Government of Canada
Canadian Food Inspection Agency
Plant Health and Production Division

Gouvernement du Canada
Agence canadienne d'inspection des aliments
Division de la production et de la protection des végétaux

Decision Document DD2002-37

Determination of Environmental Safety of SEMT15-02, SEMT15-15, and RBMT15-101 Colorado Potato Beetle and Potato Virus Y Resistant Potato Lines Developed by Monsanto Canada Inc.

This Decision Document has been prepared to explain the regulatory decision reached under the guidelines Dir94-08: *Assessment Criteria for Determining Environmental Safety of Plants with Novel Traits* and its companion document T-1-09-96: *The Biology of* Solanum tuberosum *L. (potato)*, and the guidelines Dir95-03: *Guidelines for the Assessment of Livestock Feed from Plants with Novel Traits*.

The Canadian Food Inspection Agency (CFIA), specifically the Plant Biosafety Office (PBO) of the Plant Health and Production Division, and the Feed Section of the Animal Health and Production Division, have evaluated information submitted by Monsanto Canada Inc. regarding SEMT15-02, SEMT15-15 and RBMT15-101 (also known as NewLeaf-Y™.) These plants were modified to express resistance to Colorado potato beetle and potato virus Y. The CFIA has determined that these plants with novel traits (PNTs) should pose no concerns with respect to environmental safety or the safety to livestock consuming feed derived from the PNTs, and are considered substantially equivalent to potato products currently approved for commercial production in Canada.

Unconfined release of SEMT15-02, SEMT15-15 and RBMT15-101 into the environment is therefore authorized as of April 20, 1999. Additionally, any *Solanum tuberosum* line derived from SEMT15-02, SEMT15-15 and RBMT15-101 may be released provided that: 1. no interspecific crosses are performed; 2. the intended use of the plant is the same; and 3. it is known following thorough characterization that these plants do not display any additional novel traits and are substantially equivalent to currently grown potato, in terms of their potential environmental impact and livestock feed safety.

(publié aussi en français) **February 2002**

This bulletin is published by the Plant Health and Production Division, Canadian Food Inspection Agency. For further information, please contact the Plant Biosafety Office, Plant Health and Production Division or the Feed Section, Animal Health and Production Division at the following address:

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

Designation of the PNTs:	SEMT15-02, SEMT15-15 and RBMT15-101
Applicant:	Monsanto Canada Inc.
Species:	Potato (*Solanum tuberosum* L.)
Novel Traits:	Resistance to: Colorado potato beetle, potato virus Y and kanamycin
Trait introduction method:	*Agrobacterium tumefaciens*-mediated transformation
Intended use of the PNT:	Production of potatoes for human consumption (table and processing) and livestock feed, including potato processing residue. These PNTs will not be grown outside the usual production area for potatoes in Canada.

II. Background Information

Monsanto Canada Inc.'s SEMT15-02, SEMT15-15 and RBMT15-101 potato lines were developed by transforming the parental lines *Shepody* (SE) and *Russet Burbank* (RB) with three genes: 1. the *cry3A* gene from *Bacillus thuringiensis* (Bt) which confers resistance to Colorado potato beetle (CPB); 2. the coat protein gene, *PVYcp*, from PVY which confers resistance to the virus; and 3. the *nptII* gene which confers resistance to kanamycin and was used as a selectable marker to facilitate the selection of transformed cells in the laboratory. These lines have been tested in Canada under confined conditions in British Columbia (1998-99), Alberta (1997-99), Saskatchewan (1998-99), Manitoba (1995-99), Ontario (1997-99), Quebec (1997-99), New Brunswick (1995-99) and Prince Edward island (1995-99).

Colorado potato beetle (*Leptinotarsa decemlineata* Say.) is the most damaging insect pest of potato in Canada and, to date, no traditionally bred cultivars have been produced that are resistant to CPB. Both larval and adult stages of CPB feed on potato foliage and, when left unchecked, can completely defoliate potato plants resulting in yield reductions of as much as 85%. Current control of CPB is primarily achieved through the use of insecticides that are variably effective depending on insect sensitivity and environmental factors. Crop rotation, vacuum suction, propane flaming, polyethylene-lined trenches and trap plots are alternative but less effective management strategies for CPB.

