Risk Assessment Summary Conducted Pursuant to the
***New Substances Notification Regulations (Organisms)* of the**
Canadian Environmental Protection Act, 1999
NSN 17329: *Pichia* species strain

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 1999[1], when manufactured/imported for introduction in a contained facility. Therefore, the manufacture/importation of *Pichia* species strain for this purpose may proceed after 25 October 2013.

However, a Significant New Activity (SNAc) Notice (SNAc No. 17329) was issued, pursuant to section 110 of CEPA 1999, based on uncertainties regarding possible environmental/human health impacts of the notified organism in activities outside the scope of this assessment. This SNAc Notice outlines information requirements for those activities. The SNAc Notice was published in the *Canada Gazette* Part I, Vol. 148, No. 3 on January 18, 2014 and can be found at the following URL: https://www.ec.gc.ca/subsnouvelles-newsubs/default.asp?lang=En&n=C14DD417-1. Any activity not identified in the Notice may proceed after 25 October 2013.

NSNR(O) Schedule: 2
Organism Identity: *Pichia* species strain
Notifier: Bioamber Sarnia Inc., Montreal, Quebec.
Date of decision: October 25, 2013
Proposed use: Industrial production of bio-based succinic acid in a contained facility.

IDENTIFICATION / STRAIN HISTORY/GENETIC MODIFICATION

The notified strain is genetically-modified yeast which has undergone multiple gene deletions and insertions in order to increase bio-based succinic acid production and decrease production of by-products. The inserted genes were stably integrated into the chromosomes of the microorganism. The genetic stability was verified for several generations and the enhanced production of bio-based succinic acid was verified in large scale fermentations in the United States.

The parental or host strain was isolated from a corn wet milling facility in the United States and BioAmber used this strain to develop the notified strain to optimize production of succinic acid. Identification of the notified strain was based on genetic analysis by comparing the 26S rDNA

[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.

sequence of the strains with a set of 22462 fungal 26S DNA sequences from Genbank. The strain showed 100% identity to several strains of this species. The morphology, conditions for growth and nutritional requirements of the notified strain were consistent with what is known for this species.

HAZARD CONSIDERATIONS

The following hazard considerations were based on information on the wild host *Pichia* species found in the scientific literature as well as information provided on the genetic modifications done to the notified organism.

Environmental Hazard

The sexual and asexual forms of this species are widely distributed in nature and have been isolated from many habitats such as fruits, trees, animals, soil, sewage and fresh water as well as fermented food products**.** The wild *Pichia* species is known to survive many months in urban icicles and has been isolated in Antarctic freshwater; thus, it could likely overwinter in Canada. In addition, the wild species has been found in treated domestic sewage, indicating that it could survive through some types of wastewater treatments.

The potential for airborne transmission of the notified strain is unknown. Certain birds harbour the wild species in their intestinal tracts, and are suggested to act as vectors for transmission of this organism.

Although rare, there are a few reports of opportunistic infections caused by the wild species in terrestrial mammals. The species has been implicated in causing yeast mastitis in cattle and may infect certain insects. The species is also suspected to be a weak plant pathogen. Specific testing data relating to the toxicity/pathogenicity to animals and plants for the notified strain is not required in this regulatory schedule, so a high level of uncertainty can be ascribed to the hazard determination to the environment.

The effects of the genetic modifications are not expected to enhance the hazard profile of this strain to the environment. Since the organism was notified for manufacture in a contained facility, there were no regulatory requirements for pathogenicity/toxicity testing. However, because of literature reports indicating that that the wild type species can cause opportunistic infections in some terrestrial mammals, the potential for the notified organism or its genetic material to cause adverse effects on the environment, its conservation or its biological diversity was, therefore, considered medium.

