

New substances: Risk assessment, New Substances Notification no. 20598
Schedule 1 to the *New Substances Notification Regulations (Organisms)*

Notified organism	<i>Bacillus amyloliquefaciens</i> subsp. <i>amyloliquefaciens</i> strain P6T48 (hereinafter referred to as P6T48)	
Schedule to the NSNR (Organisms)	Schedule 1 – Information Required in Respect of Micro-organisms	
First day of assessment period	October 29, 2020	
Last day of assessment period	February 25, 2021	
Organism type	Bacterium	
Use	For use in a commercial formulation to maintain grease traps and drains in commercial and community kitchens	
Anticipated quantity	The maximum manufacturing quantity for years 1 and 3 is estimated to be 1.75×10^{15} colony-forming units (CFU) of the notified organism (or 291 666 litres of commercial formulation). There will be no production in year 2. The cumulative quantity for the first 3 years of production is therefore estimated to be 3.5×10^{15} CFUs of the notified organism (or 583 332 litres of commercial formulation). The concentration of the notified organism in the commercial formulation will be 6×10^6 CFU/mL.	
Assessment level of concern	Human health hazard	Low
	Human exposure	Moderate
	Environmental hazard	Moderate
	Environmental exposure	Moderate
Assessment conclusion under section 64 of the <i>Canadian Environmental Protection Act, 1999</i>		Not suspected of being toxic for the notified uses; suspected of being toxic to the environment for certain potential uses
Recommended action	A Significant New Activity Notice for certain potential uses based on environmental concerns.	
Waiver	None	

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Synopsis

P6T48 is a naturally occurring bacterium derived from a soil sample collected in Quebec that was notified to the New Substances program for use in degreasing products to maintain grease traps and drains in commercial and community kitchens.

The risk assessment concluded that the hazard potential to the environment is moderate and the hazard potential to human health is low, and that the exposure potential for the environment and human health from the manufacture of the notified organism is moderate.

It is determined that the notified strain is not toxic according to the criteria under section 64 of the Act for the notified uses. However, there is a possible risk to aquatic and terrestrial invertebrates from certain potential uses. A Significant New Activity Notice is therefore recommended.

Background information

The notified organism is naturally occurring and has not been genetically modified. It belongs to the *Bacillus subtilis* species complex. Strain P6T48 is notable for its ability to rapidly degrade a wide range of edible oils and fats (animal and vegetable) as well as the fatty acids resulting from their hydrolysis.

The organism was notified for use in a commercial formulation to maintain grease traps and drains in commercial and community kitchens. Potential uses include the elimination of contaminants, pollutants and toxins from soil and water, and the biodegradation and cleaning of unwanted organic matter.

The risk assessment is based on the evaluation of information submitted by the notifier and publicly available literature. The following sections give more details on the hazard and exposure.

Hazard

The environmental hazard potential of the notified organism is determined to be moderate because:

- 1) The notified organism has been identified as a strain of *Bacillus amyloliquefaciens* subsp. *amyloliquefaciens*, and some of its characteristics are comparable to those of other known strains of this subspecies.
- 2) The notified organism comes from a soil sample collected from a semi-natural ecosystem in Quebec and has not undergone any genetic modification. Strains of *B. amyloliquefaciens* subsp. *amyloliquefaciens* have been isolated from soil, aquatic environments (river, hot water tank), and droppings from field mice and pigs. Its ability to form spores contributes to its ubiquitous nature.
- 3) The notified organism is highly adaptable to a variety of nutritional and environmental conditions. It produces spores that can be transported over long distances by wind, water and animals.
- 4) Although the *B. amyloliquefaciens* subsp. *amyloliquefaciens* strain is present in soils and aquatic environments and has long been used in industrial settings for enzyme production, no data on its ecological effects could be found in the scientific literature in the public domain. However, adverse effects on terrestrial invertebrates (defoliating caterpillars, aphids, mosquitoes) have been observed in the laboratory with strains of *B. amyloliquefaciens* of an unidentified subspecies.
- 5) In a facility with controlled conditions, tests performed by the notifier suggest that the notified organism has no harmful effect on aquatic plants, aquatic vertebrates, terrestrial plants and terrestrial vertebrates when it is present in the microbial mixture. However, concentrations above 123 CFU/mL (when present in the microbial mixture) in the aquatic environment could significantly inhibit reproduction in local aquatic invertebrates (toxicity) and may propagate (pathogenicity). There is uncertainty associated with the test results, since it is not possible to determine whether the adverse effects observed are attributable to the notified organism, another strain in the microbial mixture or the combination of strains in the mixture. There is also uncertainty as to the mechanism of action generating the harmful effects observed during the tests.