Potato virus Y (PVY) is the causal agent of rugose mosaic. Symptoms vary widely with virus strain and potato cultivar, ranging from the barely perceptible to severe foliage necrosis that ultimately causes plant death. Yield losses may be as high as 80% in susceptible cultivars. PVY is transmitted by over 30 species of aphids or mechanically via infected plant sap. PVY is currently managed by planting virus-free seed stock for commercial potato production, and with insecticides applied to control the aphid vectors. However, for varieties such as *Shepody*, that are asymptotic when infected with PVY, it is difficult to visually assess and remove infected seed potato plants in the field and consequently to produce virus-free seed stock.

Monsanto Canada Inc. has characterized the host and donor organisms, the plasmid vectors, the inserted genes and gene products including the number of insertion sites and copy numbers for SEMT15-02, SEMT15-15 and RBMT15-101. The novel proteins were identified, characterized, and compared to the original bacterial and viral proteins, including their potential toxicity to livestock and non-target organisms with particular attention given to beneficial arthropods. Agronomic performance was evaluated by measuring yield, stand, vigour, hollow heart, brown center and specific gravity. Stress adaptation was evaluated, including susceptibilities to various potato pests and pathogens.

The environmental consequences of the introduction of SEMT15-02, SEMT15-15 and RBMT15-101 were evaluated in accordance with the following assessment criteria for determining environmental safety of plants with novel traits, as described in the regulatory directive Dir94-08:

- Potential of SEMT15-02, SEMT15-15 and RBMT15-101 to become weeds of agriculture or to be invasive of natural habitats
- Potential for gene-flow from SEMT15-02, SEMT15-15 and RBMT15-101 to wild relatives
- Potential for SEMT15-02, SEMT15-15 and RBMT15-101 to become plant pests
- Potential impact of SEMT15-02, SEMT15-15 and RBMT15-101 or their gene products on non-target species, including humans
- Potential impact of SEMT15-02, SEMT15-15 and RBMT15-101 on biodiversity

The CFIA has consulted with the Pest Management Regulatory Agency of Health Canada on issues related to potential development of CPB populations resistant to the insecticidal protein produced by plants transformed with Bt genes. The potential development of CPB resistance to the insecticidal protein Cry3A requires that Monsanto Canada Inc. implement an insect resistance management plan to mitigate against the potential development of CPB resistance to the Cry3A insecticidal proteins

Health Canada has determined that food derived from these potatoes is substantially equivalent to that derived from currently commercialized potatoes (May, 1999;

http://www.hc-sc.gc.ca/food-aliment/english/subjects/novel_foods_and_ingredient /novel_foods_and_ingredient.html). SEMT15-02, SEMT15-15 and RBMT15-101 have also received approval for commercialization in the U.S. (for more information go to http://www.aphis.usda.gov/biotech/dec_docs/9733901p_det_ea.HTM).

The Feed Section of the Animal Health and Production Division, CFIA, has assessed the feed safety of SEMT15-02, SEMT15-15 and RBMT15-101 in accordance with the following criteria for determining safety and efficacy of livestock feed, as described in Dir95-03:

- Potential impact of SEMT15-02, SEMT15-15 and RBMT15-101 to livestock
- Potential impact of SEMT15-02, SEMT15-15 and RBMT15-101 on livestock nutrition.

Nutritional composition and proximate analyses of tubers were performed. Data to support the suitability of SEMT15-02, SEMT15-15 and RBMT15-101 potato lines as livestock feed were provided.

III. Description of the Novel Traits

1. Colorado Potato Beetle Resistance

The novel lines SEMT15-02, SEMT15-15 and RBMT15-101 produce a version of the insecticidal protein, Cry3A, derived from *Bacillus thuringiensis*, as well as the coat protein (CP) from the ordinary (O) strain of potato virus Y (PVY-O). Delta-endotoxins, such as the Cry3A protein expressed in SEMT15-02, SEMT15-15 and RBMT15-101 potatoes, act by selectively binding to specific receptors localized on the brush border midgut epithelium of susceptible insect species. Following binding, cation-specific pores are formed that disrupt midgut ion flow and thereby cause paralysis and death. Cry3A is insecticidal only to Coleopteran insects and its specificity of action is directly attributable to the presence of specific receptors in the target insect. There are no receptors for delta-endotoxins of *B. thuringiensis* on the surface of mammalian intestinal cells, therefore, livestock animals and humans are not susceptible to these proteins.

