

Determination of environmental safety of Monsanto Canada Inc.'s Roundup herbicide-tolerant brassica napus canola line GT73.

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Plant Products Division
Food Production and Inspection Branch
Agriculture and Agri-Food Canada

Decision Document

DD95-02

Determination Of Environmental Safety Of Monsanto Canada Inc.'s Roundup® Herbicide-Tolerant *Brassica napus* Canola Line GT73

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*. This is in association with the companion document Dir94-09 *The Biology of Brassica napus L. (Canola/Rapeseed)* and the proposed guidelines Pro94-04 *Guidelines for the Assessment of Plants with Novel Traits as Livestock Feed*.

Agriculture and Agri-Food Canada (AAFC), specifically the Plant Biotechnology Office and the Feed Section of the Plant Products Division, have evaluated information submitted by Monsanto Canada Inc. regarding the canola line GT73. This line has Roundup-Ready™ genes that express novel tolerance to glyphosate, the active ingredient of Roundup® herbicide. The Department has determined that this plant does not present altered environmental interactions when compared to existing commercialized canola varieties in Canada, and is considered substantially equivalent to canola currently approved as livestock feed.

Unconfined release into the environment, including feed use of GT73, and other *B. napus* lines derived from it, but without the introduction of any other novel trait, is therefore considered safe.

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Table of Contents

I.	Brief Identification of the Plant with Novel Traits (PNT)	1
II.	Background Information	1
III.	Description of the Novel Glyphosate Tolerance	2
1.	The Roundup-Ready™ Genes	2
2.	Development Method	3
3.	Stable Integration into the Plant's Genome	3
IV.	Assessment Criteria for Environmental Safety	4
1.	Potential of the GT73 to Become a Weed of Agriculture or be Invasive of Natural Habitats	4
2.	Potential for Gene Flow to Wild Relatives Whose Hybrid Offspring May Become More Weedy or Invasive	5
3.	Altered Plant Pest Potential	5
4.	Potential Impact on Non-Target Organisms	6
5.	Potential Impact on Biodiversity	6
V.	Nutritional Assessment Criteria As Livestock Feed	6
1.	Anti-Nutritional Factors	6
2.	Nutritional Composition of GT73	7
VI.	Regulatory Decision	7

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

Designation of the PNT:	GT73
Applicant:	Monsanto Canada Inc.
Plant Species:	<i>Brassica napus</i> canola
Novel Trait:	Novel tolerance to glyphosate, the active ingredient of Roundup® herbicide, expressed by the Roundup-Ready™ genes
Trait Introduction Method:	<i>Agrobacterium tumefaciens</i> mediated transformation
Proposed Use of the PNT:	Production of seed oil for human consumption and seed oil and meal for livestock feed. This line will not be grown outside the normal production area for canola in Canada.

II. Background Information

Monsanto Canada Inc. has developed a *Brassica napus* canola line, GT73, derived from the variety "Westar," which expresses their proprietary Roundup-Ready™ genes. These confer novel tolerance to glyphosate, the active ingredient of Roundup® herbicide, which can control or suppress economically important weeds in canola production.

The development of GT73 was based on recombinant DNA technology, by the introduction into the variety "Westar" of a genetic construct containing two bacterial derived genes (the Roundup-Ready™ genes). One gene imparts reduced sensitivity to Roundup® at the biochemical site of the herbicide's activity and the other expresses an enzyme that enables the plant to degrade glyphosate. The expression of both genes is directed to the chloroplasts, the site of the herbicide activity, by the addition of genetic coding sequences of a plant derived transit peptide. No antibiotic resistance marker genes were introduced into GT73.

GT73 has been field tested in Canada under confined conditions since 1992, in Alberta, Saskatchewan, Manitoba and Ontario.

Monsanto has submitted information that includes data generated from AAFC-managed cooperative trials, and information primarily submitted to Health Canada, made available to AAFC. This information described:

- the identity of GT73, including a detailed description of the modification method;
- the stability of the gene insertions;
- the role of these genes and their regulatory sequences in the donor organisms;

- the molecular characterization of the genes;
- the identification and characterization of the novel proteins, including their potential toxicity to livestock; and
- non-target organisms, allergenicity, and levels of expression in the plant.

Scientific references were listed to support information where available. In addition, Monsanto has provided information, data and recorded observations comparing GT73 to "Westar" or other varietal counterparts. These comparisons have addressed characteristics that included seed production (yield), days to flowering, days to maturity, silique shattering, susceptibility to the fungal pathogen blackleg and the effects of plant residues on growth and productivity of subsequent crops such as barley and peas.

Data to support the efficacy of GT73 as a livestock feed was provided. A proximate analysis to include crude protein, crude fat (oil), crude fibre, ash and gross energy were supplied for the whole seed and processed meal.

AAFC, specifically, the Plant Biotechnology Office and the Feed Section of the Plant Products Division, has reviewed the above information, considering 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 to become a plant pest,
- potential affects of the PNT or its gene products on non-target species, including humans, and
- potential impact on biodiversity.

