Risk Assessment Summary Conducted Pursuant to the
New Substances Notification Regulations (Organisms) of the
Canadian Environmental Protection Act, 1999
NSNs 16596 and 16597: Bacillus species strains 01 and 02

Regulatory Decision

Under part Part 6 of the *Canadian Environmental Protection Act, 1999* (CEPA) 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 organisms, and determined that the organisms are not suspected of being harmful to the Canadian environment or human health as defined in section 64 of CEPA[1], when manufactured for introduction into the environment anywhere in Canada. Therefore, the manufacture of *Bacillus* species strains 01 and 02 for this purpose may proceed after March 17, 2012, corresponding to the last day of the assessment periods.

NSNR (O) Schedule 1: Manufacture of micro-organisms for introduction in the environment anywhere in Canada
Organism Identity: *Bacillus* species strains 01 and 02
Notifier: Choisy Laboratories Ltd.
Date of decision: March 17, 2012
Proposed use: Components in industrial and commercial products for odour-control and waste degradation

IDENTITY / STRAIN HISTORY:

The notified micro-organisms are naturally-occurring bacteria belonging to the genus *Bacillus*, which, as of 2012, included 11 phylogenetic subclusters and 142 members (Bergey and Boone, 2009). Since species within the *Bacillus* genus exhibit a wide diversity of physiological traits, a polyphasic approach consisting of morphological, biochemical, metabolic and genotypic analyses were used to identify the notified strains. *Bacillus* species strains 01 and 02 are motile, Gram-positive, spore-forming bacilli occurring singly or in pairs and with an aerobic metabolism; the precise species name is not disclosed at the request of the company. They are capable of growing at temperatures between 10°C and 50°C with an optimal range between 28°C and 37°C. As well, *Bacillus* species strains 01 and 02 have a salt tolerance range of 0-10% and a pH range of 5-10.

Bacillus species strains 01 and 02 were isolated from environmental samples collected in the region of Louiseville, QC in 2003. *Bacillus* species strains 01 and 02 were selected for their capacity to express useful metabolites and to exert key metabolic traits, as well as their ability to

[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.

germinate, grow, and sporulate in industrial fermentation processes. Both will be manufactured in Canada for use in biodegradation and odour-control products.

HAZARD CONSIDERATIONS:

Environmental Hazard

Bacillus species strains 01 and 02 belong to a remarkably diverse bacterial species that is capable of growth in many different environments, including both terrestrial and aquatic environments. This ubiquitous species can form resilient spores in response to environmental stressors, and exists in the spore form under most conditions, allowing the organism to disperse via wind and water.

While *Bacillus* species strains 01 and 02 do not contain any plasmids, there is the potential for the strains to take up DNA by horizontal gene transfer through transduction, conjugation or transformation. There are reports in the literature of horizontal gene transfer occurring within the species; however, there is no indication of it leading to any adverse effects. The potential of the notified strains to acquire genes encoding virulence factors is no greater than for any other naturally occurring strains of the species.

No signs of pathogenicity or significant toxicity have been reported for different strains of the species proposed for various uses in other regulatory jurisdictions. In addition, information in the scientific literature provides a large body of evidence indicating that the species is highly unlikely to be involved in adverse environmental effects, and is widely accepted as an organism of no concern. As such, waivers were granted for data regarding the effects of the micro-organisms on plant, invertebrate and vertebrate species under subsection 106(8)(a) of CEPA. The potential for the notified organisms to cause adverse effects on the environment, its conservation or its biological diversity is, therefore, considered low.

Human Health Hazard

Strains 01 and 02 belong to a species of *Bacillus* that is not regarded as a frank human pathogen, does not possess genes encoding virulence factors, and does not produce extracellular enzymes that predisposes it to cause infections in humans. However, the species is ubiquitous and it has been occasionally isolated from wound drainage sites, prostheses, and from immunocompromised patients. Nevertheless, surrogate data on other naturally occurring strains of the species have shown no significant toxicological effects when tested for acute oral, pulmonary and dermal effects as well as for dermal and eye irritation and hypersensitivity. As well, following a review of the literature and based on information provided by the notifier, there was no indication that *Bacillus* species strains 01 and 02 would demonstrate any allergenic properties that would differ from those of strains of the species already in commercial use.

Since no evidence was found to indicate any pathogenic potential for the notified strains, waivers were granted under CEPA 106(8)(a) for data from tests of pathogenicity that are valid for related micro-organisms that are pathogenic to humans. In addition, antibiotic susceptibility testing showed the notified micro-organisms to be susceptible to a variety of clinically relevant antibiotics. Treatment options would be available in the unlikely event of an infection. The use

of *Bacillus* species strains 01 and 02 is not expected to cause adverse effects to the general population. Therefore, their potential hazard to human health is considered low.

EXPOSURE CONSIDERATIONS

Bacillus species strains 01 and 02 are owned exclusively by Choisy Laboratories Ltd. (Louiseville, Quebec) and will be incorporated as components in a variety of end-use products containing other species of bacteria. The manufacturing process meets the requirements of Containment Level 1 for large scale production as set out in the Public Health Agency of Canada's Laboratory Biosafety Guidelines (3rd ed., 2004). Strain solutions and end-use products will be prepared and handled under sterile conditions by trained employees to ensure appropriate biosafety procedures are applied at all times. Standard operating procedures are in place for each step of the material preparation, fermentation, packaging, and post-fermentation sterilization. Each batch will undergo a quality assurance program which includes identification, contamination and quantification controls.

