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Plant Health and Production Division

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Division de la production et de la protection végétaux

Decision Document DD98-28

Determination of the Safety of AgrEvo Canada Inc.'s Glufosinate Ammonium Herbicide-Tolerant *Brassica rapa* Canola Line HCR-1

This Decision Document has been prepared to explain the regulatory decision reached under the guideline Dir94-08 *Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits*, the companion document T-1-12-96 *The Biology of Brassica rapa L. (Canola/ Rapeseed)* and Dir95-03 *Guidelines for the Assessment of Plants with Novel Traits as Livestock Feed*.

The Canadian Food Inspection Agency (CFIA), specifically the Plant Biotechnology Office and the Feed Section of the Plant Products Division with input from the Plant Health Risk Assessment Unit, CFIA, has evaluated information submitted by AgrEvo Canada Inc. regarding the canola line HCR-1. This hybrid was obtained through inter-specific crosses with *B. napus* plants transformed with genes that express tolerance to glufosinate-ammonium. The CFIA has determined that the canola line HCR-1 should not pose a concern to environmental safety and is considered to be substantially equivalent to canola currently approved as livestock feed.

Unconfined release into the environment of the *B. rapa* hybrid line HCR-1 is authorized in British Columbia, Alberta, Saskatchewan and Manitoba. Its use as livestock feed is also authorized. Also, any *B. rapa* lines derived from HCR-1, resulting from the same transformation event or transformed with the same genetic construct, may be considered substantially equivalent, provided it is known following thorough characterization that: no inter-specific crosses are performed; the intended use is similar; and these plants do not display any additional novel traits.

Please note that, while determining the environmental and livestock feed safety of plants with novel traits is a critical step in the commercialization of these plant types, other requirements still need to be addressed, such as Variety Registration (CFIA).

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I. Brief Identification of the Plants with Novel Traits (PNT's)

Designation of the PNT's: HCR-1

Applicant: AgrEvo Canada Inc.

Plant Species: *Brassica rapa* canola

Novel Trait: Novel tolerance to glufosinate ammonium herbicide

Trait Introduction Method: Inter-specific cross with transgenic *B. napus*

Proposed Use of the PNT's: Production of *B. rapa* for seed oil for human consumption and seed oil and meal for livestock feed. These materials will not be grown outside the normal production area for canola.

II. Background Information

AgrEvo Canada Inc. has developed a *Brassica rapa* canola hybrid, HCR-1, derived from an inter-specific cross with the *B. napus* transformation event T45, that expresses a novel tolerance to glufosinate ammonium. This tolerance will allow the use of glufosinate ammonium as a post-emergence herbicide, thus providing an alternative for weed control in canola production, and reducing reliance on soil-incorporated herbicides. *B. napus*, transformed with the gene conferring glufosinate ammonium tolerance has been approved in May 1996 (see DD96-11.)

A bacterial gene which codes for phosphinothricin acetyltransferase, an enzyme that inactivates glufosinate ammonium through acetylation, was introduced into the HCR-1 line by an interspecific cross with the *B. napus* transformant T45. The inactivation of glufosinate ammonium by the bacterial enzyme confers the herbicide tolerance onto the novel plant.

Glufosinate ammonium tolerant *B. rapa* has been field tested in Canada under confined conditions in Saskatchewan and Alberta in 1994, 1995 and 1996.

AgrEvo Canada Inc. has submitted information on the identity of the HCR-1 hybrid line, the description of the development method and provided the expression levels of the novel protein. They have also referred to the information submitted for the environmental release and livestock feed approval of the *B. napus* line HCN28, a line derived from transformant T45.

Scientific references to support submitted information were cited, when available. In addition, AgrEvo has provided information, data and recorded observations comparing line HCR-1 to unmodified Polish canola counterparts. These comparisons have addressed characteristics that included days to flower, days to maturity, seed production (yield), crop height, lodging, disease tolerance, protein, oil content, fibre and fatty acid composition.

The Plant Biotechnology Office of the Plant Products Division, CFIA, has reviewed the above information, according to the Regulatory Directive Dir94-08, for determining environmental safety of plants with novel traits, which lists the following assessment criteria:

- potential of the PNT to become a weed of agriculture or be invasive of natural habitats
- potential for gene flow to wild relatives whose hybrid offspring may become more weedy or more invasive
- potential for the PNT's to become plant pests,
- potential effects of the PNT or it's gene products on non-target species, including humans, and
- potential impact on biodiversity.

The Feed Section of the Plant Products Division, CFIA, has also reviewed the above information in light of the assessment criteria for determining safety and efficacy of livestock feed, as described in Dir95-03. The following assessment criteria were considered:

- potential impact to livestock
- potential impact to livestock nutrition

III. Description of the Novel Herbicide Tolerance

1. Glufosinate Ammonium Tolerance:

- Phosphinothricin (L-PPT), the active ingredient of glufosinate ammonium, inhibits glutamine synthetase, which results in the accumulation of lethal levels of ammonia in susceptible plants within hours of application.