2. Potato Virus Y Resistance

PVY is the type member of the potyvirus group and is an aphid-transmissible RNA virus that commonly infects potato, causing serious disease and economic loss. The introduced viral sequences in SEMT15-02, SEMT15-15 and RBMT15-101 do not result in the formation of any infectious particles, nor does their expression result in any

disease pathology. The genetically modified potato cultivars exhibit the trait of resistance to infection and subsequent disease caused by PVY through a process that is related to viral cross-protection.

3. Kanamycin Resistance

The kanamycin-resistance gene, isolated from the bacterium *E. coli*, codes for an enzyme (NPTII) that phosphorylates kanamycin, thereby rendering it inactive and imparting resistance to the antibiotic. The *nptII* gene was used as a selectable marker to facilitate the selection of transformed cells in the laboratory.

4. Development Method

The transgenic *Shepody* (SEMT15-02 and SEMT15-15) and *Russet Burbank* (RBMT15-101) potato lines were created through two separate *Agrobacterium*-mediated transformation events in which the transfer DNA (T-DNA) contained the *cry3A* gene encoding the Cry3A protein from *B. thuringiensis* subsp. *tenebrionis* and the *PVYcp* gene encoding CP from PVY-O. In addition, the T-DNA contained the *npt II* gene, encoding the enzyme neomycin phosphotransferase II (NPTII). The expression of NPTII activity was used as a selectable trait for screening transformed plants for the presence of the *Cry3A* and PVY-O CP genes. Additional DNA outside of the T-DNA border sequences was incorporated into the genome of *Shepody* lines SEMT15-02 and SEMT15-15. These lines also contain the *aad* gene that encodes the enzyme 3''(9)-O-aminoglycoside adenylyltransferase, which confers bacterial resistance to spectinomycin and streptomycin. The *aad* gene was not expressed in plant tissue, but was present on the Ti plasmid as a selectable trait for screening bacterial colonies for the presence of plasmid vector.

5. Stability of the Traits

The constitutive expression of Cry3A protein was demonstrated for each of the transgenic *Shepody* and *Russet Burbank* lines. On average, the amounts of Cry3A protein produced in the leaves and tubers of SEMT15-02, SEMT15-15 and RBMT15-101 were comparable to the concentrations previously reported for NewLeaf *Atlantic* and *Russet Burbank* cultivars (see DD96-06, DD97-20.) The expression of PVY-O CP in either leaves or tubers from SEMT15-02, SEMT15-15 and RBMT15-101 was undetectable at a threshold of 2 ng/mg fresh weight tissue. In contrast, the accumulation of CP in plants naturally infected with PVY-O is readily detectable using enzyme linked immunosorbent assay (ELISA) or Western immunoblot analysis. It is not uncommon for commercial potato plantings to be significantly infected by PVY-O and thus the livestock (and human) consumption of viral CP from these

sources is likely to be much higher than the exposure due to consumption of potatoes derived from SEMT15-02, SEMT15-15 and RBMT15-101.

Data was provided that showed that the expression of the traits was stable over four generations removed from the original transformation event.

IV. Assessment Criteria for Environmental Safety

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

The CFIA evaluated data submitted by Monsanto Canada Inc. on the reproductive and survival biology of the potato lines SEMT15-02, SEMT15-15 and RBMT15-101, and determined that vegetative vigour, overwintering capacity, insect and disease susceptibility (other than to CPB and PVY), and tuber yield and quality, were within the normal range of expression currently displayed by commercial varieties. SEMT15-02, SEMT15-15 and RBMT15-101 have no specific added genes for cold tolerance or winter hibernation.

