Decision Document DD2005-54

# Determination of the Safety of Monsanto Canada Inc. and KWS SAAT AG's Roundup Ready® Sugar Beet (*Beta vulgaris* ssp *vulgaris L.*) Event H7-1

This Decision Document has been prepared to explain the regulatory decision reached under Directive 94-08 (Dir94-08), entitled "*Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits",* its companion biology document Bio2002-01, *The Biology of Beta vulgaris L. (Sugar Beet)*, and Directive 95-03 (Dir95-03), entitled "*Guidelines for the Assessment of Novel Feeds: Plant Sources".*

The Canadian Food Inspection Agency (CFIA), specifically the Plant Biosafety Office (PBO) of the Plant Products Directorate and the Feed Section of the Animal Health and Production Division have evaluated information submitted by Monsanto Canada Inc. and KWS SAAT AG. This information is in regard to the glyphosate tolerant sugar beet event H7-1. The CFIA has determined that this plant with a novel trait (PNT) and novel feed does not present altered environmental risk nor does it present livestock feed safety concerns when compared to currently commercialized sugar beet varieties in Canada.

**Unconfined release into the environment and use as livestock feed of the sugar beet event H7-1 are therefore authorized as of September 13, 2005. Any other sugar beet events and intraspecific hybrids resulting from the same transformation events and all their descendants, may also be released into the environment and used as livestock feed, provided no inter-specific crosses are performed, provided the intended use is similar, provided it is known, following thorough characterization, that these plants do not display any additional novel traits and are substantially equivalent to currently grown sugar beet, in terms of their potential environmental impact and livestock feed safety.**

**The sugar beet event H7-1 is subject to the same phytosanitary import requirements as its unmodified counterpart.**

Please note, that the livestock feed and environmental safety of PNTs and novel feeds are critical steps in the potential commercialization of these plant types. Other requirements, such as the evaluation of food safety by Health Canada, have been addressed separately from this review.

(*publié aussi en français*)

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

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| **Designation(s) of the PNT:** | Event H7-1<br>OECD identifier KM-000H71-4 |
| **Applicant:** | Monsanto Canada Inc. and KWS SAAT AG |
| **Plant Species:** | *Beta vulgaris* ssp *vulgaris L.* |
| **Novel Traits:** | Herbicide tolerance (glyphosate). |
| **Trait Introduction Method:** | *Agrobacterium*- mediated transformation |
| **Proposed Use of PNT:** | Production of *B. vulgaris* for processing into sugar for human consumption and by-product for livestock feed. This plant will not be grown outside the normal production area for sugar beet in Canada. |

## II. Background Information

Monsanto Canada Inc. and KWS SAAT AG have developed a sugar beet line from event H7-1 containing an *epsps* gene that codes for a 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which imparts tolerance to glyphosate, the active ingredient in the agricultural herbicide Roundup®, a novel trait in sugar beet in Canada . This trait allows for the control or suppression of economically important weeds in sugar beet production.

Event H7-1 was developed using *Agrobacterium tumefaciens* transformation, resulting in the introduction of the *epsps* gene from the bacterium *Agrobacterium* sp. strain CP4 (ie. *cp4 epsps*) into the sugar beet genome. This version of EPSPS has a reduced affinity for glyphosate in comparison with endogenous plant EPSPSs, thus imparting glyphosate tolerance to the sugar beet.

Monsanto Canada Inc. and KWS SAAT AG have provided data on the identity of H7-1, a detailed description of the transformation method, data and information on the gene insertion site, gene copy number, and levels of gene expression in the plant and the role of the inserted genes and regulatory sequences. The novel protein was identified, characterized and compared to the original bacterial protein, including an evaluation of its potential toxicity to livestock and non-target organisms.

These materials had been field tested in North America from 1998 to 2003 and in Europe, France and Germany, from1998 to 1999.

Agronomic characteristics of sugar beet hybrids derived from H7-1 such as emergence, seedling vigour, bolting, yield, recoverable sugar, seed germination rate, seed dormancy, time for vernalization, bolting date, onset of flowering and seed harvest date were compared to those of unmodified sugar beet counterparts.