The human health hazard potential of the notified organism is determined to be low because:

- 1) The notified organism is a strain of *B. amyloliquefaciens* subsp. *amyloliquefaciens* that is designated as a Risk Group 1 (RG1) bacterial species by the Public Health Agency of Canada (PHAC) and is considered generally non-pathogenic for humans (PHAC, 2023).
- 2) The notified organism has been identified and characterized by the notifier. It is not part of the *B. cereus* group, which includes human and insect pathogens such as *Bacillus anthracis* and *Bacillus thuringiensis*.
- 3) The notified organism has not been genetically modified to introduce genes that could give it a selective advantage or alter its host range, pathogenicity or toxicity to humans.
- 4) The notifier has submitted antibiotic susceptibility data for the notified organism that shows resistance to bacitracin, ceftazidime, quinupristin and spectinomycin. However, the notified organism is sensitive to several other antibiotics, which could be used as effective treatments in the unlikely event of infection by this strain.
- 5) The notifier submitted data from toxicity tests on terrestrial vertebrates using rats in an attempt to determine the effects of strain P6T48 in a mixture containing three other strains of *Bacillus*. The results showed no observable adverse effects or mortality. However, there is uncertainty associated with the test results, since it is not possible to determine whether the lack of adverse effects observed is attributable to the notified organism or inhibition caused by another strain in the

microbial mixture or the combination of strains in the mixture. Nevertheless, reports from scientific publications on pathogenicity and toxicity tests carried out on other strains of *B. amyloliquefaciens* have not identified any undesirable effects or mortality in the animal models tested.

Exposure

The environmental exposure potential of the notified organism arising from its manufacture and use is determined to be moderate because:

- 1) The notified organism has the ability to form spores (reproductive units) adapted to dispersal and survival, often for long periods under unfavourable conditions. Moreover, it is able produce biofilm, which allows it to attach to surfaces where it will have greater resistance to changing and adverse environmental conditions. Thus, following its release, the notified organism could be dispersed and survive in the environment. However, with very limited data in the scientific literature in the public domain, the assessment cannot predict whether the notified organism may have a competitive advantage over populations of aquatic and terrestrial microorganisms.
- 2) Following its notified and potential use, the notified organism will reach natural aquatic and terrestrial environments via wastewater treatment plants discharging into waterways and by means of landfilling, field spreading and composting of solid waste at low quantities.
- 3) The manufacture of the notified organism will take place under controlled conditions and will not constitute a significant source of exposure in relation to its intended use. In the unlikely event of a scenario in which an entire manufacturing lot of the notified organism is accidentally released into the water treatment plant, the maximum quantity in the purified water from this treatment plant would be greater than that required to have an adverse effect on the reproduction of aquatic and terrestrial invertebrates. However, this type of release would be a one-off, non-recurring event, and the concentration in the environment would not be maintained.
- 4) The notified organism has several potential uses, such as in cleaning products for the reduction of odours and noxious gases from pig slurry, purification of aquaculture water through the adsorption of nitrite ions and displacement of unknown undesirable microorganisms, biological treatment of wastewater, bioremediation of metal-contaminated soils and production of biomolecules (enzymes, surfactants, antibiotics). Some of these uses could significantly increase exposure compared with the notified uses.

