Environment Canada Environnement Canada

Risk Assessment Summary Conducted Pursuant to the *New Substances Notification Regulations (Organisms)* of the *Canadian Environmental Protection Act, 1999*

NSN 16850: *Nitrosomonas* species 804

Canada

This document has been prepared to explain the regulatory decision taken under Part 6 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999) and its *New Substances Notification Regulations (Organisms)* [NSNR (O)] regarding the manufacture or import of *Nitrosomonas* species 804* by Novozymes Biologicals Inc. that is intended for export only. *The species name is not provided as it has been claimed to be confidential by the proponent and this masked name has been accepted by the Government.

The New Substances Assessment and Control Bureau of Health Canada and the Emerging Priorities Division of Environment Canada have assessed the information submitted by Novozymes Biologicals Inc. and other available scientific information in order to determine whether *Nitrosomonas* species 804 is toxic[1] or capable of becoming toxic as described in section 64 of CEPA 1999.

Regulatory Decision

Based on the hazard and exposure considerations, the risk assessment conducted by Environment Canada and Health Canada concluded that *Nitrosomonas* species 804 does not meet the criteria as described in section 64 of the CEPA 1999. Therefore, the importation of *Nitrosomonas* species 804 for subsequent export only activities may proceed after November 11, 2012.

This evaluation does not include an assessment of human health risk in the occupational environment.

NSNR(O) Schedule: 2
Organism Identity: *Nitrosomonas* species 804
Notifier: Novozymes Biologicals Inc. 5400 Corporate Circle, Salem, VA, USA
Date of decision: November 11, 2012
Proposed use: Component in a water conditioning product for use in aquaculture holding tanks

IDENTIFICATION / STRAIN HISTORY

The notified organism, *Nitrosomonas* species 804 is a naturally-occurring bacterium that was originally isolated in the mid-1980s from a petroleum wastewater treatment plant in the United Kingdom. The strain was obtained by Novozymes Biologicals in 2002. Identification of *Nitrosomonas* species 804 was based on cell and colony morphology, as well as genotypic analyses (16S rDNA sequence alignment). *Nitrosomonas* species 804 is a Gram-negative, non-spore forming, slightly pear-shaped motile rod with rounded ends. Colonies on standard *Nitrosomonas* growth medium are characteristically round, light brown with a shiny or slightly slimy appearance. It is an aerobic, ammonia-oxidizing chemolithotroph that grows optimally at 28°C. This optimal growth is achieved when

[1] In accordance with section 64 of the *Canadian Environmental Protection Act, 1999* (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.

there is sufficient ammonia for energy and CO_2 as a carbon source. In anoxic environments, this organism can metabolize acetate to support anaerobic growth. It is not proficient at urea metabolism, therefore it is unlikely to flourish in acidic and low-nitrogen environments (Koops and Pommerening-Roser, 2001).

The notifier plans to import *Nitrosomonas* species 804 in order to use it as a component of a water conditioning product that will be applied to aquaculture holding tanks within a contained facility. The role of the strain in the product is to oxidize ammonia waste into nitrite, which will then be oxidized by other micro-organisms. *Nitrosomonas* Species 804 has been used for over 25 years as part of a water conditioning product in shrimp farms in South East Asia and is currently used for transporting seafood in the Middle East.

HAZARD CONSIDERATIONS

Environmental Hazard

Nitrosomonas species 804 is an organism that has a long history of safe use and is not genetically modified. In the environment, this species has been shown to exhibit some potential for horizontal gene transfer. A whole genome analysis revealed the presence of two large plasmids, multiple transposons, and several families of insertion sequence (lS) elements. However, because the notified organism will be used only in contained conditions in this case, the potential for the organism to donate or acquire genetic elements coding for detrimental traits is considered minimal.

The potential environmental hazard of *Nitrosomonas* species 804 is assessed as low because it is a Biosafety Level 1 organism with no indication in the literature that it is toxic or pathogenic to target organisms in the environment. This conclusion is also supported by the Swiss Federal Office for the Environment, of the Federal Department of Environment, Energy and Communications, which reports that this organism is not pathogenic to plants, vertebrates or invertebrates.

Human Health Hazard

There are no known recorded cases of *Nitrosomonas* species 804 infection in healthy humans despite its natural presence in the environment and its long history of use worldwide in water treatment products.

Many micro-organisms are known to contain components, such as lipopolysaccharides, antigens, toxins and enzymes, which may act as potential sensitizers. Sensitization or allergic reactions to micro-organisms could occur via dermal and respiratory routes in susceptible individuals or workers through frequent and prolonged occupational exposure (Martel et al., 2010; Ring et al., 1992). However, no cases of adverse immune reactions in humans to *Nitrosomonas* species 804 have been reported. While the notified strain was found to be resistant to many antibiotics tested, treatment options are available in the unlikely event of an infection. The notified organism has been found to be susceptible to cefazolin with intermediate sensitivity to chloramphenicol and erythromycin. Based on

these considerations, *Nitrosomonas* species 804 is deemed to represent a low human health hazard.

EXPOSURE CONSIDERATIONS

The notified micro-organism will not be manufactured in Canada. A maximum of 15,000 L of the product containing *Nitrosomonas* species 804 will be imported primarily from Novozymes Biologicals in Salem Virginia, USA into a contained facility in Canada by express air cargo. The notifier plans to import *Nitrosomonas* species 804 in order to use it as a component of a water conditioning product that will be applied to aquaculture holding tanks within a contained facility. The holding tanks will then be shipped to Europe.

The environmental exposure potential of *Nitrosomonas* species 804 is assessed as low because: (i) the strain is intended for import only to a contained facility certified by the Canadian Food Inspection Agency (CFIA) to handle Biosafety Level 1 organisms, (ii) the strain will not be manufactured therefore there will not be continuous production of the microorganism or waste released containing the micro-organism, (iii) the only possible release of the micro-organism into the environment would be from accidental release (for which there are appropriate treatment and clean-up measures in place) in the facility or en route to or from airports.

Taking into account the proposed use and the procedures in place to limit any potential release, as well as effective treatments to inactivate the notified organism, no significant environmental release is expected which may result in general population exposure to the notified microorganism. Therefore, general population exposure to *Nitrosomonas* species 804 is expected to be low.

RISK CHARACTERIZATION

Nitrosomonas species 804 is well characterized, and has a history of safe use in water conditioning products. It is a naturally-occurring bacterium originally found in petroleum waste-water and is not considered to be a plant, animal or human pathogen. The notified organism will not be manufactured in Canada and will be used in a contained facility for export only, where contingency measures are in place in case of accidental spills. Based on the proposed use, *Nitrosomonas* species 804 is not considered to be harmful to the Canadian environment or human health and thus does not meet any of the criteria described in section 64 of CEPA 1999.

REFERENCES

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

Koops, H.P., and Pommerening-Roser, A (2001). Distribution and ecophysiology of the nitrifying bacteria emphasizing cultured species. FEMS Microbiol. Ecol. 37, 1-9.

Martel, C., Nielsen, G.D., Mari, E., Licht, T.R., and Poulsen, L.K. (2010). Scientific *I* Technical Report Submitted to EFSA - Bibliographic Review on the Potential of Microorganisms, Microbial Products and Enzymes to Induce Respiratory Sensitization. *CFPIEFSAIFEEDAP/2009/02.* http://www.efsa.europa.eulen/supportinglpub/75e.htm (viewed October 2012).

Ring, J., Abeck, D., and Neuber, K. (1992). Atopic eczema: Role of microorganisms on the skin surface. Allergy Eur J Allergy Clin Immunol. 47, 265-269.