Monsanto Canada Inc. and KWS SAAT AG has provided an agronomic stewardship plan for Roundup Ready® sugar beet in the Canadian environment. This plan includes information regarding a safe and sustainable deployment of Roundup Ready® suger beet and an efficient mechanism for growers to report agronomic problems with this product to Monsanto Canada Inc.

Nutritional components of H7-1, such as proximates, carbohydrates, and amino acids were compared with those of unmodified sugar beet counterparts.

The PBO of the Plant Products Directorate, CFIA, has reviewed the above information, in light of the assessment criteria for determining environmental safety of PNTs, as described in the Directive 94-08 (Dir94-08), entitled "*Assessment Criteria for Determining Environmental Safety of Plants With Novel Traits*". The PBO has considered:

- potential of sugar beet event H7-1 to become a weed of agriculture or invasive of natural habitats,
- potential for gene flow from sugar beet event H7-1 to wild relatives whose hybrid progeny may become more weedy or more invasive,
- potential for sugar beet event H7-1 to become a plant pest,
- potential impact of sugar beet event H7-1 or their gene products on non-target species, including humans, and
- potential impact of sugar beet event H7-1 on biodiversity.

The Feed Section of the Animal Health and Production Division, CFIA, has also reviewed the above information with respect to the assessment criteria for determining the safety and efficacy of livestock feed, as described in Directive 95-03 (Dir95-03), entitled "*Guidelines for the Assessment of Novel Feeds: Plant Sources*". The Feed Section has considered:

- potential impact of sugar beet event H7-1 on livestock health and
- potential impact of sugar beet event H7-1 on livestock and workers/by-standers.

## III. Description of the Novel Traits

### 1. Development Method

Roundup Ready® sugar beet event H7-1 was generated using KWS multigerm sugar beet line 3S0057 which was transformed using the disarmed binary vector PV-BVGT08 carrying the *cp4 epsps* gene. The *cp4 epsps* cassette was introduced by *Agrobacterium tumefaciens* transformation into cultured sugar beet cells. Transformants containing the *cp4 epsps* gene were selected based on glyphosate tolerance. Plants were regenerated and a suitable line was designated as event H7-1.
The event H7-1 is a diploid (18 chromosomes (2n=18)) belonging to genus and species *Beta vulgaris* ssp. *vulgaris*.

## 2. Glyphosate Tolerance

A gene derived from *Agrobacterium* sp. strain CP4 (*cp4 epsps*), which imparts field level tolerance to glyphosate, the active ingredient in the agricultural herbicide Roundup®, was introduced into sugar beet event H7-1. An optimized chloroplast transit peptide, isolated from *Arabidopsis thaliana* was fused to the *cp4 epsps* coding sequence. This peptide facilitates the import of the newly translated EPSPS enzyme into the chloroplast, the site of amino acid biosynthesis.

EPSPS is an enzyme involved in the shikimic acid metabolic pathway which is essential for the production of the aromatic amino acids. Typically glyphosate binds to the plant EPSPS enzyme and blocks the biosynthesis of aromatic amino acids, thereby depriving the plant of essential compounds. The CP4 EPSPS enzyme from *Agrobacterium* sp strain CP4 has a reduced affinity for glyphosate and continues to function in presence of glyphosate. Thus the sugar beet event H7-1, which expresses the CP4 EPSPS enzyme, is tolerant to glyphosate.

