

**Risk Assessment Summary Conducted Pursuant to the
New Substances Notification Regulations (Organisms) of the
Canadian Environmental Protection Act, 1999
NSN 17179: *Arcobacter* SP. Strain**

Regulatory Decision

Under Part 6 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999) 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 organism, and determined that the organism is not suspected of being harmful to the Canadian environment or human health as defined in section 64 of the CEPA 1999¹, when manufactured for introduction into an ecozone in accordance with confinement procedures and into an ecozone to which the micro-organism is indigenous. Therefore, the manufacture of *Arcobacter* SP. Strain for this purpose may proceed after August 2, 2013.

NSNR(O) Schedule: 1 (introduction in accordance with confinement procedures and in an ecozone where it is indigenous)

Organism Identity: *Arcobacter* SP. Strain

Notifier: Confidential

Date of decision: August 2, 2013

Proposed use: Microbial enhanced oil recovery

IDENTITY

The notified micro-organism is a naturally occurring bacterium isolated from the production water in an oil reservoir in Alberta in 2009. The organism was identified as belonging to the genus *Arcobacter* based on both phenotypic and genotypic criteria. Sequence analysis of the 16S rDNA gene showed the greatest similarity to *Arcobacter* sp. Solar Lake, followed by *Arcobacter marinus* and *Arcobacter halophilus*. Phylogenetic analysis compared the notified strain with that of the type strain of 16 recognized *Arcobacter* species, *Arcobacter* sp. Solar Lake, and two other *Arcobacter* sp. strains isolated from the same oilfield. Multiple sequence alignment produced a phylogenetic tree placing the notified strain in a clade containing only environmental isolates. The remaining species, including known pathogens, were grouped into two other clades. Phenotypically, the bacterium is characterized as an obligate halophile as the notified strain was found to be much more tolerant to salt than the type strain of *A. marinus*. On microscopic examination, cells are Gram-negative, motile, curved or helical rods with no evidence of sporulation. These features are consistent with other species of the

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.

Arcobacter genus. Based on the phylogenetic and phenotypic characterization, the notifier concluded that *Arcobacter* SP. Strain is likely a new species of *Arcobacter*.

HAZARD CONSIDERATIONS

Environmental Hazard

The notified strain will be used in the ecozone and the type of habitat from which it was isolated and without any genetic modification. These are important factors in estimating the consequences of horizontal gene transfer of any sequences of concern as no new sequences will be introduced into the environment. There is no specific information on the capacity of *Arcobacter* SP. Strain to contribute to horizontal gene transfer, but there is nothing to indicate that the potential is greater for this strain than for any other naturally occurring species of *Arcobacter*.

The potential of the notified strain for pathogenicity or toxicity to terrestrial and aquatic non-human species is not known. Species of *Arcobacter* occupy a large diversity of habitats and show very varied capacities to cause disease in mammals (Collado and Figueras, 2011). There is no literature documenting infections that can be attributed to any of the species in the clade to which the notified strain belongs. In addition, members of this clade were observed by the notifier to be less motile than type strains of known pathogenic species of *Arcobacter*. Contrary to those pathogenic species, members of the notified strain's clade show no invasion of mammalian cells *in vitro* (Levican et al., 2013).

However, there is some uncertainty; *Arcobacter* SP. Strain belongs to a new and as yet uncharacterized species, in a genus that contains pathogenic species. As well, it was notified under Schedule 1 with confinement whereby test results for ecological effects, including pathogenicity and toxicity testing on aquatic and terrestrial organisms are not a requirement. Therefore, there could be some potential for the notified micro-organism to cause adverse effects in the environment, its conservation or its biological diversity and so its environmental hazard is considered medium.