Human Health Hazard

This species can grow at elevated temperatures up to 45°C and in low pH conditions. The wild species also has a high propensity to adhere to non-biological surfaces and possesses the ability to form biofilms. These features could be considered key virulence characteristics enabling colonization of this species in humans that use non-biological apparatuses such as intravascular catheters. The asexual form of this species is known to have intrinsic resistance to triazole antifungal drugs. Although no information was found suggesting involvement of the notified

micro-organism in adverse immune reactions, the asexual form has been associated with invasive fungal infections in deep-seated tissue and other normally sterile sites including the blood stream, other body fluids, and internal organs. Epidemiological evidence also indicates increasing rates of morbidity and mortalities in high risk patients due to the increasing resistance of the species to azole drugs. However, it is important to note that the notified strain has shown susceptibility to most antifungals tested including the azole group i.e. fluconazole, ketoconazole, as well as to amphotericin B, and flucytosine. According to the information provided by the Public Health Agency of Canada (PHAC), the characteristics of the notified microorganism fit the description of a risk group 1 organism, however, laboratory containment level 2 is required if the notified organism will be handled in close proximity to immunocompromised patients (Government of Canada, 2013). The genetic elements inserted and the method used to achieve the genetic modification for the notified strain posed no additional human health concerns except in specific cases of immunocompromised individuals.

The notified organism has been used for two years in research and large-scale facilities. In the course of these fermentations, there has been no indication of phenotypic or genotypic instability.

The use of the notified strain is not expected to cause adverse effects to the general population. However, because of sufficient literature information describing clinical infections in immunocompromised individuals, its potential hazard to human health was considered to be medium.

EXPOSURE CONSIDERATIONS

The notified microorganism will be imported and manufactured in Canada, in order to use it for the production of bio-based succinic acid which in turn has applications in bioplastics, plasticizers, and solvents as well as food additives and personal care products. A batch of no more than 50 ml or 50g (dry cell weight) will be imported from the United States to a contained facility in Sarnia, Ontario where a bank of seed microorganism cultures will be established. The facility has a production capacity of 37,000 metric tonnes of bio-based succinic acid.

The notified strain does not have a long history of use except for the small-scale fermentations conducted in BioAmber's research facility in Minnesota, USA since 2012. During these fermentations, there was no indication of genetic drift or phenotypic /genotypic instability.

The notified strain will be manufactured in a contained facility that meets the containment level 1, as described in the *Laboratory Biosafety Guidelines* (Health Canada, 2004). The waste containing live production strain will be adequately inactivated. Also, a programme will be in place to monitor the efficacy of inactivation in the biomass and the disposal of the biomass will be to landfill where the strain is not expected to survive; the strain is considered to be adequately contained. The genetic modifications made to the BioAmber *Pichia* strain result in it requiring very specific growth and nutrient conditions which would thus likely decrease its capacity to survive in the natural environment should any releases occur. The environmental exposure potential of the notified strain was therefore assessed as low.

Potential scenarios of concern may arise if the notified strain can survive the inactivation process of the biomass waste. In cases where monitoring for treatment efficacy by the notifier lapses for any reason, or if waste is disposed of in any location where the notified strain is known to survive, release to the environment could be significant. It is expected that potential release quantities could be as low as zero and as high as 10^7 CFU/g in treated waste biomass, where the maximum amount is in excess of the threshold needed to cause infection in animals and potentially immunocompromised individuals.

Taking into account the proposed use and the procedures in place to limit any potential release, as well as effective treatments to inactivate the notified organism, no significant release is expected leading to exposure of by-standers or the general public. Therefore, general population exposure to the notified strain is expected 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 the notified strain is not expected to cause harm to the Canadian environment or human health as described in section 64 of the CEPA 1999.

Failure to adequately inactivate the fermentation off-gas, the process waste water, and the solid waste biomass may result in an environmental release of a large number of propagules at concentrations sufficient to potentially cause infection in animals, plants and/or humans, particularly in immunocompromised individuals or those with underlying medical conditions. Therefore, the Significant New Activity (SNAc) powers in section 110 of CEPA 1999 will be recommended to address this possibility by covering all potential deviations in the proposed containment measures described by the notifier.

The substance is not eligible for addition to the Domestic Substances List on the basis of this risk assessment.

REFERENCES

The following is a partial reference list due to confidentiality reasons.

Government of Canada. (2013). Canadian Biosafety Standards and Guidelines (First Edition) for Facilities Handling Human and Terrestrial Animal Pathogens, Prions, and Biological Toxins. Government of Canada *(2013).2013, 343-28.*

Health Canada. (2004). The Laboratory Biosafety Guidelines: 3rd Edition 2004 -Public Health Agency of Canada, Reproduced with the permission of the Minister of Public Works and Government Services Canada, *2008.2013.*