The *cp4 epsps* gene expressed in sugar beet event H7-1 is linked to a constitutive promoter. Samples of leaf and root were collected from five representative US field trial sites. Average protein expression in micro-grams protein per gram fresh weight tissue, as evaluated by ELISA, are as follows:

| **Field Site** | **CP4 EPSPS Mean protein level (ug/g fwt)** | |
|---|---|---|
| | Leaf Tissue | Root Tissue |
| Idaho | 107 | 91 |
| Michigan | 142 | 92 |
| Minnesota | 125 | 93 |
| Nebraska | 92 | 120 |
| North Dakota | 143 | 124 |

The EPSPS protein is present in many foods with a long history of safe use in Canada, and therefore would not be expected to be toxic or allergenic. Unlike known food allergens, CP4 EPSPS is heat sensitive and susceptible to digestion. When baked at 200°C for 24 minutes, to simulate the heat processing step used to produce dried beet pulp for consumption by livestock, CP4 EPSPS levels were essentially non-detectable. Additionally, the CP4 EPSPS enzyme was completely digested within 15 seconds in simulated gastric fluid as demonstrated by Western blots**.** Unlike many known allergens, the CP4 EPSPS protein is not glycosylated, and is present at low levels (< 1.44% of the total protein) in the root tissue of H7-1 sugar beet.

Amino acid sequence comparisons made between the CP4 EPSPS protein and known allergens, using a database assembled from the public domain databases SwissProt, GenPept, TrEMBL, GenBank, EMBL, PIR and NRL3D, revealed no significant amino acid sequence homologies (based on sequence identity of eight or more contiguous amino acids). A comparison with a similarly constructed database of known toxins indicated no biologically relevant similarities amino acid sequence homologies between known toxins and the CP4 EPSPS protein. A previously submitted acute mouse gavage study demonstrated that the CP4 EPSPS is not toxic. No treatment-related adverse effects were observed in animals administered CP4 EPSPS protein by oral gavage at doses up to 475 mg/kg.

Due to the low levels of CP4 EPSPS protein expressed in the sugar beet plant it was necessary to produce CP4 EPSPS protein by bacterial fermentation to obtain sufficient quantities to conduct some of the safety studies (acute oral mouse toxicity study, simulated gastric fluid digestion study and simulated intestinal fluid digestion study). The bacterial-produced protein was compared to the plant-produced protein and shown to be of similar molecular weight, immunological reactivity and to have similar functional activity as the plant produced protein. Matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) and N-terminal sequencing demonstrated amino acid sequence equivalency of the plant and bacterial produced proteins.

### 3. Stable Integration into the Plant's Genome

Southern blot analysis of event H7-1 indicated that there is one site of integration of the introduced *cp4 epsps* gene cassette which includes a single copy of the *cp4 epsps* gene. The data demonstrates that the *cp4 epsps* coding region and associated promoter and terminator sequences are intact.

Southern blot analysis over three generations demonstrated the stability of the *cp4 epsps* gene cassette . Segregation analysis was performed on four generations of event H7-1 and demonstrated that the insert is inherited as a single locus in an expected Mendelian pattern.

## IV. Criteria for the Environmental Assessment

### 1. Potential to Become a Weed of Agriculture or be Invasive of Natural Habitats

The biology of sugar beet *(Beta vulgaris* L.*)* as described in Biology Document Bio2002-01 , "*The biology of Beta vulgaris L.*" shows that unmodified plants of this species are not invasive of unmanaged habitats in Canada. *Beta vulgaris* is not a primary colonizer in unmanaged ecosystems. There is no evidence in Canada that *B. vulgaris* has weed or pest characteristics. In Canada, they do not survive outside of cultivation for significant periods of time due to cold sensitivity and poor competitiveness.

The CFIA evaluated data submitted by Monsanto Canada Inc. on the reproductive and survival biology of sugar beet hybrids derived from H7-1, and determined that

germination, flowering, root yield, susceptibility to plant pests and diseases typical to sugar beet and bolting percentage were within the normal range of expression of these traits currently displayed by commercial sugar beet hybrids. No genes were inserted for cold tolerance or winter survival.

No competitive advantage was conferred to these plants, other than that conferred by tolerance to glyphosate herbicide. Resistance to Roundup® agricultural herbicides will not, in itself, render sugar beet weedy or invasive of natural habitats since none of the reproductive or growth characteristics were modified.

The above considerations, together with the fact that the novel traits have no intended effects on weediness or invasiveness, led the CFIA to conclude that the H7-1 sugar beet event has no altered weed or invasiveness potential compared to currently commercialized sugar beet.

