Risk Assessment Summary Conducted Pursuant to the
***New Substances Notification Regulations (Organisms)* of the**
Canadian Environmental Protection Act, 1999
NSNs 16908 and 16909: *Bacillus* species strains 06 and 03

Regulatory Decision

Under 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 the CEPA[1], when manufactured for introduction into the environment anywhere in Canada. Therefore, the manufacture of *Bacillus* species strains 06 and 03 for this purpose may proceed after October 16, 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 06 and 03
Notifier: Choisy Laboratories Ltd
Date of decision: October 16, 2012
Proposed use: Biodegradation of organic residues and odour-control in commercial and industrial settings

IDENTITY AND STRAIN HISTORY

The notified micro-organisms are both naturally occurring environmentally isolated bacteria. Strain 06 (NSN 16908) and strain 03 (NSN 16909) were obtained in 2002 from environmental samples collected in the regions of Louiseville, QC and Trois-Rivières, QC, respectively. The precise species name is not disclosed at the request of the company. Species 06 and 03 were selected based upon their capacity to express useful metabolites and to exert key metabolic traits, as well as their efficient growth and sporulation in industrial fermentation, capacity to remain viable during extended storage and ability to efficiently germinate. The notified strains were identified as belonging to the genus *Bacillus* based on morphological, physiological, biochemical and genotypic characteristics. Vegetative cells of strains 06 and 03 are Gram-positive rods, with aerobic metabolism, occurring singly or in pairs, and are motile. Permissive growth conditions include temperatures between 10 °C and 50 °C, pH 5 to 10, and 0 to 10% (w/v) NaCl. Strain 06 can be distinguished from strain 03 based on different carbohydrate utilization profiles using API 50 CH carbohydrate strips. Both will be manufactured in Canada as liquid formulations of spores for use in products dedicated to odour-control and waste degradation in various industrial

[1] In accordance with section 64 of CEPA, 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.

devices such as septic tanks, grease traps, refuse containers, compactors, garbage trucks, bathrooms, hydrocarbon waste traps and wastewater of agri-food industries.

HAZARD CONSIDERATIONS

Environmental hazard

Strains 06 and 03 belong to a diverse bacterial species that is capable of growth within many environments, including both aquatic and terrestrial, and is naturally present on fresh produce. This ubiquitous species is capable of forming highly resistant spores in response to environmental stressors and exists in the spore form under most conditions allowing the organism to spread by wind and water. Among *Bacillus* species, it is not recognized as a frank pathogen to insects or other animals and is not taxonomically related to any known plant pathogens (Logan and de Vos, 2009).

Strains 06 and 03 do not contain any plasmids and are not expected to be a source of genes for pathogenic traits or antibiotic resistance. While there is the potential for the notified micro-organisms to take up DNA by horizontal gene transfer, this potential is no greater for strains 06 and 03 than for any other strains that are naturally present in the environment.

Literature searches confirmed that for the proposed and potential uses, strains 06 and 03 do not pose any hazards to terrestrial and aquatic plants and animals. Therefore, the potential for the notified organisms to cause adverse effects on the environment, its conservation or its biological diversity is considered low.

Human health hazard

Bacillus species strains 06 and 03 belong to a member of the *Bacillus* family that is classified as a Biosafety Level 1 organism and that is not considered to be a frank human pathogen. Despite a ubiquitous distribution, where frequent exposure to members of this species is likely to occur, there are no documented cases of the species causing infection in healthy humans. Test data from animal studies using acceptable surrogate strains showed no indication of toxicity, infectivity or pathogenicity. As with most micro-organisms, strains 06 and 03 have the potential to act as moderate skin sensitizers; however, there were no reports of hypersensitivity incidents during product development and there is no indication they are capable of causing more serious adverse immune effects. Antibiotic susceptibility tests found the notified strains to be sensitive to certain clinically relevant antibiotics. Therefore, treatment options would be available in the unlikely event of an infection. The use of *Bacillus* strains 06 and 03 is not expected to cause adverse effects to the general population. Their potential hazard to human health is therefore considered low.

EXPOSURE CONSIDERATIONS

Exposure to the notified micro-organisms can occur through the manufacturing process and/or end-use product usage. *Bacillus* species strains 06 and 03 will be manufactured by a batch-fermentation process, concentrated and stored in liquid form at the corporate headquarters of the notifier in Louiseville, QC. The facility meets the standards of Containment Level 1 for large scale production of micro-organisms as set out in the Laboratory Biosafety Guidelines (3rd

edition, 2004). Each batch will go through a quality assurance program which includes identification, contamination and quantification controls.

