**Risk Assessment Summary Conducted Pursuant to the New Substances Notification Regulations of the *Canadian Environmental Protection Act, 1999***
***Trichoderma reesei* P210A**
**NSN # 12961**

This document has been prepared to explain the regulatory decision taken under Part 6 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999) regarding the manufacture or import of *Trichoderma reesei* P210A by Iogen Corporation in a contained facility located in Ottawa.

*Trichoderma reesei* P210A was notified pursuant to subsection 29.11(4) of the CEPA 1999 New Substances Notification Regulations (NSNR).

The New Substances Branch of Environment Canada and the New Substances Assessment and Control Bureau of Health Canada have assessed the information submitted by Iogen Corporation and other available scientific information in order to determine whether *T. reesei* P210A is *toxic*[1] or capable of becoming *toxic* as defined in section 64 of CEPA 1999.

## Regulatory Decision:

Based on the hazard and exposure considerations, the joint risk assessment conducted by Environment Canada and Health Canada concluded that *Trichoderma reesei* P210A is not considered to be *toxic* to the Canadian environment or human health as described in section 64 of the CEPA 1999.

Therefore, the manufacture in or import to a contained facility of *T. reesei* P210A for use in the contained facility or for export only, may proceed after February 13, 2004.

This evaluation does not include an assessment of human health risk in the occupational environment nor does it include an assessment of the potential exposure and risk to humans associated with the use of the organism in or as an item that falls under the purview of the *Food and Drugs Act*.

[1] In accordance with section 64 of the 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.

**NSN Schedule:** XVI (manufacture or import to a contained facility a micro-organism that is not for introduction outside a contained facility or is for export only)[2]

**Organism Identity:** *Trichoderma reesei* P210A

**Notifier:** Iogen Corporation, 310 Hunt Club Rd. East Ottawa, Ontario K1V 1C1 Canada

**Date of decision:** February 12, 2004

**Proposed use:** Commercial production, in a contained facility, of a novel thermophilic and alkalophilic xylanase II *(xln2)* enzyme by genetically engineered *Trichoderma reesei* P210A.

## Strain History/Genetic Modification:

*Trichodema reesei* P210A was derived from an auxotrophic mutant of the parental strain M2C38 (ATCC 74252) by the introduction of a fragment of the transformation vector pc/xITX1-TV. The selection cassette used in the construction of the transformation vector contains a *Neurospora crassa* gene functioning as a selectable marker. The expression cassette consisted of the modified version *T. reesei* xylanase II structural gene (*xln2)* under the control of *T. reesei* regulatory sequences. Strain M2C38 is a derivative of the *T. reesei* strain RUTC30. RUTC30 (ATCC 56765) was obtained from the American Type Culture Collection (ATCC), and is a mutagenic derivative of the founder strain QM6a (ATCC 13631) which was isolated in the Solomon Islands from cotton canvas during World War II (Kuhls *et al.,* 1996).

## Hazard considerations:

In addition to the information provided by the notifier, a review of in-house reference material and a comprehensive search of the scientific literature were conducted to gather information on potential harmful environmental and human health effects attributable to *T. reesei*.

*Trichoderma* species are common soil saprophytes and are metabolically versatile, aerobic, mesophilic, imperfect fungi (Nevalainen *et al*., 1994). The *Trichoderma* species are differentiated primarily by patterns of conidiophore branching and conidia morphology. They are widespread in nature, quick-growing, easy to culture and they can produce large amounts of conidia with long lifetime (Manczinger *et al*., 2002).

In general, large scale industrial manufacture of *T. reesei* enzyme preparations have a history of safe use in many industries including starch and animal feed processing, grain alcohol fermentation, malting and brewing, extraction of fruit and vegetable juices, in pulp and paper, and in textiles (Hjortkjaer *et al*., 1986). Based on the criteria outlined in the Organisation for Economic Co-operation and Development guidelines entitled *Recombinant DNA Safety Considerations* (OECD, 1986) and the European Communities Council (ECC) Directive 90/219/EEC on the contained use of genetically

[2] Provisions relating to organisms previously contained in Part II.1 of the NSNR are now contained within the NSNR (Organisms). These came into force on October 31, 2005, and included changes to Schedule numbering. Under the NSNR (Organisms), Schedule XVI is now referred to as Schedule 2.

modified micro-organisms (ECC, 1990), *Trichoderma* species can be regarded as safe host organisms.

*Trichoderma reesei* has been shown to be non-pathogenic and non-toxic to healthy laboratory animals (Hjortkjaer *et al*., 1986). *Trichoderma reesei* is not reported to be a frank pathogen of plants or animals including humans. However, this species can act as an opportunistic pathogen to immunodepressed animals under extreme experimental conditions (Hjortkjaer *et al*., 1986). Some *Trichoderma* species have been cited as rare and newly emerging fungal pathogens (Fleming *et al*., 2002).

