New substances: risk assessment summary, New Substances Notifications 21737, 21738, 21739, and 21740

Official title: New substances: risk assessment summary, New Substances Notifications 21737, 21738, 21739, and 21740 – Schedule 2 of the *New Substances Notification Regulations (Organisms)*

Notified organisms:

- *E. coli* BL21 *ΔrhaB M1RI_pLMAR* (NSN-21737)
- *E. coli* BL21 *ΔrhaB A1RI_pLMAR* (NSN-21738)
- *E. coli* BL21 *ΔrhaB M2RI_pLMAR* (NSN-21739)
- *E. coli* BL21 *ΔrhaB LacZ::Taq* (NSN-21740)

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: Bacteria

Use: Manufacture of the notified organisms for the production of recombinant proteins, namely, ribonuclease inhibitors (RI) and a DNA polymerase protein, Taq, for use in molecular diagnostic and research applications.

Anticipated quantity: Approximately 7.5 kg each of the first three notified organisms is expected to be manufactured in a 12-month period, producing a maximum of 10 g of each respective modified RI per year. Approximately 15 kg of the fourth notified organism is expected to be manufactured in a 12-month period, producing a maximum of 24 g of the modified DNA polymerase per year.

Assessment level of concern:

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

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

Category: Not Eligible for Domestic Substances List

Recommended action: None

Waiver: A waiver to submit the data from tests of antibiotic susceptibility under paragraph 4(b) of Schedule 2 of the *New Substances Notification Regulations (Organisms)* was granted under paragraph

106(8)(a) *of the Canadian Environmental Protection Act, 1999* [CEPA] due to the fact that *E. coli* BL21, the ancestor of the notified organisms, is a common laboratory strain with a long history of safe use in production of modified proteins. In addition, the inserted genetic materials or the expressed proteins in the notified strains are not expected to alter the susceptibility of the notified strains to commonly used antibiotics.

Synopsis

The notified organisms are genetically modified *E. coli* strains that were notified to the New Substances program for the production of recombinant proteins that will be used to detect the virus causing COVID-19 (SARS-CoV-2), other pathogens, or any DNA or ribonucleic acid (RNA) target sequence of interest related to molecular diagnostic or research applications.

There is no evidence to suggest a potential risk of adverse environmental and indirect human health effects at the exposure levels predicted to the environment and general population in Canada from the production of the notified organisms.

It is determined that the notified strains are not toxic or capable of becoming toxic according to the criteria under section 64 of CEPA as there is no evidence to suggest that the notified organisms may enter the environment in a quantity or concentration or under conditions that:

- have or may have an immediate or long-term harmful effect on the environment or its biological diversity,
- constitute or may constitute a danger to the environment on which life depends, or
- constitute or may constitute a danger in Canada to human life or health.

No risk management is recommended.

Background information

The notified organisms are genetically modified *E. coli* bacteria. They have been modified using expression plasmids encoding the proteins of interest, namely, the recombinant ribonuclease inhibitors (RI) and a DNA polymerase protein, Taq. The resulting proteins will be purified and used for molecular diagnostic or research applications.

Hazard

The environmental and human hazard potential of the notified organisms is determined to be low because:

1) The four notified organisms are genetically modified from a parental strain which is derived from *E. coli* BL21. *E. coli* BL21 has a long history of safe use in industrial and laboratory applications, including production of specialty proteins and human drugs, as well as routine use in molecular biology research and genetic studies worldwide. The only phenotypic differences between the notified strains and the strain *E. coli* BL21 are their resistance to kanamycin (used as a marker), the inability to use certain sugars as a carbon source, and the production of recombinant RI or Taq proteins under specific conditions.
2) The four notified organisms are genetically modified by transforming *E. coli* BL21 with plasmids for production of modified proteins, RIs or a DNA polymerase. The genetic modifications are well-defined and the plasmids do not contain any genetic elements that are known to confer any virulence properties to the notified strain.
3) *E. coli* BL21 has lost all virulence genes commonly found in pathogenic *E. coli* strains, because of its high level of mutation and extended growth under laboratory conditions. Whole genome sequence analysis of *E. coli* BL21 did not reveal any genes for virulence or toxin production. Therefore, any reversion to pathogenicity is unlikely.
4) *E. coli* BL21 is classified as a Risk Group (RG) 1[1] human and terrestrial animal pathogen by the Public Health Agency of Canada (PHAC). It is considered nonpathogenic, noninfectious, and nontoxic. Moreover, it is not expected to survive in the environment because this strain of *E. coli* has undergone various manipulations and is now much more suited to the laboratory environment than the natural environment.
5) No pathogenicity or toxicity testing were conducted on the notified organisms as testing is not required for notifications under Schedule 2 of the NSNR (Organisms). However, neither the presence of the inserted plasmids nor the production of the RI or DNA polymerase proteins is anticipated to alter the infectivity, pathogenicity, toxicity, toxigenicity, and survival properties of *E. coli* BL21.
6) There are no reports suggesting the potential for *E. coli* BL21 to cause any harm to aquatic and terrestrial plants, and terrestrial vertebrates. No information was found in the literature regarding the use of *E. coli* BL21 strains on terrestrial invertebrates, aquatic invertebrates, and aquatic vertebrates. However, no negative effects are anticipated to occur on these species as the notified strain does not possess virulent or pathogenic factors.
7) No antibiotic susceptibility testing was conducted on the notified organisms. The notified organisms are resistant to the antibiotic, kanamycin, due to presence of the kanamycin resistance (marker) gene. However, *E. coli* BL21 is known to be susceptible to a wide range of antibiotics. Therefore, in the unlikely event of an infection with the notified strains, there are several clinically relevant antibiotics available to treat the infection.

[1] RG1 pathogens pose a low risk to the health of individual humans and animals, and low or no risk to public health and animal populations. RG1 pathogens may pose harm to immunocompromised or immunosuppressed individuals (e.g., through medical therapy, pregnancy, diabetes, or other conditions).

Exposure

The environmental and human exposure potential of the notified organisms is determined to be low because:

1) The notified strains will be manufactured in two facilities in Canada under containment conditions that meet the requirements for Containment Level 1 (CL-1) of the PHAC's Canadian Biosafety Standard, Third Edition. They will not be sold or distributed in Canada.
2) The manufacturing facilities are designed to prevent releases of the notified organisms (e.g., use of process equipment, closed systems). All personnel have adequate training, and standard operating procedures are in place to deal with any accidental spills.
3) A maximum of 30 L (7.5 kg) of each of the first three notified organisms will be manufactured for any 12-month period. They will produce no more than 10 g per RI protein per year. A maximum of 60 L (15 kg) of the fourth notified organism will be manufactured for any 12-month period. It will produce no more than 24 g of the Taq DNA polymerase protein per year.
4) During the manufacturing process, no live cells are expected to be released into the environment. Procedures for decontamination and disposal of wastes were provided and deemed acceptable by the New Substances program. The release of the live organism and the modified proteins from liquid and solid wastes is estimated to be low. The waste gas generated due to manufacturing of the notified organisms is expected be negligible.
5) In case of loss of the containment allowing the release of live cells of the notified strains in wastewater, the potential adverse effect on the aquatic environment is expected to be low because most water treatment plants in Canada have in place the technology to reduce the concentration of *E. coli*.
6) The recombinant proteins will be extracted and the quantity of the modified proteins reaching aquatic or terrestrial environment is expected to be extremely low.
7) The parental strain is not expected to survive, persist, disperse, or become established in the environment and the notified strains can be expected to behave similarly in case of a potential release to the environment.
8) Should the containment level decrease to the Good Large-Scale Practice of Appendix K of the NIH Guidelines, human exposure to the notified strains of *E. coli* or their production proteins is still expected to be minimal. This level of containment requires discharges containing viable modified or synthetic organisms to be handled in accordance with applicable governmental environmental regulations. In this case, the Quebec Ministry of Sustainable Development, Environment, and Fight Against Climate Change regulates the releases of contaminants, which includes micro-organisms, into the environment under the *Environmental Quality Act*.

Risk characterization

Owing to the low potential hazard and the low potential exposure, the environmental and human health risks associated with the use of the notified organisms to produce RI and DNA polymerase proteins are both low.

Risk assessment conclusion

There is no evidence to suggest a potential risk of adverse environmental and human health effects at the exposure levels predicted to the environment and general population from the manufacture of the notified organisms. The risk to the environment and human health associated with the notified organisms is not suspected to meet the criteria in paragraphs 64(a), (b), or (c) of CEPA. No further action is recommended.