AAFC have also reviewed the above information in light of the assessment criteria for determining safety and efficacy of livestock feed, as described in Pro94-04:

- potential impact on livestock, and
- potential impact on livestock nutrition.

III. Description of the Novel Glyphosate Tolerance

1. The Roundup-Ready™ Genes

- Two genes have been introduced into the variety "Westar", which in combination provide field level tolerance to glyphosate the active ingredient in Roundup® herbicide. The exact nature of these genes is considered confidential business information by Monsanto.

- The first gene expresses a bacterial derived version of a plant enzyme involved in the shikimate biochemical pathway for the production of the aromatic amino acids phenylalanine, tyrosine and tryptophan. The plant version of this enzyme, ubiquitous in nature, is sensitive to glyphosate - the herbicide disrupts this essential pathway, leading to growth suppression or death of the plant. However, the bacterial derived version of this enzyme is highly insensitive to glyphosate and fulfils the aromatic amino acid needs of the plant.
- The second gene, also bacterial derived from a strain of a species ubiquitous in nature, expresses an enzyme that degrades glyphosate, thereby deactivating the herbicidal effect. The coding sequence of this gene has been altered to enhance the efficiency of glyphosate degradation, compared to the original bacterial version.
- A plant-derived coding sequence expressing a chloroplast transit peptide was co-introduced with each of the Roundup-Ready™ genes. This peptide facilitates the import of the newly translated Roundup-Ready™ enzymes into the chloroplasts, the site of both the shikimate pathway and glyphosate mode-of-action.
- Both genes associated with their transit peptide coding sequence, are linked to the same constitutive promoter. Roundup-Ready™ protein expression was monitored in leaf and seed tissue of plants in two years of confined field trials.
- The Roundup-Ready™ proteins showed no significant homology with any known toxins or allergens. The novel enzyme associated with the shikimate pathway, is a version of an enzyme that is ubiquitous in nature, and therefore would not be expected to be toxic or allergenic to non-target organisms. For the second novel enzyme, which degrades glyphosate, Monsanto describes experiments with a variety of substrates that show that it has a narrow substrate specificity, and appears not to affect any plant specific pathways. Both proteins were inactivated during processing of canola seed into feed ingredients.

2. Development Method

Brassica napus variety "Westar" was transformed using a disarmed non-pathogenic *Agrobacterium tumefaciens* vector, i.e., the T-DNA region of an *A. tumefaciens* plasmid from which virulence and disease causing genes were removed, and replaced with the Roundup-Ready™ genes. 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, as was shown to be the case in GT73.

3. Stable Integration into the Plant's Genome

Monsanto has provided information on segregation and Southern blot analysis that demonstrates that GT73 has a single genetic insert, consisting of single copies of the Roundup-Ready™ genes.

GT73 is several generations removed from the original transformant. Mendelian inheritance of the herbicide tolerance, and Southern and PCR analyses of third-generation material field tested in 1992 show the stability of the introduced traits.

Monsanto has backcrossed GT73 to *B. rapa*, and the herbicide tolerance has been successfully transferred. This suggests that the genetic insert is stably 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 GT73 to Become a Weed of Agriculture or be Invasive of Natural Habitats

AAFC has evaluated the data and information submitted by Monsanto Canada Inc., on the reproductive and survival biology of GT73. From this, the Department has found that vegetative vigor, overwintering capacity, days to flowering, time to maturity, seed production or yield, germination and dormancy were within the normal range of expression found in single seed selected unmodified "Westar." GT73 has no specific added genes for cold tolerance or winter hibernation. No overwintered plants were observed by Monsanto's cooperators in post-harvest years of field trials. The number of volunteers in the years following a field trial was comparable between plots of GT73 and "Westar". Similar comparisons on the degree of silique shattering, seed dispersal and seed germination, demonstrated no change due to the gene inserts, showing that seed dispersal potential was not altered.

Based on the submitted data, AAFC has found that GT73 did not show any stress adaptation other than its tolerance to glyphosate. Tests for resistance to the fungal pathogen *Leptosphaeria maculans* (blackleg) showed that GT73's susceptibility falls within the ranges currently displayed by "Westar". Recorded observations by Monsanto's cooperators over two years of field trials consistently state that no differences in susceptibility to other diseases or insect infestations were found between GT73 and "Westar". Similarly, no differences were observed for abiotic stress factors such as heat, drought and frost.

The biology of *B. napus*, described in Dir94-09, shows that this species normally is not invasive of unmanaged habitats in Canada. According to the information and data provided by Monsanto, GT73 was found to be no different from "Westar" in this respect. Invasiveness was studied in an undisturbed habitat, roadside non-crop land. Data showed that GT73 was neither more invasive nor more persistent than "Westar". AAFC concurs that no competitive advantage was conferred by the insertion of the Roundup-Ready™ genes, other than tolerance to Roundup® herbicide. Glyphosate tolerant *B. napus* volunteer plants 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, have led AAFC to conclude that GT73 has no altered weed or invasiveness potential compared to currently commercialized canola varieties such as "Westar".