While certain species of the genus *Trichoderma* can be used as biocontrol agents in agriculture for their ability to produce antifungal compounds against several plant pathogenic fungi, *T. reesei* P210A is not one of them. Some species of *Trichoderma* may also produce toxins under certain conditions; however, experience with *T. reesei* indicates that it is not likely to be toxigenic (Hjortkjaer *et al*., 1986). Tests conducted on commercial enzyme preparations confirm that neither antibiotics nor inhibitory substances are produced during the growth of industrial *T. reesei* strains (Hjortkjaer *et al*., 1986). A carbohydrase enzyme product manufactured by the notifier using the parental strain M2C38 was tested for aflatoxin with negative results. The native xylanase as well as the novel thermophilic/alkalophilic xylanase II belong to a large family of structurally and biochemically related xylanases. Xylanases have been reported as allergens in industrial settings; however, studies on toxicity and mutagenic effects of native xylanases to humans did not reveal any positive results (Pico *et al*., 1999; Perderson and Broadmeadow, 2000; Dersjant-Li *et al*, 2001; Harbak and Thygesen, 2002).

Toxicity studies on native xylanases from *Aspergillu*s and *Thermomyces* administered orally to rats and mice did not result in adverse effects (Pederson and Broadmeadow, 2000). Native xylanases were not found to be mutagenic in the *Salmonella typhimurium* reverse mutation assay, nor did they cause chromosomal aberrations in cultured human lymphocytes (Pederson and Broadmeadow, 2000).

*Neurospora crassa*, the fungal source of the selectable marker gene used in the construction of the transformation vector, is not reported to be a frank pathogen. The *N. crassa* gene product makes selection of *T. reesei* strain P210A, from a mixture of other microorganisms, easier and is unlikely to pose a risk to the environment since it has many functional equivalents in most living organisms.

Both *T. reesei* and *N. crassa* are listed as ‘Biosafety Level 1’ organisms by the American Type Culture Collection (ATCC). In addition, *T. reesei* has been designated as a ‘Risk Group 1’ organism by the Office of Laboratory Security of the Public Health Agency of Canada.

The DNA fragments used in the construction of the transformation vector are well characterized and do not contain any large undefined fragments. It is unknown whether the ampicillin resistance gene present on the transformation vector was integrated onto the host genome. Nevertheless, the ampicillin resistance gene used in the construction of the transformation vector is under the control of a bacterial promoter that will not function in *T. reesei*. In addition, the vector DNA has been shown to be stably integrated into the chromosome without loss or rearrangement of the sequence even after several generations on non-selective media. Therefore, the potential for lateral gene transfer

from this organism to humans, animals or other microbes in the environment is extremely low.

Genetic modifications performed to develop *T. reesei* P210A do not give rise to concerns of altered virulence or pathogenicity to humans, animals, plants or altered hazards to the environment. The phenotype resulting from the modification is well characterized and is not likely to influence the normal behavior of *T. reesei*.

## Exposure considerations:

*Trichoderma* species, including *T. reesei* are common soil saprophytic fungal species found in all climate zones and are particularly prevalent in the litter of humid, mixed hardwood forests (Nevalainen *et al*., 1994).

*Trichoderma reesei* P210A is manufactured solely as an intermediate in the production of a novel thermophilic/alkalophilic xylanase II enzyme in a contained facility. The notifier indicated that the manufacturing process meets the standards for the *Good Large Scale Practice* (GLSP) level as defined in Appendix K of the *NIH Guidelines for Research Involving Recombinant DNA Molecules* (NIH, 2002*)*. The notified strain is not intended for release outside the contained facility. Consequently, the potential exposure to the general population and the environment is expected to be low.

The notifier describes procedures which will limit potential worker exposure. These include the use of protective equipment, such as the National Institute for Occupational Safety and Health (NIOSH) approved respiratory masks with particulate filters, face shield, or safety goggles with side shields, rubber gloves, lab coats or overalls for workers who are chronically exposed to enzyme dusts or aerosols during such procedures as transfers of fermentation broths.

Precautions are in place and used by the notifier to ensure that exhaust and aerosols from the fermentor are decontaminated by UV irradiation to kill any organisms and volatiles and odours are removed by a cyclone and scrubber system. The fermentor is equipped with an alarm to indicate high pressure, foam-over and low-level and is diked in case of massive leakage. *Trichoderma reesei* P210A is not intrinsically hazardous, thus, inadvertent release from the manufacturing facility is not expected to pose significant risk to the environment and human health.

When enzyme production is complete, the spent cell mass is chemically inactivated using a quaternary ammonium compound with 99.999% effectiveness prior to disposal in a registered landfill or composting sites in accordance with provincial regulations. Given that *T. reesei* P210A lacks pathogenicity and toxicity potential, the likelihood of significant harm to the environment or human health resulting from the disposal route of exposure is expected to be minimal.

## References:


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