Summary Report of Risk Assessment Conducted Pursuant to subsection 108(1) of the *Canadian Environmental Protection Act, 1999*

New Substances Notification 21300: *Escherichia coli* strain SYNB1934v1

Regulatory decision

Under the provisions for Animate Products of Biotechnology in Part 6 of the *Canadian Environmental Protection Act, 1999* (CEPA), and pursuant to section 108 of the Act, the Minister of the Environment and the Minister of Health have assessed information in respect of the substance that is a living organism. It was determined that *Escherichia coli* strain SYNB1934v1 (hereafter SYNB1934v1) is not suspected or anticipated to 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 to human life or health in Canada. Therefore, no further action is recommended as a result of this assessment.

Identity, notified and potential uses

SYNB1934v1 is a genetically modified bacterium that is derived from *E. coli* Nissle 1917 (EcN) which itself was isolated during the First World War from the faeces of a German soldier. Insertion of genes encoding several key enzymes into the genome of SYNB1934v1 enables effective uptake of the amino acid phenylalanine in the gastrointestinal tract of phenylketonuria (PKU) patients and converts it into non-toxic metabolites. Therefore upon oral administration, SYNB1934v1 is anticipated to minimize accumulation of harmful levels of phenylalanine in PKU patients that could otherwise lead to severe symptoms in those individuals.

SYNB1934v1 was notified as an investigational drug product for management of PKU in adult patients. Other potential uses could include its use in commercial drug product for management of PKU and/or other therapeutic uses.

Exposure assessment

<u>The environmental exposure potential of SYNB1934v1 from notified activities is assessed to be low because:</u>

1. SYNB1934v1 is a genetically modified bacterium with special nutritional requirements as it lacks the ability to synthesize an essential compound needed for its growth. As a result, SYNB1934v1 cannot survive and grow on its own in the environment without an exogenous source of the essential compound that is provided during strain manufacture.

2. The planned target dose of SYNB1934v1 cells will be imported into Canada during the course of the clinical trial is to treat patients in healthcare settings or outpatient home by properly trained health care professionals.
3. SYNB1934v1 will likely shed from treated patients. However, when released to the environment, it is not expected to survive since the essential compound needed for its growth will not be available. In addition, the genetic modifications are not expected to provide a selective survival advantage over its parental organism *E. coli* Nissle 1917(EcN). Taken together, exposure to the environment is expected to be low.
4. General biosafety measures in healthcare settings during handling and administration of the notified organism, as well as contingency plans for accidental spills are expected to be in place.

The indirect human exposure potential of SYNB1934v1 through the environment is assessed to be low because:

1. Even if SYNB1934v1 sheds from treated patients or is released to the environment as waste, exposure to the general population is less likely as it is not expected to survive in the environment due to its special nutritional requirements.

The following were also taken into account in the human health and environmental exposure considerations for SYNB1934v1:

1. SYNB1934v1 is intended to be used as an investigational drug product for management of PKU in adult patients. Other potential uses include the use of SYNB1934v1 as a commercial drug product and/or other therapeutic uses as a probiotic. Regardless, SYNB1934v1 is not expected to grow in the environment. Therefore, exposure to the environment or general population is not expected to significantly increase from commercial use of SYNB1934v1.
2. The notifier does not intend to manufacture SYNB1934v1 in Canada. Should it be manufactured in Canada, the environmental exposure would not be expected to significantly increase due to its special nutritional requirements for growth.

Hazard assessment

The environmental potential of SYNB1934v1 is assessed to be low because:

1. Similar to its parental organism, EcN, SYNB1934v1 does not contain plasmids carrying genes for antibiotic resistance. In addition, harmful genes inherited from the parental organism were deleted in SYNB1934v1.
2. The information provided by the notifier and an updated search of the scientific literature yielded no results for potential negative impacts (pathogenicity or toxicity) of SYNB1934v1 on aquatic plant, invertebrate or vertebrate species, or on terrestrial plant and invertebrate or vertebrate species.

The human health hazard potential of SYNB1934v1 is assessed to be low because:

1. Similar to its parental organism EcN, SYNB1934v1 does not contain plasmids carrying genes for antibiotic resistance. In addition, it lacks other harmful genes usually present in the parental organism that are known to cause adverse effects in humans as they were deleted as part of its development.
2. Some cases indicated that the parental strain EcN might promote sepsis in immunocompromised patients or in individuals with underlying chronic diseases. However, the results of previous clinical studies conducted in other countries using two *E.coli* strains (SYNB1618 and SYNB1934) that are closely related to SYNB1934v1 showed that those strains are safe[1].
3. SYNB1934v1 is susceptible to several members of the β-lactam family of antibiotics, which could be used as effective treatments in the unlikely event of infection with the strain.

Hazards related to micro-organisms used in the workplace should be classified accordingly under the Workplace Hazardous Materials Information System (WHMIS)[2].

<u>The following considerations were also taken into account in the assessment of human health and environmental hazard potential for SYNB1934v1:</u>

1. SYNB1934v1 is a risk group 1 human and animal pathogen as per Public Health Agency of Canada. Its parent strain EcN is a naturally occurring commensal strain of *E. coli* that has been granted market authorization in Canada since 2013.
2. The whole genome sequencing analysis of SYNB1934v1 provided by the notifier confirmed all expected genetic modifications in the genome of SYNB1934v1.
3. The animal study conducted by the notifier to determine the effects of a closely related strain (*E.coli* SYNB 1618) on mice did not indicate any mortality or adverse health effects on the animals tested.

Risk characterization

Risk is typically described as the probability of an adverse effect occurring based on hazards and a particular scenario of exposure. In the present case, the organism will be imported and used as an investigational drug product for management of PKU and/or other therapeutic uses as a probiotic. Owing to the low potential hazard and the low potential exposure, the environmental and human health risks associated with the use of SYNB1934v1 as an investigational or commercial drug product for management of PKU and/or other therapeutic uses as a probiotic is assessed to be low.

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[1] In Canada, biologic drugs are assessed for safety, quality, and efficacy under the *Food and Drugs Act* and Regulations, administered by the Biologic and Radiopharmaceutical Drugs Directorate of Health Canada.

[2] 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.

The assumptions made in the assessment are considered to be adequately protective for the general population as well as for subpopulations who may be more susceptible or highly exposed.

Risk assessment conclusion

There is no evidence to suggest a potential risk of adverse environmental effects at the exposure levels predicted for the Canadian environment from the use of SYNB1934v1 as an investigational or a commercial drug product for management of PKU and/or other therapeutic uses as a probiotic. The risk to the environment associated with SYNB1934v1 is not suspected to meet criteria in paragraphs 64(a) or (b) of CEPA. No further action is recommended.

Similarly, 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 SYNB1934v1 as an investigational or a commercial drug product for management of PKU and/or other therapeutic uses as a probiotic. The risk to human health associated with SYNB1934v1 is not suspected to meet criteria in paragraphs 64(c) of CEPA. No further action is recommended.