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

B. napus plants are known to outcross up to 30% with other plants of the same species, and potentially with plants of the species *B. rapa*, *B. juncea*, *B. carinata*, *B. nigra*, *Diplotaxis muralis*, *Raphanus raphanistrum*, and *Erucastrum gallicum* (see Dir94-09). Studies show that potential introgression of the herbicide tolerance is most likely to occur with *B. rapa*, the other major canola species, which occasionally is a weed of cultivated land, especially in the eastern prairies of Canada.

If glyphosate tolerant individuals did arise through interspecific or intergeneric hybridization, the tolerance would not confer any competitive advantage to these plants unless challenged by Roundup® herbicide. This would only occur in managed ecosystems where Roundup® is applied for broad spectrum weed control, or in plant varieties developed to exhibit Roundup® tolerance and in which Roundup® is used to control weeds. As with glyphosate tolerant *B. napus* 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 Roundup® as a tool to control these species. This however, can be avoided by the use of sound crop management practices.

The above considerations led AAFC to conclude that gene flow from GT73 to relatives is indeed possible, but would not result in increased weediness or invasiveness of these relatives.

3. Altered Plant Pest Potential

The intended effects of the novel herbicide tolerance trait is unrelated to plant pest potential, and *B. napus* is not a plant pest in Canada (see Dir94-09). In addition, agronomic characteristics, stress adaptation, and qualitative and quantitative

composition of GT73 were demonstrated by Monsanto to be within the range of values displayed by varieties such as "Westar". AAFC concurs with the conclusion that plant pest potential has not been inadvertently altered.

4. Potential Impact on Non-Target Organisms

Data presenting the effect of plant residue from GT73 on agronomic performance of succeeding crops, barley and peas, were evaluated by AAFC. No significant differences in either plant counts or grain yield were identified between field plots where GT73 and counterpart "Westar" had been grown the previous year. AAFC considers this as indirect evidence that soil microorganisms involved in maintaining soil fertility, are not negatively affected by GT73 plant residues in comparison to "Westar" residues.

According to extensive data initially submitted to Health Canada, using simulated digestive fluids and acute mouse gavage studies, the enzymes expressed by the Roundup-Ready™ genes are rapidly inactivated and degraded in the gastric and intestinal systems. A search of the FAST amino-acid sequence database revealed no significant homology with known toxins or allergens entered in that database.

Based on the above, AAFC has determined that the unconfined release of GT73 will not result in altered impacts on interacting organisms, including humans, compared to current varieties such as "Westar".

5. Potential Impact on Biodiversity

GT73 has no novel phenotypic characteristics which would extend its use beyond the current geographic range of canola production in Canada. Data and information submitted by Monsanto have demonstrated to AAFC that GT73 is not invasive of natural habitats, and that it is not more competitive than its counterparts, both in natural and managed ecosystems. Since outcross species are only found in disturbed habitats transfer of the novel herbicide tolerance would not impact unmanaged environments.

AAFC has therefore concluded that the potential impact on biodiversity of GT73 is equivalent to that of currently commercialized canola varieties, such as "Westar".

V. Nutritional Assessment Criteria as Livestock Feed

1. Anti-Nutritional Factors

Ninety-five percent Confidence Intervals were determined for glucosinolate and erucic acid content of the meal and oil produced from GT73 grown under a variety of conditions. These confidence intervals demonstrated that GT73 contained levels of these anti-nutritional factors below the prescribed standards for both the meal and

oil fractions, i.e., <30 micromoles glucosinolates per gram of dry meal and, <2% erucic acid in the oil.

2. Nutritional Composition of GT73

No statistical differences in nutritional composition (i.e., crude protein, crude fat, crude fibre, ash and gross energy content), were noted between the whole seed nor processed meal of GT73 and current commercial canola varieties. No statistical differences were observed in the levels of the shikimate pathway aromatic amino acids phenylalanine, tyrosine and tryptophan. These results collectively demonstrate that the introduction of this construct into *B. napus*, resulting in GT73, should not result in any secondary effects impacting on its composition or nutritional quality. Accordingly, GT73 was judged to be substantially equivalent to traditional canola varieties in terms of nutritional composition.

VI. Regulatory Decision

Based on the review of Monsanto Canada Inc.'s data and information submitted to the Plant Biotechnology Office and the Feed Section of the Plant Products Division, AAFC concludes that the Roundup-Ready™ genes and their corresponding novel Roundup® herbicide tolerance do not in themselves confer any intended ecological advantage to the plant or to its relatives, should gene flow occur. In addition, this novel herbicide tolerance does not alter the characteristics of GT73 with regards to the assessment criteria for environmental safety. Thorough comparisons of GT73 and unmodified *B. napus* canola counterparts such as "Westar", showed no unexpected effects.

Unconfined release into the environment and the feeding of livestock with GT73 (and other *B. napus* lines derived from it, provided no other novel traits are introduced) is therefore considered safe.