The fermentation system is equipped with filters upstream and downstream to avoid air contamination. Solid wastes are chemically disinfected or autoclaved before disposal. Liquid wastes will be transferred into a retention tank containing 12% sodium hypochlorite and disinfected for a minimum of 24 hours prior to discharge into the municipal sewer system. Air filters will be cleaned by submerging them in a 40% isopropanol solution for 2 - 16 hours, followed by heat drying at 40°C to 50°C, then sterilization in an autoclave. The premises of the manufacturing facility are surrounded by drainage gutters. These gutters are directed to the retention tank and prevent any large spills from directly entering into the municipal sewage. Unintentional release into the environment of the notified micro-organisms from the manufacturing facility is unlikely.

Bacillus species strains 01 and 02 will be released into the environment through product usage but only to specific sites and only when required. Methods of product application are expected to vary depending on the intended use. Some products will be sprayed directly onto surfaces, some will be injected using a dispensing system to control odours in grease and hydrocarbon waste traps, or in agri-food industrial wastewater, and others will be poured directly into the site of use.

As *Bacillus* species strains 01 and 02 were notified under Schedule 1, uses for other purposes are possible. Other potential uses for *Bacillus* species strains 01 and 02 include the production of biosurfactants and enzymes for use in bioremediation, biotransformation, and wastewater treatment (Sammai et al., 2011; Plaza et al., 2008; Brooksbank et al., 2007). Given that *Bacillus* species strains 01 and 02 are environmental isolates, other potential habitats of introduction should not be very different from the species' natural habitat.

Environmental Exposure

Given the intended and potential uses, exposure of *Bacillus* species strains 01 and 02 may occur by inhalation, dermal contact, ocular, and ingestion routes. *Bacillus* species strains 01 and 02 have the potential to survive and disperse, but only in the spore state and when not biologically active. While the populations of the notified strains may increase due to product usage and

accidental release, it is expected there will be a natural reversion of the species to background population levels due to competition with the other bacteria already present at the site of introduction. Any living organisms coming into contact with *Bacillus* species strains 01 and 02 will likely have come into contact with other strains of that species of *Bacillus*, given its ubiquitous distribution in nature. Given their ability to form spores and potential usage in a large spectrum of activities, the potential environmental exposure to *Bacillus* species strains 01 and 02 is therefore considered to be medium.

Human Exposure

Exposure to *Bacillus* species strains 01 and 02 during manufacture and product usage could possibly occur through all four main routes (dermal, pulmonary, ocular, and oral); however, the likely routes of direct and bystander exposure is via dermal and inhalation. The species is not recognized as a wound pathogen and there is no indication it has ability to penetrate intact skin. Inhalation exposure to fine mist or spray during product usage is possible; however, the extent of the exposure will depend on the intended use, volume, concentration, rate of particle aerosolization and proximity to source of application. This can be minimized through the proper use of personal protective equipment. Exposure to the notified strains is also possible if product usage results in the contamination of surfaces and the formation of biofilms. Human exposure to *Bacillus* species strains 01 and 02 is not expected through environmental media as introduced populations of the notified strains in soil are expected to decline due to microbiostasis. As the intended uses are for commercial and industrial applications, exposure to the general population from the formulation, handling and application of the products containing *Bacillus* species strains 01 and 02 is estimated to be low to medium.

RISK ASSESSMENT CONCLUSION / REGULATORY OUTCOME

Bacillus species strains 01 and 02 are environmental isolates that belong to a bacterial species with a ubiquitous distribution. The species is not recognized as a plant, animal or human pathogen and is not considered to be an organism of concern. *Bacillus* species strains 01 and 02 will be manufactured at one facility in Canada in accordance with large-scale CL 1 requirements and will be incorporated into various commercial products for biodegradation and odour-control. Based on the hazard and exposure considerations described above, the risk assessments conducted by Environment Canada and Health Canada concluded that *Bacillus* species strains 01 and 02 are not expected to cause harm to the Canadian environment or human health as described in section 64 of the CEPA.

The substances are eligible for addition to the Domestic Substances List on the basis of these risk assessments.

REFERENCES

Please note that the following is only a partial reference list due to confidentiality reasons.

Bergey, D.H., and Boone, D.R. (2009). Bergey’s manual of systematic bacteriology. Springer Verlag.

Brooksbank, A.M., Latchford, J.W., and Mudge, S.M. (2007). Degradation and modification of fats, oils and grease by commercial microbial supplement. World J. Microb. Biot. 23, 977-985.

Plaza, G.A., Jangid, K., Lukasik, K., Nalecz-Jawecki, G., Berry, C.J., and Brigmon, R.L. (2008). Reduction of petroleum hydrocarbons and toxicity in refinery wastewater by bioremediation. Bull. Environ. Contam. Toxicol. 81, 329-333.

Sammai, A., Sobhon, V., and Maneerat, S. (2011). Molasses as a whole medium for biosurfactants production by Bacillus strains and their application. Appl. Biochem. Biotechnol. 165, 315-335.