Human Health Hazard

Some species of *Arcobacter* are emerging as human pathogens as *A. cryaerophilus*, *A. skirrowii* and most commonly, *A. butzleri* have all been associated with enteritis and occasionally bacteremia (Snelling et al., 2006). The pathogenicity and virulence mechanisms of *Arcobacter* species remain poorly understood but adhesion, invasion, and cytotoxicity have been observed in *in vitro* studies (Collado and Figueras, 2011). Host characteristics such as age and immune status likely also have a role in determining severity of *Arcobacter* infections (Collado and Figueras, 2011; Shah et al., 2011). Based on phylogenetic analysis, these three species are placed on a different clade from the notified strain. An acute oral toxicity/pathogenicity study conducted on the notified strain saw no mortalities or other treatment-related clinical signs throughout the study period and concluded *Arcobacter* SP. Strain to be non-toxic, non-infectious, and non-

pathogenic. Antibiotic susceptibility tests found the notified strain to be sensitive to certain clinically relevant antibiotics. The use of *Arcobacter SP*. Strain is not expected to cause adverse effects to the general population. Its potential hazard to human health is considered low.

EXPOSURE CONSIDERATIONS

The ownership and responsibility for the notified strain will remain with the notifier who will be the sole provider as part of a service provided to oil field operators. The introduction of *Arcobacter SP*. Strain into the Canadian environment will be confined to oil reservoirs in the Prairie ecozone, to which it is indigenous. This use conforms to the scope of activities that can take place within a notification under Schedule 1 of the NSNR(O) with confinement procedures.

A small quantity of the frozen notified strain will be imported from the notifier's facility in the United States for manufacture in a fermentation facility in Canada under the notifier's supervision. Information provided indicated that the manufacture will, at minimum, take place in accordance with large-scale BSL 1 physical and operational requirements, as described in the 3rd edition of the Laboratory Biosafety Guidelines published by the Public Health Agency of Canada. As such, measures are in place to prevent release of the micro-organism into the environment during manufacture and to ensure the integrity of the culture. All biological wastes produced during the fermentation process are inactivated by autoclaving or treatment with either bleach or 70% ethanol solution.

Fermentation broth containing *Arcobacter SP*. Strain will be injected into oil wells via steel piping over a period of several hours up to about 2000 metres deep. Periodic feeding with nutrients will be done to maintain the population of the notified strain in the reservoir. Once nutrients are exhausted, numbers of *Arcobacter SP*. Strain are expected to return to background levels that correspond to its indigenous habitat. The strain has the capacity to attach to solid surfaces within a biofilm in hydrocarbon-rich environments. The oil well itself will act to confine the micro-organism as oilfields operate using a closed water handling system where oil is removed from the production water exiting the well. Oil extraction from the water is carried out by heat treatment at temperatures *Arcobacter SP*. Strain cannot survive which significantly decreases the possibility of environmental release of the notified organism from the wells. The normal conditions prevailing in the Prairies ecozone, outside of the oil fields, are outside of the conditions required for optimal growth of the notified strain. In the unlikely event of a confinement breach, *Arcobacter SP*. Strain is not expected to survive and persist at high levels in the environment. The potential environmental and human exposure to *Arcobacter SP*. Strain is therefore considered to be low.

RISK ASSESSMENT CONCLUSION / REGULATORY OUTCOME

Arcobacter SP. Strain is a naturally-occurring bacterium that is not considered to be a human, animal, or plant pathogen. No adverse effects were seen in an acute toxicity study

and phylogenetic analyses place the notified strain in a different clade from known pathogenic *Arcobacter* species. The notified micro-organism will be manufactured in Canada in one facility in accordance with large-scale BSL 1 requirements. *Arcobacter* SP. Strain will be introduced into the same ecozone from which it was first isolated where it will be confined in subterranean oil reservoirs. Based on the proposed use, the risk assessments conducted by Environment Canada and Health Canada concluded that *Arcobacter* SP. Strain is not harmful to the Canadian environment or human health and therefore does not meet any of the criteria described in section 64 of CEPA 1999. The substance is not eligible for addition to the Domestic Substances List on the basis of this risk assessment.

REFERENCES

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Levican, A., Alkeskas, A., Gunter, C., Forsythe, S.J., and Figueras, M.J. (2013). Adherence to and invasion of human intestinal cells by *Arcobacter* species and their virulence genotypes. Appl. Environ. Microbiol. 79, 4951-4957.

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