Risk Assessment Summary Conducted Pursuant to the *New Substances Notification Regulations (Organisms)* of the *Canadian Environmental Protection Act, 1999*
EAU-439 : *Lactococcus lactis* subsp. *cremoris* strain sAGX0037

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 *Lactococcus lactis* subsp. *cremoris* strain sAGX0037 by ActoGenix that is intended to be used during a clinical trial of a therapeutic drug against inflammatory bowel disease. However, under the above regulations, this intended use also allows for this strain to be introduced into the environment anywhere in Canada.

Lactococcus lactis subsp. *cremoris* strain sAGX0037 was notified pursuant to subsection 3(1) of the NSNR (O)].

The New Substances Assessment and Control Bureau of Health Canada has assessed the information submitted by ActoGenix and other available scientific information in order to determine if *L. lactis* subsp. *cremoris* strain sAGX0037 meets the criteria set out in section 64 of CEPA 1999[1].

Regulatory Decision

Based on the hazard and exposure considerations, the risk assessment conducted by Health Canada concluded that *L. lactis* subsp. *cremoris* strain sAGX0037 does not cause harm to the Canadian environment or human health as described in section 64 of the CEPA 1999. Therefore, the import of *L. lactis* subsp. *cremoris* strain sAGX0037 for introduction anywhere in Canada may proceed after October 6, 2008.

This evaluation does not include an assessment of human health risk in the occupational environment nor does it include an assessment for the substance which is already prescribed under the purview of the *Food and Drugs Act*.

NSNR(O) Schedule: 1 (manufacture or import of micro-organisms for introduction anywhere in Canada)
Organism Identity: *Lactococcus lactis* subsp. *cremoris* strain sAGX0037
Notifier: ActoGenix N.V., Technologiepark 5, 9052 Zwijnaarde, Belgium
Date of decision: October 6, 2008
Proposed use: Vehicle for the intestinal delivery of human interleukin-10 (hIL-10) during phase 2 clinical trial of a therapeutic drug against inflammatory bowel disease.

[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.

STRAIN HISTORY/GENETIC MODIFICATION

The identification of *Lactococcus lactis* subsp. *cremoris* strain sAGX0037 was based on morphological characteristics, 16S rRNA analysis and full genome sequencing.

Lactococcus lactis subsp. *cremoris* sAGX0037 was developed using homologous recombination to replace the thymidylate synthase gene *(thyA*) gene of the parental strain *L. lactis* subsp. *cremoris* strain MG1363with a synthetic expression cassette that encodes for a human interleukin-10 (hIL-10). The genetic modifications resulted in the extracellular expression of the hIL-10 protein and the organism's dependence on thymine or thymidine supplementation to survive.

Transformed cells were isolated based on their ability to grow on selective media containing a thymidine supplement. To ensure proper integration of the hIL-10 expression cassette into the *L. lactis* subsp. *cremoris* sAGX0037 genome and deletion of the *thyA* gene, various PCR amplifications and sequence analyses were performed. In addition, the presence of hIL-10 was confirmed by using standard Western blot and ELISA assays.

Genetic stability of the integrated hIL-10 expression cassette was demonstrated for over 55 generations of *L. lactis* subsp. *cremoris* sAGX0037. The absence of growth on thymine-deficient media, the secretion of hIL-10, and the DNA analysis of the *thyA* locus corroborate genetic stability is maintained over generations.

After a thorough analysis of the integrated DNA and the well characterized new phenotype, it was determined that the modifications to the micro-organism do not give rise to concerns of altered virulence.

HAZARD CONSIDERATIONS

Environmental Hazard

Information provided by the notifier and an updated search of the scientific literature indicated that there is little evidence of any pathogenic potential of *L. lactis* and hIL-10 protein in aquatic plants, fish or marine mammals. Pre-clinical studies on *L. lactis* subsp. *cremoris* sAGX0037 in mice and *Cynomolgus* monkeys showed no toxic effects after repeated exposures. The reports of infections to wildlife species due to *L. lactis* appear to be rare. Consequently, the notifier's request for waiver on the pathogenicity data requirements for aquatic plants, invertebrates and vertebrates and for terrestrial plants and invertebrates was granted.

Since strain sAGX0037 is deficient of the Tn916 and Tn919 enterococcal conjugative transposons which encode for resistance to tetracycline and represent an important mode of horizontal gene transfer to a broad range of microbial recipients (Alpert *et al.,* 2003; Bringel *et al.,* 1992), the notified strain is not expected to undergo genetic transfer in the

environment. Also, *L. lactis* is not known to competently take up exogenous DNA from the environment. The ability of the notified strain to spontaneously acquire foreign *thyA* genes from an exogenous source through homologous recombination is expected to be significantly low as demonstrated by Steidler *et al.* (2003).