The agronomic stewardship plan, which contains a herbicide tolerance management plan, submitted by Monsanto Canada Inc. and KWS SAAT AG was evaluated by the CFIA and determined to be satisfactory. The herbicide tolerant stewardship plan includes recommendations on agricultural practices concerning Roundup Ready® sugar beet and provides an efficient mechanism for growers to report agronomic problems with this product to Monsanto Canada Inc., facilitating the ongoing monitoring of Roundup Ready® sugar beet.

A longer term consideration, if there is general adoption of several different crop species and specific herbicide weed management systems (ie. numerous combinations of crop species and tolerances to different herbicides), is the potential development of crop volunteers with a combination of novel tolerances to different herbicides. This could result in the loss of the use of these herbicides and any of their potential benefits. Therefore, Monsanto Canada Inc. will make their stewardship plan readily available to growers and agriculture extension personnel, in both private and public sectors, to promote the careful management practices, such as use of alternate control tools as appropriate to achieve complete weed and volunteer control, recommended to help minimize the development of resistant weed populations.

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

The biology of sugar beet, as described in Bio2002-01, indicates that there are no wild relatives in Canada that can hybridize with *Beta vulgaris*.

The CFIA therefore concludes that gene flow from H7-1 to sugar beet relatives is not possible in Canada.

**3. Altered Plant Pest Potential**

The intended effects of the novel trait is unrelated to plant pest potential, and sugar beet is

not a plant pest in Canada (Bio2002-01). In addition, agronomic characteristics of the modified sugar beet hybrids were shown to be within the range of values displayed by currently commercialized sugar beet hybrids, and indicate that the growing habit of sugar beet was not inadvertently altered. Glyphosate is commonly used for chemical fallow production and resistant sugar beet volunteer plants will not be controlled. Despite the tolerance to glyphosate, H7-1 volunteers can still be managed by growers using alternative herbicides with different modes of action. Field observations did not indicate modifications of disease and pest susceptibilities.

The CFIA has therefore determined that the H7-1 sugar beet event does not display any altered pest potential.

### 4. Potential Impact on Non-Target Organisms

The detailed characterization of the novel gene and resulting enzyme, as briefly summarized in Part III of the present document, has led to the conclusion that the expression of the novel protein does not result in altered toxic or allergenic properties. The EPSPS protein is not a known toxin and is commonly found in a wide variety of plants and micro-organisms with a long history of safe use. Additionally, the lack of acute oral toxicity of the CP4 EPSPS protein was demonstrated in mice.

Based on the above, the CFIA has determined that the unconfined release of the H7-1 sugar beet event will not result in altered impacts on interacting organisms, including humans, compared to current sugar beet varieties.

### 5. Potential Impact on Biodiversity

H7-1 has no novel phenotypic characteristics which would extend its use beyond the current geographic range of sugar beet production in Canada. Since sugar beet does not outcross to wild relatives in Canada, there will be no transfer of novel traits to unmanaged environments. In addition the novel trait was determined to be safe to non-target organisms.

The CFIA has therefore concluded that the potential impact on biodiversity of H7-1 will not be altered.

## V. Nutritional Assessment Criteria for Use as Livestock Feed

### 1. Potential Impact on Livestock Nutrition

#### Nutritional Composition and Anti-Nutritional factors

In one study, nutritional composition of H7-1 processed sugar beet roots (brei) and sugar beet tops were compared to the isogenic null segregant line (control) and eight commercial varieties. Compositional data was obtained from replicated trials in 5 European sites. Proximates (crude protein, crude fat, crude fibre, ash), saponins, 18

amino acids, sucrose, invert sugar, sodium (Na), potassium (K), alpha-amino nitrogen were determined for roots. Tops were analysed for proximates, saponins, and amino acids. In this trial, some differences between H7-1 and control in amino acids were reported:

- in roots: differences in alanine (Ala) (higher in H7-1) and glutamic acid (Glu) (lower in H7-1);
- in tops: differences in Ala (higher in H7-1), histidine (His), phenylalanine (Phe), tyrosine (Tyr) (lower in H7-1). All amino acid levels were within the commercial ranges.

