

New substances: risk assessment summary, new substances notifications 21838, 21840, 21841 and 21842

Official title: New substances: risk assessment summary, New Substances Notifications 21838, 21840, 21841 and 21842 – Schedule 2 of the *New Substances Notification Regulations (Organisms)*

Notified organisms:

- *Saccharomyces* species YS219-A3 (NSN-21838, hereinafter referred to as YS219-A3)
- *Saccharomyces* species YS372-C8 (NSN-21840, hereinafter referred to as YS372-C8)
- *Saccharomyces* species YS392-F7 (NSN-21841, hereinafter referred to as YS392-F7)
- *Saccharomyces* species YS344-D1 (NSN-21842, hereinafter referred to as YS344-D1)

Schedule of the NSNR(O): Schedule 2 - Information Required in Respect of Micro-organisms Manufactured in or Imported to a Contained Facility That Are Not for Introduction Outside the Contained Facility or That Are for Export Only

Organism type: Yeast

Use: Import and manufacture of the notified organisms for export to the United States for use in ethanol production.

Anticipated quantity: confidential and not for disclosure.

Assessment level of concern:

- Human health hazard: Medium
- Human exposure: Low
- Environmental hazard: Medium
- Environmental exposure: Low

Assessment conclusion under section 64 of the *Canadian Environmental Protection Act, 1999 (CEPA)*: Low risk, not suspected to be toxic

Recommended action: None

Waiver: None

Synopsis

Saccharomyces species strains YS219-A3, YS372-C8, YS392-F7 and YS344-D1 were notified for import and manufacture in a single contained facility in Canada for export to the United States for use in the ethanol industry. There is no evidence to suggest a risk of adverse human health effects at the exposure levels predicted for the general Canadian population from the use of this organism in a contained facility and export to the United States. Despite the medium potential for human hazard due to the potential allergenicity of the notified strain compared to the parental strain, there is low potential for exposure. There is no evidence to suggest a potential risk of adverse environmental effects at the exposure levels predicted for the Canadian environment from manufacture and export activities. Therefore, *Saccharomyces* strains YS219-A3, YS372-C8, YS392-F7 and YS344-D1 are not suspected to meet criteria in section 64 of CEPA.

Background information

The notified organisms are genetically modified derivatives of *Saccharomyces* strain MBG 5318 that was developed by mass mating of several commercial yeast strains used in the baking and wine industries. The notified strains contain genetic modifications that enhance its ethanol production capacity during fermentation. The notifier plans to manufacture the notified organisms in a single contained facility for export to the United States for use in the ethanol industry.

Hazard

The environmental hazard potential of *Saccharomyces* strains YS219-A3, YS372-C8, YS392-F7 and YS344-D1 is determined to be **medium** because:

1. There is no history of safe use for the notified strains, nor all intermediates used to generate the recipient strain MBG 5318 in Canada, apart from the safe manufacturing of the strain in the same contained facility by the notifier .
2. Growth at elevated temperature is a known potential virulence-associated trait of *Saccharomyces* species in terrestrial vertebrates. The parental strains were developed through mass mating of strains that are stress and thermo- tolerant. However, there is a lack of data related to growth conditions and resistance to stresses in the notified strains. Generally, there is a lack of information on whether mass-mated strains exhibit any differences in pathogenicity and toxicity towards

plants, vertebrates and invertebrates compared to known strains of this species, increasing the uncertainty in the hazard assessment of these strains.

3. The potential transfer of integrated genetic material from the notified strains to other *Saccharomyces* species is very low given the proposed containment measures and horizontal gene transfer has rarely been reported in yeast.
4. The ability of the transgenes to contribute to virulence varies across species and genetic backgrounds. One of the inserted proteins has been shown to contribute to virulence, to a greater or lesser extent, in a variety of different species. However, that virulence potential has not been tested in the notified strains, leading to some uncertainty as to their effects.

The human hazard potential of *Saccharomyces* strains YS219-A3, YS372-C8, YS392-F7 and YS344-D1 is determined to be medium because:

1. The notified organisms are genetically modified derivatives of strain MBG5318 that was produced by mass mating several commercial strains of *Saccharomyces* species used in baking and wine industries.
2. The notified organisms contain genes derived from non-pathogenic fungal and bacterial species.
3. The genetic modifications in the notified organisms are well-defined and stable. Antifungal/antibiotic resistance markers used in the construction of the notified strains were removed and are not present in the notified strain.
4. Analysis of the amino acid sequences of some of the inserted genes indicates the potential for allergenicity. In addition, there are reported cases in the literature of sensitization to glucoamylase. Consequently, the notified organisms have the potential to lead to allergic reactions in those sensitized individuals who may be exposed.
5. Although human infection from wild-type *Saccharomyces* species are rare, in-house searches of the scientific literature reported some infections. The majority of these were reported in individuals with compromised immunity or underlying medical conditions, and were treated using antifungal therapy.
6. Given the notified organisms will be maintained under fermentation conditions at temperatures between 25°C and 40°C, there is potential that they can grow at normal and febrile human body temperatures. However, it is unknown if the notified strains could harbor other potential virulence factors such as pseudohyphal growth and high phospholipase activity.

