

**Risk Assessment Summary Conducted Pursuant to the
New Substances Notification Regulations (Organisms) of the
Canadian Environmental Protection Act, 1999
NSN 17140: *Trichoderma* strain P1640A**

Regulatory Decision

Under Part 6 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999) and its *New Substances Notification Regulations (Organisms)* [NSNR (O)], the Minister of the Environment and the Minister of Health have assessed information in respect of the notified organism, and determined that the organism is not suspected of being harmful to the Canadian environment or human health as defined in section 64 of the CEPA 1999¹, when manufactured in a contained facility. Therefore, the manufacture of *Trichoderma* strain P1640A for this purpose may proceed after March 3, 2013.

NSNR (O) Schedule: 2 (micro-organisms manufactured in or imported to a contained facility that are not for introduction outside the contained facility or are for export only).

Organism Identity: *Trichoderma* strain P1640A

Notifier: Novozymes Canada, 300 Hunt Club Rd., Ottawa, ON K1V 1C1

Date of decision: March 3, 2013

Proposed use(s): Production of cellulase/hemicellulase enzyme mixture consisting of the HTX47A xylanase.

IDENTITY / STRAIN HISTORY / GENETIC MODIFICATION:

Trichoderma strain P1640A is a genetically modified derivative of a *Trichoderma* strain, whose parental strain M2C38 (ATCC 74252) is listed on the DSL. All these strains are descendants of a wild type *Trichoderma* strain that was originally isolated from the Solomon Islands. It was identified based on colony and spore morphology and genotypic analyses. The notifier has demonstrated that P1640A has growth, sporulation and hyphal properties similar to that of all of its ancestral strains. *Trichoderma* strain P1640A has green, thin colonies that are approximately 30 mm in diameter, with conidiophores in small fascicles or tufts. It can be distinguished from its predecessors through its unique ability to grow on selective minimal media and its ability to produce a modified HTX47A xylanase. Its identification was confirmed by sequence analysis of the ITS1, ITS2 and tef1 regions using the molecular barcode diagnostic method available online as TrichoKey (Druzhinina et al., 2005). An in-house sequence comparisons of the ITS1, ITS2 and tef1a sequences found no evidence that would indicate that the notified strain was related to any

¹ In accordance with section 64 of CEPA 1999, a substance is toxic if it is entering or may enter the environment in a quantity or concentration or under conditions that (a) have or may have an immediate or long-term effect on the environment or its biological diversity; (b) constitute or may constitute a danger to the environment on which life depends; or (c) constitute or may constitute a danger in Canada to human life or health.

of the strains belonging to the *T. longibrachiatum* clade/aggregate and other related species such as *T. harzianum*, *T. koningii*, and *T. viride*, which have been identified as etiologic agents of infections in immunocompromised patients and susceptible individuals, due to their ability to grow at 37°C and neutral pH (Kredics et al., 2003; Druzhinina et al., 2012).

Strain P1640A was developed through a series of genetic modification and selection processes to produce high levels of HTX47A xylanase. The strain history of *Trichoderma* strain P1640A provides a clear chain of events from the parental strain to the notified strain P1640A. With the given direct lineage, history of use, and consistency in the maintenance of morphological, growth and reproductive characteristics, and confirmation of the identity through morphological characteristics and genotypic analyses, the taxonomic identification was deemed adequate for risk assessment.

HAZARD CONSIDERATIONS:

Environmental Hazard

Trichoderma species are common soil saprophytes particularly prevalent in the litter of humid, mixed hardwood forests (Eveleigh, 1985). They are metabolically versatile, aerobic, mesophilic, imperfect fungi common in all climatic zones (Nevalainen, 1994). With the wild type strain used in the production of hemicellulase in Japan since the 1960s (Toyama, 1969), the strong cellulolytic abilities of *Trichoderma* are well recognized.