The biology of *Solanum tuberosum*, described in T-1-09-96, is such that unmodified plants of this species are not invasive of unmanaged habitats in Canada. According to the information provided by Monsanto Canada Inc., SEMT15-02, SEMT15-15 and RBMT15-101 were determined not to be different from their counterparts in this respect. No competitive advantage was conferred to SEMT15-02, SEMT15-15 and RBMT15-101; resistance to CPB and PVY will not render these potatoes weedy or invasive of natural habitats as the typical vegetative reproductive characteristics of these potatoes were not modified. Although limited distribution is possible through dispersal of tubers, volunteers will not persist in cultivated in the presence of normal agronomic practices or compete in uncultivated habitats.

Based on the above considerations, the CFIA has concluded that SEMT15-02, SEMT15-15 and RBMT15-101 potatoes have no altered weed or invasiveness potential compared to currently commercialized potato varieties.

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

There are no wild relatives in Canada that can naturally hybridize with *S. tuberosum* (see T-1-09-96) and the novel traits have not altered the inability of SEMT15-02, SEMT15-15 or RBMT15-101 to outcross to wild relatives. The CFIA therefore

concludes that gene flow from SEMT15-02, SEMT15-15 and RBMT15-101 to relatives of *S. tuberosum* is not possible in Canada.

3. Altered Plant Pest Potential

The intended effects of the novel traits in SEMT15-02, SEMT15-15 and RBMT15-101 are unrelated to plant pest potential. The agronomic characteristics of SEMT15-02, SEMT15-15 and RBMT15-101 were shown to be within the range of values displayed by conventional *Shepody* and *Russet Burbank*. Susceptibilities to diseases such as early blight, late blight, verticillium, and potato leaf roll virus were unchanged, leading to the conclusion that plant pest potential was not inadvertently altered.

Recombination: Recombination is defined as an exchange of nucleotide sequences between two nucleic acid molecules. Recombination between viral genomes results in heritable, permanent change. The persistence of a recombined viral genome will depend upon its fitness with respect to its ability within the original host cell, its ability to replicate in the presence of parental viruses, its ability to spread systemically within the host, or its successful transmission to other host plants. Based upon the following information provided by Monsanto Canada Inc., the CFIA concluded that since the viral transgene is derived from a virus that naturally infects the potato host, is produced in less concentration than during natural potato infections, and if a recombination was formed it would have to be competitive with other potato-infecting viruses, the likelihood of novel interactions and formation of recombinant virus is no more likely than its occurrence during natural mixed infections. In addition, recombination would not be more competitive with other potato viruses and could be managed through normal agronomic practices for new viruses.

Synergism: Synergism is noted when two viruses simultaneously naturally infect a plant and the symptoms are more severe than when either of the viruses infects the plant singly. Synergy was first described and is best studied with natural infections of potato (potex) virus X (PVX) and potato (poty) virus Y (PVY). Monsanto did not observe synergistic symptoms during field testing or in artificial inoculation of the plants with PVX.

Transencapsidation: When a single plant cell is simultaneously naturally infected by two different strains of a virus (or two viruses), it may be possible for the genome of one virus to become encapsidated by coat protein of the second virus. If the virus is encapsidated by only one of the coat proteins, it is termed genomic masking or transencapsidation. Any changes are not inherited if such transencapsidated virions move to another host, so any effects are transient and pose no plant pest risk. Confined field experiments noted that expression levels of PVY coat protein remain extremely

low even in the presence of an invading potyvirus (PVA) and analysis concluded that the transgenic PVY coat protein was not incorporated into the PVA virion at a detectable level. Since PVA and PVY are found naturally within the same cells of potato plants, much greater concentrations of PVY and PVA coat protein accumulate during natural infections, therefore the risk for transencapsidation due to agronomic use of PVY resistant potatoes is much less likely than natural co-infections with PVA and PVY and present no increase in plant pest risk.

Given the above, and that potato has no history as a plant pest in Canada, the CFIA has concluded that SEMT15-02, SEMT15-15 and RBMT15-101 do not display any altered pest potential.