7. The notified organisms are sensitive to clinically relevant antifungal agents including 5-fluorocytosine, caspofungin, and voriconazole. Treatments are available in the unlikely event of infections caused by the notified strain.

Hazards related to micro-organisms used in the workplace should be classified accordingly under the Workplace Hazardous Materials Information System (WHMIS)¹.

Exposure

The environmental exposure potential of the notified *Saccharomyces* strains YS219-A3, YS372-C8, YS392-F7 and YS344-D1 is determined to be **low** because:

1. The notified organism will be manufactured in a single contained facility that meets Biosafety level 1-Large Scale requirements, ensuring adequate monitoring and treatment of all solid, liquid, and gaseous wastes.
2. Aqueous waste that may potentially contain the notified organisms will be heat-treated at 65°C for at least 10 minutes prior to disposal or release into the environment. This inactivation procedure demonstrated a high kill rate in a culture with a starting concentration in the range of 10⁸ to 10⁹ CFU/mL. It should be noted that the notifier stated little to no organisms will be present in the postproduction supernatant and as such, the treatment should kill what few organisms may be present in the spent media. The potential for release of viable organisms or the introduced genetic material to the environment is low, and no live cells should be released in the environment.
3. Consideration was given to a potential loss of containment (i.e., to Good Large-Scale Practice of the NIH Guidelines). In case of accidental release of the notified strain to the environment, the notifier has an adequate emergency response procedure in place.
4. Existing oversight by Ontario's Ministry of the Environment under the Environmental Protection Act (R.S.O 1990, c. E.19) and regulation 347, and the City of Ottawa under the Sewer Use By-Law (No 2003-514) would minimize the releases of

¹ A determination of whether one or more of the criteria of section 64 of CEPA are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposure in the general environment. For humans, this includes, but is not limited to, exposure from air, water and the use of products containing the substances. A conclusion under CEPA is not relevant to, nor does it preclude, an assessment against the criteria in the *Hazardous Products Regulations*, which is part of the regulatory framework for the Workplace Hazardous Materials Information System (WHMIS) for products intended for workplace use.

hazardous waste into the environment and limit liquid discharges to sewers and sewage works.

5. The intended use of the strain YS219-A3 is to produce commercial quantities of the live organism for export to the United States for industrial production of ethanol. Therefore, there is no potential for introduction into the Canadian environment.

The human exposure potential of *Saccharomyces* strains YS219-A3, YS372-C8, YS392-F7 and YS344-D1 is determined to be low because:

1. The notified organisms will be manufactured within a single contained facility in accordance with Appendix K of the NIH Guidelines Biosafety Level 1- Large Scale requirements.
2. Small quantities of the notified organisms will be imported for manufacture and export to the United States for use in ethanol production.
3. A closed system will be maintained throughout the manufacturing process. All manufacturing plant personnel have adequate training, and standard operating procedures are in place to deal with any accidental spills or loss of culture.
4. Releases of the notified organisms into the environment are expected to be negligible since the harvested cells will be exported to the United States and all liquid, solid and gaseous wastes containing the notified organisms will be inactivated prior to disposal or release into the environment and containment measures are in place in the event of a spill.
5. Should there be a release into the environment, favourable conditions are possible for survival and establishment of the notified organisms. However, given the specific conditions required for growth, the likelihood of human exposure is expected to be low.
6. Based on the intended use, containment measures and the stable integration of introduced genetic material in the notified organisms, the potential for dissemination of the introduced genes to other *Saccharomyces* strains or other organisms is low.
7. Should the containment level change to Good Large Scale Practice (GLSP), no significant change in human exposure to the notified organisms is expected since the GLSP level of containment requires discharges containing viable recombinant or synthetic organisms to be handled in accordance with applicable provincial regulations (Ontario Water Resources Act) and municipal by-laws (Ottawa Sewer Use By-law No. 2003-514).

Risk characterization

Owing to the medium potential hazard and the low potential exposure, the environmental risk posed by the notified organisms for manufacture and export is characterized as low. Hence, we propose to conclude that *S. Saccharomyces* strains YS219-A3, YS372-C8, YS392-F7 and YS344-D1 are not suspected of being “toxic” for the environment, as defined in section 64 (a) and (b) CEPA, when manufactured for export.

Despite the medium potential hazard due to the potential for allergenicity of some of the inserted enzymes, there is low potential exposure. Therefore, the human health risk associated with the manufacture of *Saccharomyces* strains YS219-A3, YS372-C8, YS392-F7 and YS344-D1 in a contained facility for export to the United States for use in bioethanol production is assessed to be low.

Risk assessment conclusion

Based on an evaluation of the notifier’s submission and relevant literature, there is no evidence to suggest a potential risk of adverse environmental effects or human health at the exposure levels predicted for the Canadian environment or the general population in Canada from the manufacture and export. The risk to the environment and human health associated with *Saccharomyces* strains YS219-A3, YS372-C8, YS392-F7 and YS344-D1 is not suspected to meet criteria in section 64 of CEPA.