The removal of the thymidylate synthase function generates a dependence of the notified strain on thymine or thymidine supplementation as thymine starvation leads to rapid cell death. This was confirmed by the significant decrease in viability of sAGX0037 within 36 hours in thymine-free growth medium. No viable cells were observed after a prolonged incubation period of 120 hours at 30°C.

Since there is no selective advantage in the environment for *L. lactis* subsp. *cremoris* sAGX0037, the potential for the organism or its genetic materials to cause adverse effects on the environment and on the conservation and on biological diversity is, therefore, considered low.

Human Health Hazard

L. lactis is not considered a human pathogen given its history of safe use in the food industry. *L. lactis* infections generally occur in patients with co-morbidities and are often associated with the consumption of unpasteurized dairy products. Overall, reported cases are scarce and infectivity is not severe in patients with underlying conditions. There are no reported cases of allergic reaction linked to any strain of *L. lactis*.

IL-10 is known to initiate a wide range of activities for the regulation of the immune system. The notifier provided evidence from the human clinical trials on patients with Crohn's disease and healthy volunteers to demonstrate the therapeutic effects and safety of the *L. lactis* derived hIL-10.

The notifier submitted data on antibiotic testing for *L. lactis* subsp. *cremoris* sAGX0037 which showed that it is resistant to nalidixic acid, sulfamethoxazole, and metronidazole, but sensitive to the following antibiotics: penicillin G, ampicillin, amoxicillin + clavulanic acid, tetracycline, erythromycin, vancomycin, gentamicin, chloramphenicol, bacitracin, levofloxacin, cefepime, imipenem, and linezolid. Therefore, in the unlikely event of *L. lactis* sAGX0037 infection to humans, effective antibiotic treatments are currently available.

Since *L. lactis* has a safe history of use and is usually considered as non pathogenic to the general population and that the recombinant hIL-10 has been shown to be safe for humans, the use of *L. lactis* subsp. *cremoris* sAGX0037 is not expected to cause adverse effects to the general population. Its potential hazard to human health is considered low.

EXPOSURE CONSIDERATIONS

The notified micro-organism will be imported to Canada from Belgium and Germany as a vehicle for the intestinal delivery of hIL-10 during Phase 2 clinical trial of a therapeutic

drug against inflammatory bowel disease. The drug will contain a minimum viable *L. lactis* subsp. *cremoris* sAGX0037 concentration of 9.0×10^9 cfu/g formulation.

The drug containing sAGX0037 will be distributed by health care professionals where containment procedures are in place to minimize worker, bystander and wildlife exposure. All waste generated during the clinical trial will be discarded in approved biological waste containers and disposed according to provincial regulations. Contingency plans, including the use of detergent or sodium hypochlorite to decontaminate spills, exist in case of accidental release during transport and administration. If the clinical trial needs to be terminated, unused product containing sAGX0037 will be appropriately destroyed using guidelines for medical waste treatment.

The release of the notified strain into the environment will occur mainly due to faecal shedding. Braat *et al.* (2006) reported that 10^4 viable sAGX0037 cells/g stool were present two days after the treatment period of patients with Crohn's disease who received a daily dose of 2.0×10^{10} cfu for seven days. It is expected that any *L. lactis* subsp. *cremoris* sAGX0037 entering the sanitary sewer system will be inactivated and/or removed by the physical, biological, and/or chemical treatments in place in wastewater treatment plants.

Given the metabolic limitations of *L. lactis* subsp. *cremoris* sAGX0037, as well as the existence of effective safety measures and waste management practices to prevent or minimize its release into the environment, the potential environmental and human exposure to *L. lactis* subsp. *cremoris* sAGX0037 (apart from humans exposed as part of the clinical trial) is therefore considered to be significantly low.

RISK CHARACTERIZATION

Based on the hazard and exposure considerations, the risk assessment conducted by Health Canada concluded that *L. lactis* subsp. *cremoris* strain does not cause harm to the Canadian environment or human health as described in section 64 of the CEPA 1999.

REFERENCES

Alpert CA, Mater DD, Muller MC, Ouriet MF, Duval-Iflah Y, and Corthier G. (2003). Worst-case scenarios for horizontal gene transfer from *Lactococcus lactis* carrying heterologous genes to *Enterococcus faecalis* in the digestive tract of gnotobiotic mice. Environ Biosafety Res. 2(3):173-180.

Braat H, Rottiers P, Hommes DW, Huyghebaert N, Remaut E, Remon JP, van Deventer SJ, Neirynck S, Peppelenbosch MP, and Steidler L. (2006). A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease. Clin Gastroenterol Hepatol. 4(6):754-759.

Bringel F, Van Alstine GL, and Scott JR. (1992). A host factor absent from *Lactococcus lactis subspecies lactis* MG1363 is required for conjugative transposition. Mol Microbiol. 5:2983-2993.

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