4. Potential Impact on Non-Target Organisms

Colorado potato beetle, the target insect, was controlled in test plots of SEMT15-02, SEMT15-15 and RBMT15-101 throughout the growing season. Monsanto Canada Inc. demonstrated that Cry3A protein is not toxic to non-target organisms represented by larval and adult honeybee, ladybird beetle, green lacewing, parasitic wasp, *Collembola* sp., earthworm, mice and bobwhite quail.

The novel proteins were rapidly inactivated in simulated mammalian stomach fluids by enzymatic degradation and pH-mediated proteolysis. The proteins expressed in SEMT15-02, SEMT15-15 and RBMT15-101 potatoes were shown to be equivalent to the native proteins. PVY coat proteins are found naturally within the cells of potato plants, at much greater concentrations than in potato lines SEMT15-02, SEMT15-15 and RBMT15-15. As previously stated, there are no receptors for delta-endotoxins of *B. thuringiensis* on the surface of mammalian intestinal cells, therefore, livestock animals and humans are not susceptible to these proteins.

Screening the sequence of the expressed proteins against a database of known allergens revealed no significant homology to any known allergenic protein.

Based on the above, the CFIA has concluded that the unconfined release of SEMT15-02, SEMT15-15 and RBMT15-101 will have no significant impact on non-target organisms, including humans.

5. Potential Impact on Biodiversity

SEMT15-02, SEMT15-15 and RBMT15-101 have no novel phenotypic characteristics that would extend their use beyond the current geographic range of potato production in Canada. Since potato does not outcross to wild relatives in Canada, there will be no transfer of novel traits to unmanaged environments.

The use of SEMT15-02, SEMT15-15 and RBMT15-101 potato plants could reduce the need for the insecticidal treatment of CPB and aphids, resulting in a reduction of chemicals released into the environment, an increase of non-target insect populations, and an increase in potential for the biological control of harmful insect pests. The CFIA has therefore concluded that the potential impact of SEMT15-02, SEMT15-15 and RBMT15-101, would not be significant and may be positive.

V. Potential for Development of CPB Resistance to the PNTs

The potential for CPB to develop resistance to conventional chemical insecticides is well documented, and many of the chemical foliar sprays currently registered for CPB control are no longer effective. Bt foliar insecticides containing the Cry3A protein are registered in Canada for use on both potatoes and tomatoes. To date, resistance of CPB to Bt sprays has not been observed under field conditions. Resistance could develop as a result of increased use of Bt foliar sprays or as a consequence of the widespread adoption of Bt potatoes such as SEMT15-02, SEMT15-15 and RBMT15-101.

SEMT15-02, SEMT15-15 and RBMT15-101 potatoes produce high levels of Cry3A protein in all plant tissues throughout the growing season, resulting in extremely high mortality in CPB feeding on these plants. Target insects are consequently exposed to significantly higher levels of Bt than with the current foliar spray treatments, leading to high selection pressure for resistant CPB. In order to mitigate the eventual development of Cry3A resistant CPB, resistance management plans have been developed and implemented. A critical component of accepted resistance management strategies is the mandatory planting of refugia – proximal fields of non-Bt potatoes where CPB populations susceptible to Cry3A are maintained. Any resistant CPB that survive in Bt potato fields will be very rare and it is presumed will mate with susceptible CPB to produce heterozygotes susceptible to the Cry3A. The efficacy of the high dose-refugia strategy for managing the advent of Cry3A resistant CPB has not been conclusively determined. All Bt potato lines should therefore be responsibly managed and CPB populations monitored for development of resistant individuals in a regular and consistent manner.

The CFIA believes that sound management practices can delay the development of resistant CPB populations. The resistance management plan for Bt potatoes to be implemented by Monsanto Canada Inc. must include, but is not limited to, the criteria outlined below:

1. Monsanto Canada Inc. must ensure that each farm planted to potatoes has no more than 80% of its farm acreage as NewLeaf™ potatoes (NewLeaf-Y ™ potatoes and all other NewLeaf™ potatoes transformed with Bt genes) and leaves at least 20% of potato acres as a refuge for CPB.