The human exposure potential of the notified organism is determined to be moderate because:

- 1) The notified organism will be manufactured under controlled conditions in accordance with containment standards established by PHAC. As a result, exposure of the general population will be reduced to an absolute minimum during the production process.
- 2) The notified organism will be used in a commercial formulation to maintain grease traps and drains in commercial and community kitchens. It also has properties that make it potentially useful for eliminating contaminants, pollutants and toxins from soil, water and other environments and for biodegrading and cleaning unwanted organic matter.

- 3) Environmental concentrations of the notified organism were modelled according to various use scenarios. In all scenarios, the concentration at which humans would be exposed to the notified organism is lower than the concentration at the point of introduction into the environment.
- 4) The manufacture and sale of the notified organism may be restricted because it is the property of the notifier. However, the notified organism will be added to the Domestic Substances List prior to manufacture and may then be used for any purpose and produced in any quantity in Canada.
- 5) The notified organism has the ability to form spores (reproductive units) adapted to dispersal and survival, often for long periods under unfavourable conditions. As a result, exposure may occur long after a product containing the notified organism has been used. However, indirect human exposure to the notified organism through the natural environment will be lower than through the intended uses.

Risk characterization

Risk is generally described as the probability of an adverse effect occurring based on the hazards and a particular exposure scenario.

Environmental risk associated with the notified use

The estimated concentrations of the notified organism that would be released into the environment following its manufacture and use in grease traps would be well below the concentrations necessary to produce adverse effects on aquatic and terrestrial invertebrates. Therefore, the environmental risk associated with the manufacture and notified use of the notified organism in a commercial formulation to maintain grease traps and drains in commercial and community kitchens is assessed as low.

Environmental risk associated with potential uses

The estimated concentrations of the notified organism that would be released into the environment as a result of certain potential uses (water purification in aquaculture, wastewater treatment, reduction of noxious odours and gases from manure intended for field application, bioremediation of water and soil) would be higher than those that produced adverse effects on aquatic and terrestrial invertebrates in the notifier's pathogenicity and toxicity tests. The uncertainty arising from the use of a microbial mixture as a test material makes it impossible to determine whether these effects are attributable to the notified organism, another strain in the microbial mixture or the combination of microbial strains in the mixture. There is also uncertainty as to the mechanism of action generating the harmful effects. Results of multi-concentration tests with the notified strain alone will be needed to better discern the risk for these potential uses. A Significant New Activity Notice is therefore recommended. Due to the moderate hazard potential and moderate exposure potential, the environmental risk associated with certain potential uses of the notified organism is assessed as moderate.

Risk to human health

Despite the low potential hazard to human health and the moderate potential for human exposure, the risk to human health associated with use of the notified organism in a commercial formulation to maintain grease traps and drains in commercial and community kitchens and for other potential uses, such as bioremediation, biodegradation, water and wastewater treatment, drain cleaning and degreasing, and enzyme production, is assessed as low.

Risk assessment conclusion

The notified organism will be manufactured and used to maintain grease traps and drains in commercial and community kitchens. There is no indication that a potential risk of adverse environmental effects could occur for the predicted exposure levels in the environment as a result of the notified use. For this reason, the notified organism is not suspected of meeting the criteria in paragraphs 64(a) or (b) of the *Canadian Environmental Protection Act, 1999* (CEPA). However, there is the possibility of a risk to aquatic and terrestrial invertebrates from certain potential uses. *Order 2021-112-11-01 Amending the Domestic Substances List* was published on January 5, 2022, in the *Canada Gazette*, Part II, Volume 156, Number 1.

There is no evidence to suggest a potential risk of adverse human health effects at the exposure level predicted for the general population for the notified uses. The risk to human health associated with the notified organism is not suspected to meet the criteria in paragraph 64(c) of CEPA. No further action is recommended.

References

[PHAC] Public Health Agency of Canada. (2023). ePATHogen – Risk Group Database. Available at <https://health.canada.ca/en/epathogen> [2023-01-18]