Risk Assessment Summary Conducted Pursuant to the *New Substances Notification Regulations (Organisms)* (NSNR[O]) of the *Canadian Environmental Protection Act, 1999* EAU-288:*Saccharomyces cerevisiae* strain ECMo01

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 of *Saccharomyces cerevisiae* strain ECMo01 by First Venture Technologies Corp. for introduction anywhere in Canada. *Saccharomyces cerevisiae* strain ECMo01 was notified pursuant to subsection 3(1) of the CEPA 1999 New Substances Notification Regulations (Organisms).

The New Substances Assessment and Control Bureau of Health Canada has assessed the information submitted by First Venture Technologies Corp. and other available scientific information in order to determine whether *S. cerevisiae* strain ECMo01 is *toxic*[1] or capable of becoming *toxic* as defined by section 64 of CEPA 1999.

Regulatory Decision:

Based on the hazard and exposure considerations, the risk assessment conducted by Health Canada concluded that *S. cerevisiae* strain ECMo01 is not considered to be *toxic* to the Canadian environment or human health as described in section 64 of the CEPA 1999. Therefore, manufacture of *S. cerevisiae* strain ECMo01 for introduction anywhere in Canada may proceed after August 23, 2006.

The 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*.

NSNR(O) Schedule: 1 (manufacture of micro-organisms for introduction, anywhere in Canada).
Organism Identity: *Saccharomyces cerevisiae* strain ECMo01
Notifier: First Venture Technologies Corp.[2], Box 21147,
Charlottetown, PEI, C1A 9H6
Date of decision: August 23, 2006
Proposed use: Active dry yeast to reduce ethyl carbamate during commercial production of alcoholic beverages

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1 In accordance with section 64 of the *Canadian Environmental Protection Act, 1999*(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.

2 First Venture Technologies Corp. is currently known as Functional Technologies Corporation.

Strain History/Genetic Modification:

Saccharomyces cerevisiae strain ECMo01 was derived from the naturally occurring *S. cerevisiae strain* Davis 522 (ATCC 36026) that is commonly used in the wine industry.

The identification of strain ECMo01 was based on morphological characteristics, API20C AUX carbohydrate utilisation tests, and transcriptome analysis using Affymetrix GeneChip® Yeast Genome S98 Array.

Urea is a precursor of ethyl carbamate, a suspected carcinogen in humans, which is formed in wine production from the reaction of urea and ethanol. The purpose of creating *S. cerevisiae* strain ECMo01 is to increase the expression of *S. cerevisiae* urea amidolyase, an enzyme that hydrolyzes urea, in order to reduce the formation and accumulation of ethyl carbamate in wine. The introduced recombinant genetic insert is composed of the *DUR1,2* gene (encoding urea amidolyase) derived from *S. cerevisiae*TCY1 and the *PGK1* promoter and terminator sequences (ensuring proper expression of*DUR1,2* gene) derived from *S. cerevisiae* AB972.

The host strain, *S. cerevisiae* Davis 522, was transformed by electroporation with a mixture of the integration cassette and the plasmid carrying the Tn5*Ble* marker that confers resistance to phleomycin in yeast. The transformants were isolated based on their ability to grow on selective media containing phleomycin. The cells transformed with the plasmid are also more likely to have integrated the *DUR1,2* cassette. The plasmid containing the antibiotic resistance genes was removed from the strain ECMo01 during growth on non-selective media.

Genetic stability was demonstrated for more than 100 generations in the absence of selective pressure. The potential for expression of unpredicted novel traits or introduction of uncharacterized genetic materials is significantly low since examination of putative open reading frames present on the integration cassette indicates that the putative proteins would be similar to these expressed by the parental strain. Additionally, the parental strain has not previously produced unwanted products.

Hazard Considerations:

Environmental Hazard

S. cerevisiae is a saprophytic yeast that is widely distributed in nature. It has been isolated from sediments, soil, water, animals, and plants under varying ecological conditions. The nutritional requirements, along with the ability to produce ascospores under starvation conditions, enhance its ability to survive in nature. It has never been reported to negatively affect biogeochemical cycling.

Despite its ubiquitous nature and wide use in the food and wine industries, reports of *S. cerevisiae* pathogenicity to insects, birds, fish, animals, and plants in the available scientific literature are exceedingly rare. Only one case has been reported associating *S.*

cerevisiae with chronic diarrhea in a dog [1]. The Canadian Food Inspection Agency, under the *Plant Protection Act*, recognizes that non-recombinant *Saccharomyces* spp. are not plant pests and do not require a plant protection permit for import into Canada [2].

Since the inserted genetic elements in this case do not appear to possess any intrinsic hazard potential, the overall potential environmental impacts from the release of *S. cerevisiae* strain ECMo01 are not expected to be any different from other well-known *S. cerevisiae* strains commonly found in nature.

Human Health Hazard

S. cerevisiae is predominantly found in association with human activities, particularly the production of bread and alcoholic beverages. *S. cerevisiae* has also been used as a probiotic for the prevention or treatment of various diarrheal disorders.

S. cerevisiae has been isolated from human intestinal flora and is regarded as an opportunistic pathogen with low virulence. The non-recombinant *S. cerevisiae* strain is recognized as a Risk Group 1 agent, by the Public Health Agency. In spite of its ubiquity in nature, reported *S. cerevisiae* clinical infections in healthy populations appear to be rare. A review of scientific literature shows that *S. cerevisiae* has led to different type of infections such as empyema [3], liver abscess [4], peritonitis [5], urinary tract infection [6], and endocarditis [7]. The most reported infection caused by *S. cerevisiae* is fungemia [8-12]. The majority of these infections are found in individuals with compromised immunity or an underlying disease or condition.