No environmental hazard potential has been reported with the parental strain and strain M2C38 (ATCC 74252). Whereas under extreme environmental conditions (e.g. inoculation of large doses of viable spores) some species of *Trichoderma* have been shown to act as opportunistic pathogens of animals (Hjortkjaer et al., 1986), previous risk assessments of multiple *Trichoderma* strains produced by the notifier, with similar phenotypic characteristics to P1640A, have all concluded that there is little evidence to suggest that any of the strains were pathogenic, toxigenic, mutagenic or posed any hazards to healthy animals or plants. Although there is no established history of safe use particularly for strain P1640A as a production organism, most industrial production strains of *Trichoderma* have demonstrated a long history of safe use in many industries (Hjortkjaer et al., 1986). The modifications that resulted in the notified strain, including transformation using various well characterized vectors and a selectable marker gene from a non-pathogenic source, are not expected to change the environmental hazard potential of strain P1640A compared to the parental strain.

The potential for the notified organism or its genetic material to cause adverse effects on the environment, its conservation or its biological diversity is, therefore, considered low.

Human Health Hazard

The notified strain (*Trichoderma* strain P1640A) is a genetically modified fungus that traces its ancestry back to the wild type Solomon Islands parental strain. The complete DNA and amino acid sequences of the genes that were inserted and stably integrated into the strain P1640A

genome were provided by the notifier and literature searches on all the genetic elements did not reveal any potential to cause adverse health effects in humans.

An in-house literature review identified no reports of this species of *Trichoderma* having adverse effects towards humans. Nonetheless, this *Trichoderma* species is known to produce cellulases and hemicellulases which may act as potential sensitizers. This has been documented in reported cases of dermatitis, rhinitis and asthma resulting from exposure to commercial enzyme preparations (Halprin et al., 1973; Hytonen et al., 1994; Ransom and Schuster, 1981; Tarvainen et al., 1991) and delayed respiratory distress amongst maintenance workers in a pulp mill wood chip plant (Cohn et al., 1984). Sensitization to an enzyme xylanase, also produced by *Trichoderma* species, has been reported among workers in baking and animal feed industries (Vanharen et al., 1996; Vanhanen et al., 2001; Elms et al., 2003).

Apart from the reported cases of hypersensitivity related to the repeated and prolonged occupational exposures to the enzymes produced by *Trichoderma*, the organism is not considered a human pathogen. Due to this fact and that the organism will only be grown in contained facility, a waiver for antifungal susceptibility data was granted. However, most *Trichoderma* species, including the notified species, are susceptible to amphotericin B, fluconazole, itraconazole, ketoconazole, and miconazole (Kredics et al., 2003). Antifungal agents are available in the unlikely event of an infection from an inadvertent exposure. Although only a remote possibility, the presence of a selective marker gene in the genome of the notified strain could be a human health concern if the organism/gene is released into the environment in large quantities.

The use of *Trichoderma* strain P1640A is not expected to cause adverse effects to the general population. Its potential hazard to human health is considered low.

EXPOSURE CONSIDERATIONS

Trichoderma strain P1640A will be produced only in a contained facility where the manufacturing process meets the standards for the Good Large Scale Practice (GLSP) level as defined in Appendix K of the US NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines).

The sole intended use of strain P1640A is in the production of a cellulase/hemicellulase mixture containing modified xylanase. It will not be sold or otherwise distributed in Canada or internationally. All liquid, gaseous and solid wastes containing *Trichoderma* strain P1640A are adequately treated to effectively kill live cells and contingency measures are in place in case of accidental spills. The containment and inactivation procedures are in place to prevent or minimize release from the facility.

The potential environmental and human exposure to *Trichoderma* strain P1640A is therefore considered to be low.

RISK ASSESSMENT CONCLUSION / REGULATORY OUTCOME

Based on the hazard and exposure considerations described above, the risk assessment conducted by Environment Canada and Health Canada concluded that *Trichoderma* strain P1640A is not expected to cause harm to the Canadian environment or human health as described in section 64 of the CEPA 1999.

The substance is not eligible for addition to the Domestic Substances List on the basis of this risk assessment under Schedule 2 of the NSNR (Organisms).

REFERENCES

Please note that the following is only a partial reference list due to confidentiality reasons.

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