44 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* gene will not result in 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 microorganisms 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 RH44 were shown to be within the range of values displayed by currently commercialized *L. culinaris* varieties. The CFIA concluded that there were not likely to be significant unintended changes to line RH44 that could have adverse impacts on non-target organisms.

5. Potential Impact of Line RH44 on Biodiversity

As discussed previously, line RH44 has no novel phenotypic characteristics which would extend its use beyond the current geographic range of lentil production in Canada. Responses of line RH44 to common insect pests and fungal pathogens are within the range of typical commercial lentil cultivars. Since lentil 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 CFIA has therefore concluded that the impact on biodiversity of line RH44 is equivalent to that of currently commercialized lentil lines.

V. Criteria for the Livestock Feed Assessment

1. Potential Impact on Livestock Nutrition

Nutritional composition

Nutritional composition was determined for line RH44 lentil seed samples taken from various sites in Saskatchewan over three growing seasons (2000B 2002). Nutrients measured included proximates, amino acids, fatty acids, B vitamins, and minerals. Control samples from these sites included between 3 and 6 commercial varieties. In some cases the data (proximates, amino acids) were analysed by appropriate statistical methods. In other cases, simple comparisons with control commercial varieties were conducted (minerals, fatty acids, B vitamins). The applicant demonstrated that protein, fat, fibre, leucine, and valine composition for line RH44 was not different from control commercial varieties. For some nutrients there were significant differences among the lentil varieties (i.e., protein, isoleucine, and threonine) but line RH44 values were intermediate among the varieties for these nutrients. Concentrations reported for minerals, fatty acids and B vitamins were within the range reported for the commercial control varieties.

Antinutritional factors

Phytic acid and trypsin inhibitor were analysed in samples for line RH44 and 5 commercial varieties (pooled sample for each variety). Line RH44 levels were within the range of the commercial cultivars for phytic acid, and trypsin inhibitor was undetected (<2000 TIU) in all varieties.

The applicant demonstrated that the nutritional composition of line RH44 lentil is similar to commercial varieties grown in Canada.

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 RH44 is

feedback inhibited by valine and leucine as is the 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 and agronomic data of the modified plant compared to the unmodified comparator, as well as similar protein banding patterns and IgE activity from sera obtained from lentil allergic individuals between the modified and unmodified lentils) it is unlikely that secondary mutations causing unintended effects have occurred in the lentil 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 use and will re-evaluate its decision with respect to the livestock feed use and environmental release authorizations of this lentil line.

VII. Regulatory Decision

Based on the review of data and information submitted by BASF Canada, including comparisons of line RH44 with unmodified lentil counterparts, the Plant Biosafety Office, CFIA, has concluded that the modified gene and its corresponding novel trait do not confer to lentil line RH44 any characteristic that would result in an ecological advantage or plant pest risk following unconfined release.

Based on the review of data and information submitted by BASF Canada, including comparisons of Line RH44 with unmodified lentil 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 the lentil Line RH44. Lentil and lentil screenings are currently listed in Schedule IV of the *Feeds Regulations* and are, therefore approved for use in livestock feeds in Canada. Line RH44 has been assessed and found to be as safe and as nutritious as traditional lentil varieties. Lentil line RH44 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 lentil line RH44 is therefore authorized as of June 24, 2004. Any

other lentil 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 lentils, in terms of their potential environmental impact and livestock feed safety.

Line RH44 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 lentil line RH44. 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

This bulletin is published by the Canadian Food Inspection Agency. For further information, please contact the Plant Biosafety Office or the Feed Section at:

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[Top of Page](#)

[Important Notices](#)