There were no other differences in composition.

In another study, root biomass and four nutrient quality constituents in brei (sucrose, sodium, potassium, and alpha amino nitrogen) were analysed in H7-1 and control sugar beets from ten replicated trials conducted in North America. In this study H7-1 root biomass was lower than the control variety at one location, and sucrose content was higher at two locations. For potassium, H7-1 was higher in one location and lower in two locations. In these cases the values for the H7-1 event were within the established literature ranges.

Finally, a digestibility trial in sheep (7 animals per treatment) was conducted using diets containing 70% sugar beets (H7-1 vs. 5 commercial varieties grown in Europe). There were no differences in apparent digestibility of dry matter, organic matter, crude protein, acid detergent fibre and neutral detergent fibre and digestible energy.

The applicant has demonstrated that composition and nutrient digestibility of sugar beet variety H7-1 are equivalent to non-modified sugar beet varieties.

## 2. Potential Impacts on Livestock and Workers/By-standers

Sugar beet is not known for the production of endogenous allergens and the transformation event which produced H7-1 would not be expected to induce their synthesis.

The CP4 EPSPS enzyme is from *Agrobacterium* strain CP4, a soil bacterium, which is not a known human or animal pathogen. The CP4 EPSPS used in transformation of H7-1 shares 99.7% amino acid sequence identity to native Agrobacterium sp strain CP4 EPSPS. The amino acid sequence is identical to the CP4 EPSPS protein in RR crops previously approved in Canada . CP4 EPSPS shares no biologically relevant significant homology with known toxins or allergens, 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 Monsanto Canada, CP4 EPSPS is unlikely to be a novel toxin or allergen.

An acute mouse gavage study demonstrated that the CP4 EPSPS is not toxic. No

treatment-related adverse effects were observed in animals administered CP4 EPSPS protein by oral gavage at doses up to 475 mg/kg body weight.

Based on the predicted exposure levels and the results of the above tests, no significant risk to livestock and workers/by-standers is expected from exposure to the CP4 EPSPS protein.

## VI. New Information Requirements

If at any time, Monsanto Canada Inc. or KWS SAAT AG becomes aware of any information regarding risk to the environment, including risk to human or animal health, that could result from release, in Canada or elsewhere, of sugar beet event H7-1, its descendants, or products derived there from Monsanto Canada Inc. or KWS SAAT AG must 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 feed use and environmental release and will re-evaluate its decision with respect to the livestock feed use and environmental release authorizations of this sugar beet event.

## VII. Regulatory Decision

Based on the review of data, information and stewardship plan submitted by Monsanto Canada Inc. and KWS SAAT AG, and through comparisons of sugar beet hybrids derived from H7-1 with unmodified sugar beet counterparts, the PBO, CFIA, has concluded that the novel gene and its corresponding traits do not confer to these plants any characteristic that would result in intended or unintended environmental effects following unconfined release.

Based on the review of submitted data and information, the Feed Section of the Animal Health and Production Division has concluded that the novel trait does not in itself raise any concerns regarding the safety or nutritional composition of event H7-1. Sugar beet and its by-products are currently listed in Schedule IV of the *Feeds Regulations* and are therefore approved for use in livestock feeds in Canada. Event H7-1 has been assessed and found to be substantially equivalent to traditional sugar beet varieties. Event H7-1 and its by-products are considered to meet 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 sugar beet event H7-1 is therefore authorized as of September 13, 2005. Any other sugar beet events derived from it, may be imported and/or released provided no inter-specific crosses are performed, provided the intended uses are similar, and provided it is known based on characterization that these plants do not display any additional novel traits and are substantially equivalent to currently grown sugar beet, in terms of their potential environmental impact and livestock feed safety and efficacy.**

**H7-1 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 sugar beet event H7-1. The food safety decisions are available at the following Health Canada web site:
http://www.hc-sc.gc.ca/fn-an/gmf-agm/appro/index_e.html

This bulletin is published by the CFIA. For further information, please contact the Feed Section or PBO at:

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