- The phosphinothricin tolerance derives from the same gene engineered into canola line HCN28 (see DD96-11). The gene codes for PPT- acetyltransferase (PAT). This enzyme detoxifies phosphinothricin by acetylation into an inactive compound. It has extremely high substrate specificity; experimental data clearly showed that neither the L-PPT analog L-glutamic acid, D-PPT, nor any protein amino acid can be acetylated by the PAT enzyme.

- The PAT gene was originally isolated from *Streptomyces viridochromogenes*, an aerobic soil actinomycete. The PAT enzyme is therefore naturally occurring in the soil. More generally, acetyltransferases are ubiquitous in nature.

- Average PAT content of seed samples collected at three trial sites was 107 ng/g of plant tissue. The average values at the 3 locations varied from 84 to 132 ng/g of plant tissue The PAT expression levels are comparable to those determined in the approved *B. napus* lines HCN92 and HCN28 (ranges from approx 95-250 ng/g).

- The PAT protein showed no significant nucleotide sequence similarity with any known toxins or allergens. Unlike most allergens, there are no glycosylation sites on the PAT protein. There should be no health concern with the ingestion of HCR-1 by livestock as there is no activity detected from PAT in processed meal. The raw PAT protein was rapidly inactivated in dog stomach fluid (pH1.1) within 1 minute. Rapid inactivation of the enzyme was observed, even under buffered conditions (at pH 4.0 inactivation occurred in 10 minutes.) PAT protein in plant tissue was inactivated within 15 minutes in dog stomach fluid. There is no known effect of PAT on beneficial insects or pollinators and there are extensive studies showing the safety and specificity of the PAT enzyme.

2. Development Method

- The *Brassica napus* transformant T45 was developed using a disarmed non-pathogenic *Agrobacterium tumefaciens* vector; the vector contained the transfer DNA (T-DNA) region of an *A. tumefaciens* plasmid from which virulence and disease causing genes were removed, and replaced with the PAT gene. The T-DNA portion of *A. tumefaciens* plasmids are generally known to insert randomly into the plant's genome and the insertion is usually stable. A single copy of the PAT gene was inserted into the *B. napus* transformant T45 and no sequences outside the border regions of the T-DNA were detected.

- The T45 transformant was used as the pollinator and the *B.rapa* cultivar Parkland was the recipient. A single plantlet was selected and three crosses back to Parkland were performed, followed by full sib matings for selection of homozygous plants. The resulting plants formed the basis for seed multiplication.

3. Stable Integration into the Plant Genome

- The introduced gene has been sequenced and its function is well characterized. The amino acid sequence of the PAT protein is known. Southern analysis of restriction enzyme digests, using the 550 bp PAT gene as a probe, showed that a single copy of the T-DNA was stably incorporated into the *Brassica* genome (data presented for T45). Segregation data with the HCR-1 hybrid line confirmed the stable integration of the T-DNA into the genome.

- The successful introgression of the glufosinate ammonium tolerance from the T45 transformant into the HCR-1 *B. rapa* line suggests that the trait was located in the *B. rapa* portion of the amphidiploid *B. rapa/B. oleracea* genome of *B. napus*.

IV. Assessment Criteria for Environmental Safety

1. Potential of the PNT to Become a Weed of Agriculture or Be Invasive of Natural Habitats

The CFIA has evaluated the data and information submitted by AgrEvo Canada Inc., on the reproductive and survival biology of the HCR-1 hybrid. From this, the Agency has found that vegetative vigor, flowering, time to maturity and seed production were within the normal range of expression found in unmodified Polish canola hybrids. HCR-1 has no specific added genes for cold tolerance or winter hibernation. It did not show any stress adaptation other than tolerance to glufosinate ammonium herbicide.

The biology of *B. rapa*, described in T-1-12-96, shows that this species normally is not invasive of unmanaged habitats in Canada. It occasionally is a weed of cultivated land, especially in the eastern provinces of Canada. According to the information and data provided by AgrEvo Canada Inc., HCR-1 was found to be no different from unmodified canola counterparts in this respect. The CFIA concurs that no competitive advantage was conferred by the insertion of the glufosinate ammonium tolerance gene, other than tolerance to the herbicide. Resistant canola volunteer plants will not be controlled by glufosinate ammonium herbicides, however they can be managed by growers using alternative herbicides with different modes of action.

The above considerations, together with the fact that the novel traits have no intended effect on weediness or invasiveness, has led the CFIA to conclude that HCR-1 has no altered weed or invasiveness potential compared to currently commercialized canola varieties.

Note: A longer term concern, if there is general adoption of several different crop and specific herbicide weed management systems, is the potential development of crop volunteers with a combination of novel resistances to different herbicides. This could result in the loss of the use of these herbicides and any of their potential benefits. Therefore, agricultural extension personnel, in both the private and public sectors, should promote careful management practices for growers who use these herbicide-tolerant crops, to minimize the development of multiple resistance.

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

Brassica rapa is an obligate outcrosser with other plants of the same species, and potentially with plants of the species *B. napus*, *B. juncea*, *B. carinata*, *B. nigra*, *Diplotaxis muralis*, *Raphanus raphanistrum*, and *Erucastrum gallicum* (see T-1-12-96). Studies show that potential introgression of the herbicide tolerance is most likely to occur with *B. napus*, the other major canola species, that is not considered as a weed in Canada.