While *S. cerevisiae* may be found as a component of the human gastrointestinal tract, there are documented exogenous infections such as vaginitis [13-16]. A few cases of allergic reactions resulting from either oral, dermal, or inhalation exposure to *S. cerevisiae* have been reported in the literature [17-18]. However, according to a study conducted by Kortekangas-Savolainen *et al*. [19], there are not enough yeast allergens in wine or baked products to be the source of allergic reaction.

The principal virulence factor of yeasts is the secretion of phospholipases; however, compared to a wide range of fungi assayed, *S. cerevisiae* was found to have the lowest level of phospholipase activity [20].

The use of combination antifungal therapy is recommended for the treatment of *S. cerevisiae*-induced diseases, as is prolonged therapy [21]. In the unlikely event of *S. cerevisiae* strain ECMo01 infection to humans, antifungal treatments are currently available. Amphotericin B is considered the treatment of choice for serious *S. cerevisiae*infections except where underlying conditions preclude its use [1], in which case prolonged treatment with azole antifungal agents (e.g., clotrimazole, fluconazole, itraconazole, voriconazole) has also been found effective [16, 22]. Since the antibiotic resistance genes were removed from the genome of strain ECMo01 during the modification of the strain it is highly unlikely that they will be disseminated to the environment.

Aside from its enhanced urea hydrolysis ability, it is not expected that strain ECMo01 will behave differently from its non-recombinant parental strain which is traditionally used in commercial winemaking. The likelihood of significant harm to human health is therefore expected to be low. The ECMo01 yeast received the Generally Regarded as Safe (GRAS) affirmation from the US Food and Drug Administration in 2005 [23].

Exposure Considerations:

The notified micro-organism will be manufactured at the University of British Columbia's Wine Research Centre[3]. An estimated amount of 17 kg containing 3.4 x 10^{14} viable *S. cerevisiae* ECMo01 cells will be manufactured for the first 12 months and will be exported to the United States for wine production trials and to international yeast manufacturers. Future plans include annual large-scale production of up to 30 metric tons in a Canadian commercial facility and distribution to approximately 200 wineries in Ontario and British Columbia.

It is expected that the amount of strain ECMo01 in bottled wine will range from 0 to 10 cells/ml depending on the manufacturing process employed. Commonly, between 0.1 to 0.2 grams of active dry yeast is used to manufacture a litre of wine. However, clarification followed by filtration can reduce the amount of yeast cells to less than 0.5 cfu/ml of wine while ensuring the absence of any additional urea amidolyase. Membrane filtration would allow for complete removal of yeast cells.

In the unlikely event of ECMo01 release from the Wine Research Centre, the terrestrial and aquatic plants and animals that could be exposed are those in the immediate area surrounding the facility. The University is located at the fringe of the Pacific Spirit Regional Park on Point Grey. Most of this forested park contains a mixture of trees such as Hemlock, Western Red Cedar, Red Alder, Douglas-Fir and Broadleaf Maple. The park supports a diverse ecosystem that includes black-tailed deer, cougars, elk, wolves and otters. The Pacific Water Shrew is found in the park and this area is one of only three sites in Canada where Western Redbacked Vole can be found. The chestnut backed chickadee, American black oystercatcher and tufted puffin[4] are found only in this region. Northern sea lions, harbour seals, beaked whales and sea otters are common in the ocean of this ecozone. *S. cerevisiae* is non-pathogenic to terrestrial and aquatic invertebrate and vertebrate animals and plants and it is ubiquitous in nature, thus its potential release from the manufacturing facility is not expected to pose any significant ecological hazards.

As with naturally occurring *S. cerevisiae* wine strains, human exposure may occur via inhalation. The level of human exposure is expected to be comparable to those *S. cerevisiae* strains normally encountered during the manufacture of foods and beverages.

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[3] At the date of decision, the plan was to manufacture the micro-organism at the University of British Columbia's Wine Research Centre. However after the original assessment was performed, manufacturing has since relocated to Charlottetown, P.E.I.

[4] The notifiers originally reported "tufted pigeon". This change was made after the original assessment, when corrected information was brought to the attention of the New Substances Program.

The notifier provided detailed procedures to disinfect all solid and liquid wastes in order to mitigate any potential environmental release of the strain.

Exposure to the ECMo01 or to the newly introduced proteins either through disposal of unused wine or by wine consumption is considered significantly low since the processing procedures used in the winemaking will remove intact yeast cells, debris associated with the autolyzed yeast cells and proteins released during autolysis of yeast cells.

Persistence and Dispersal

There is currently limited information on the ecological characteristics of ECMo01. Valero*et al*. [24] performed a 3-year field study to track the spreading and survival of industrial yeast strains in vineyards of North Portugal and South France. Results show that commercial strains behave similarly to naturally occurring yeast strains. Strain ECMo01 is expected to have less competitive advantage in the environment, than the naturally occurring yeasts in soil, since it is adapted to well-defined media.

The behaviour of genetically modified *S. cerevisiae* strains within microbial populations of a confined wine cellar and greenhouse vineyard has also been evaluated [25] and no significant difference was found between modified strains and commercial yeast strains. The introduction of strain ECMo01 is expected to have no significant effect on the ecological balance of vineyard associated flora in the environment.

Any notified strain released into the environment as a result of the large-scale manufacturing process can be dispersed by wind, by fauna existing at the wineries, or by run-off with surface water. Considering the detailed procedures to disinfect all solid and liquid wastes provided by the notifier, it is expected that the dissemination of strain ECMo01 in the wineries and surrounding areas will be restricted to short distances and limited periods of time.

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