Standard operating procedures are in place for each step of the material preparation, fermentation, packaging and post-fermentation sterilization. Solutions and end-use products will be prepared and handled under sterile conditions by properly trained employees to ensure biosafety procedures are applied at all times. The fermentation system is equipped with filters upstream and downstream to minimize air contamination. Solid wastes are either chemically disinfected or autoclaved prior to disposal. Liquid wastes are directed to a retention tank and then chemically or thermically disinfected before disposal into the municipal sewer system. The manufacturing premises are surrounded by drainage gutters that direct the liquid wastes into the retention tank and thereby prevent large spills from directly entering the municipal sewage system. Therefore, environmental release of the notified micro-organisms through the manufacturing process is unlikely.

Strains 06 and 03 are owned exclusively by the notifier and will only be incorporated in the company's products and sold under the commercial brand "Choisy". Products may be used year round with method and frequency of application to vary based on intended use. Some may be sprayed manually on surfaces or automatically in containers. Other products may be injected using a dispensing system to control odours in grease and hydrocarbon waste traps, or in agri-food industrial wastewater or poured directly into the site of use. Repeated applications are recommended to promote the formation and maintenance of beneficial competitive microflora. Discontinuing use of the products would allow the concentrations of strains 06 and 03 at the sites of application to decrease over time and allow the indigenous microbial flora to naturally reinstate itself.

Examples of other potential uses of bacteria similar to the notified strains include use in agriculture as a biocontrol agent against phytopathogenic bacteria as well as production of biomolecules for use in soil decontamination, bioremediation, biodegradation, and wastewater treatment (Issazadeh et al., 2011; Prasad and Manjunath, 2011).

Environmental exposure

According to intended and potential uses, exposure to *Bacillus* species strains 06 and 03 may occur via inhalation, dermal contact, ocular, and ingestion routes. Although there is the potential to survive and disperse upon release, this would only be in the spore state and when not biologically active. Despite the increased concentrations of the strains from either product usage or by accidental release, the concentrations will gradually decrease over time as the spores enter the vegetative state and the indigenous microbial flora will naturally reinstate itself (Kokalis-Burelle et al., 2006). Living organisms coming into contact with the notified strains will likely have already come into contact with the species given its ubiquitous nature. Given their ability to form spores and other potential uses in a variety of activities and products, the potential environmental exposure to *Bacillus* species strains 06 and 03 is therefore considered to be medium.

Human exposure

While ocular and oral exposures are possible, dermal and respiratory exposures are more likely to occur. Literature searches do not suggest that the species is associated with the typical microbial flora of humans or that it can penetrate intact skin of healthy individuals; nor is the species a recognized wound pathogen. Respiratory exposures to a fine mist or spray by bystanders during applications in public areas are possible. The rate of exposure will depend upon the volume, concentration, rate of aerosolisation, and the proximity of bystanders to the application source. Respiratory exposure from the proposed use is predicted to be low to medium. Human exposures to the notified strains are not expected through environmental media as introduced populations in soil are expected to decline due to microbiostasis. Based on the proposed uses in commercial cleaning products and potential other uses, human exposure to *Bacillus* species strains 06 and 03 from the handling and application of products containing the notified micro-organisms is therefore considered to be low-to-medium.

RISK ASSESSMENT CONCLUSION / REGULATORY OUTCOME

Bacillus species strains 06 and 03 are naturally occurring isolates of a species of bacteria that has a ubiquitous distribution in nature. This species is not recognized to act as a human, animal or plant pathogen. The notified micro-organisms will be manufactured in Canada in one facility in accordance with large-scale Containment Level 1 requirements for use as components in various commercial and industrial biodegradation and odour-control products. Based on the hazard and exposure considerations described above, the risk assessment conducted by Environment Canada and Health Canada concluded that *Bacillus* species strains 06 and 03 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.

Issazadeh, K., Majid, M.R., Pahlaviani, K., and Massiha, A. (2011). Bioremediation of toxic heavy pollutants by Bacillus spp. Isolated from Guilan Bay sediments, North of Iran. Int. Proc. Chem. Biol. Environ. Eng. 18, 67-71.

Kokalis-Burelle, N., Kloepper, J.W., and Reddy, M.S. (2006). Plant growth-promoting rhizobacteria as transplant amendments and their effects on indigenous rhizosphere microorganisms. Appl. Soil Ecol. 31, 91-100.

Logan, N.A., and de Vos, P. (2009). Genus I: Bacillus. Bergey's Manual of Systematic Bacteriology, 2nd Ed. Volume Three, The Firmicutes. De Vos, P., Garrity, G.M., Jones, D., Krieg,N.R., Ludwig,W., Rainey, F.A., Schleifer, K.H., and Whitman, W.B. eds., (New York: Springer) pp. 21-128.

Prasad, M.P., and Manjunath, K. (2011). Comparative study on biodegradation of lipid-rich wastewater using lipase producing bacterial species. Indian J. Biotechnol. 10, 121-124.