If glufosinate ammonium tolerant individuals did arise through interspecific or intergeneric hybridization, the tolerance should not confer any competitive advantage to these plants unless challenged by the herbicide. This would only occur in managed ecosystems where glufosinate ammonium is applied for broad spectrum weed control, or in plant varieties developed to exhibit glufosinate ammonium tolerance and in which that herbicide is used to control weeds. As with glufosinate ammonium tolerant *B. rapa* volunteers, these individuals, should they arise, would be controlled using other available chemical means. Hybrids, if they developed, could potentially result in the loss of glufosinate ammonium as a tool to control these species. This however, can be avoided by the use of sound crop management practices.

The above considerations led the CFIA to conclude that gene flow from the HCR-1 hybrid line to relatives is possible, but should not result in increased weediness or invasiveness of these relatives.

3. Altered Plant Pest Potential

The intended effects of the novel herbicide tolerance trait are unrelated to plant pest potential, and *B. rapa* is not a plant pest in Canada (see T-1-12-96). In addition, agronomic characteristics, and qualitative and quantitative composition of HCR-1 were shown by AgrEvo to be within the range of values displayed by unmodified canola varieties. The CFIA concurs with the conclusion that plant pest potential has not been inadvertently altered.

4. Potential Impact on Non-Target Organisms

The detailed characterization of the novel gene and novel protein, briefly summarized in Part III, suggest that the modified *B. rapa* should not possess altered toxic or allergenic properties. AgrEvo has presented evidence that shows that the plant expressed PAT is rapidly inactivated in gastric fluids by enzymatic degradation and pH-mediated proteolysis. The PAT enzyme is highly specific and displays enzyme kinetics typical of those found in the plant kingdom. Seed protein profiles and fatty acid composition fall within the range of those of their unmodified counterparts

Based on the above, the CFIA has determined that the unconfined release of the HCR-1 hybrid will not result in altered impacts on interacting organisms, including humans, when compared with current canola varieties.

5. Potential Impact on Biodiversity

The introduced genes were determined to be safe to non-target organisms. In addition, the HCR-1 hybrid line has no novel phenotypic characteristics which would extend its use beyond the current geographic range of canola production in Canada. Since outcross species are only found in disturbed habitats, transfer of the novel herbicide tolerance would not affect unmanaged environments.

The CFIA has therefore concluded that the potential impact on biodiversity of the HCR-1 hybrid line is equivalent to that of currently commercialized canola varieties.

V. Nutritional Assessment Criteria For Use As Livestock Feed

1. Anti-Nutritional Factors

Glucosinolates in the PNT line were determined from composite samples taken from short season zone trials. The glucosinolate levels were not significantly different from the control, unmodified line and well within the established standard of 30 µM/ g. dried meal. The relative concentration of erucic acid for the PNT and the control were similar and well within the established limit of 2%.

2. Nutritional Composition of PNT

No statistical differences in nutritional composition, i.e., crude protein, crude fat, crude fibre, ash, gross energy levels, oil content and fatty acid composition were noted between the whole seed from the HCR-1 line and current commercial canola cultivars. These results demonstrate that the introduction of the novel herbicide tolerance into *B. rapa*, resulting in the HCR-1 hybrid line, did not likely result in any secondary effects on the composition or nutritional quality of the cultivar. Accordingly, the HCR-1 hybrid line was judged to be substantially equivalent to traditional canola varieties in terms of nutritional composition.

VI. Regulatory Decision

This decision takes into consideration the presence of populations of wild bird rape (*Brassica rapa*) in Quebec, and the Atlantic provinces of Canada, and to a lesser extent in the Southern part of Ontario; and the undesirable movement of glufosinate ammonium herbicide tolerance into these weedy populations where this herbicide is used for weed control. This decision has been reached after consultation with plant breeders, weed scientists, and agronomists in academia and provincial government services.

Based on the review of submitted data, the Feed Section of the Plant Products Division has concluded that the novel gene expressed by the HCR-1 hybrid line does not raise safety or nutritional concerns as livestock feed. As this hybrid line has been assessed and found to be substantially equivalent to traditional *B. rapa* varieties with respect to feed safety, the HCR-1 hybrid line and it's byproducts are approved for use as livestock feed ingredients in Canada.

If at any time, AgrEvo becomes aware of any information regarding risk to the environment, or risk to animal or human health, that could result from release of these materials in Canada, or elsewhere, AgrEvo must immediately provide such information to the CFIA. On the basis of such new information, the CFIA may re-evaluate the potential impact of the release and re-evaluate its decision.

Unconfined release into the environment of the *B. rapa* hybrid line HCR-1 is authorized in British Columbia, Alberta, Saskatchewan and Manitoba. Its use as livestock feed is also authorized. Also, any *B. rapa* lines derived from HCR-1, resulting from the same transformation event or transformed with the same genetic construct, may be considered substantially equivalent, provided it is known following thorough characterization that: no inter-specific crosses are performed; the intended use is similar; and these plants do not display any additional novel traits.

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