2. Monsanto Canada Inc. must ensure that refuges for CPB are managed in a manner which supports a viable population of CPB sufficient to maintain the functionality of the refuge as a reservoir of Bt-susceptible beetles. The use of insecticides which effectively eliminate CPB from a refuge is not compatible with the management of a refuge.

3. Education tools must be developed and provided to all growers and company field personnel. These will include information on product performance, resistance management, monitoring procedures and timetables, detection protocols for resistant CPB individuals, instructions to contact Monsanto Canada Inc. when support/information is required, and strategies to be followed if unexpected levels of CPB damage occur.

4. Monsanto Canada Inc. will have procedures in place for responding to any reported instances of unexpected CPB damage. These procedures will include, where warranted, the collection of plant tissue and CPBs, and use of appropriate bioassays to evaluate suspected Cry 3A resistant individuals, and a protocol for immediate action to control resistant individuals.

5. Detection of confirmed resistant CPB populations will immediately be reported to the Plant Biosafety Office, CFIA, and a procedure for control of resistant individuals must be ready for immediate implementation.

6. Integrated Pest Management practices must be promoted, such as crop rotation and alternative control measures for CPB.

7. Continued research in the area of resistance management of CPB using sound science will be conducted and the results will be made available to the CFIA.

The CFIA reserves the right to ask for modifications to any resistance management plan when warranted by the presentation of new information and in accordance with the *Seeds Regulations, Part V, Section 112*.

VI. Nutritional Assessment Criteria for Use as Livestock Feed

1. Nutritional Composition and Proximate Analyses of the PNTs

Nutritional composition and proximate analyses of SEMT15-02, SEMT15-15, RBMT15-101 and the control varieties *Shepody* and *Russet Burbank* were completed. Total solids, soluble protein, dextrose, sucrose, glycoalkaloids, vitamin B6, niacin, vitamin C, potassium, copper, magnesium, and amino acids were analyzed to characterize the nutritional composition of the three transgenic lines. The proximate analyses included total protein, moisture, total fat, ash, crude fibre, carbohydrates, and calories. All components analyzed were statistically identical to the controls or within the levels reported in the literature for commercially available potato cultivars.

2. Anti-Nutritional Factors

Solanine and chaconine are the principal glycoalkaloids commonly found in potato tubers. Analyses of total glycoalkaloid levels in each of the transgenic lines SEMT15-02, SEMT15-15 and RBMT15-101 demonstrated that in each case the level was below the administrative guideline of 20 mg/100g fresh weight that has previously been established for total glycoalkaloids in potato.

VII. Regulatory Decision

Based on the review of data and information submitted by Monsanto Canada Inc., and through comparisons of SEMT15-02, SEMT15-15 and RBMT15-101 with unmodified *Solanum tuberosum* counterparts, the Plant Biosafety Office of the Plant Health and Production Division, CFIA, has concluded that the novel genes and their corresponding traits will not confer any intended or unintended ecological advantage to SEMT15-02, SEMT15-15 and RBMT15-101 following unconfined release.

Based on the review of data and information submitted by Monsanto Canada Inc., the Feed Section of the Animal Health and Production Division, CFIA, has concluded that the novel trait does not in itself raise any concerns regarding the safety or nutritional composition of this line. *Solanum tuberosum* and several of its byproducts are currently listed in Schedule IV of the Feeds Regulations and are, therefore, approved for use in livestock feeds in Canada. As tubers and plants of SEMT15-02, SEMT15-15 and RBMT15-101 have been assessed and found to be nutritionally equivalent to traditional potato varieties, SEMT15-02, SEMT15-15 and RBMT15-101 and their byproducts are considered to meet the present ingredient definitions and are approved for use as livestock feed ingredients in Canada.

Unconfined release of SEMT15-02, SEMT15-15 and RBMT15-101 into the environment is therefore authorized as of April 20, 1999. Additionally, any *Solanum tuberosum* line derived from SEMT15-02, SEMT15-15 and RBMT15-101 may be released provided that: 1. no interspecific crosses are performed; 2. the intended use of the plant is the same; and 3. it is known following thorough characterization that these plants do not display any additional novel traits and are substantially equivalent to currently grown potato, in terms of their potential environmental impact and livestock feed safety.