Risk Assessment Summary Conducted Pursuant to the
***New Substances Notification Regulations (Organisms)* of the**
Canadian Environmental Protection Act, 1999
EAU 760: *Influenza virus* cold-adapted B/Massachusetts/2/2012

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 Health and the Minister of Environment 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 1999[1], when imported for introduction into the environment anywhere in Canada. Therefore, the importation of *Influenza virus* cold-adapted B/Massachusetts/2/2012 for this purpose may proceed after October 12, 2013.

NSNR (O) Schedule: 1
Organism Identity: *Influenza virus* cold-adapted B/Massachusetts/2/2012
Notifier: AstraZeneca Canada Inc, Mississauga, Ontario
Date of decision: October 12, 2013.
Proposed use(s): Preparation of FluMist® vaccine (live, attenuated) for 2013-2014 seasonal flu immunization

IDENTITY / STRAIN HISTORY / GENETIC MODIFICATION:

The notified micro-organism is a live, genetically modified, cold-adapted and temperature-sensitive influenza virus reassortant of the wild-type B/Massachusetts/2/2012, which has been derived from the seasonal influenza B. The strain is a component of the 2013-2014 FluMist® flu vaccine.

There are three types of influenza virus, A, B and C, and these are distinguished by differences between their nucleocapsid and matrix M proteins. Influenza B virus is divided into two antigenically different lineages B/Victoria-like and B/Yamagata-like based on the conformation of the hemagglutinin (HA) protein. The HA and neuraminidase (NA) proteins are implicated in the attachment of the virus to specific cell receptors and are the target of neutralizing antibodies which confer protection against influenza infection (Lamb and Krug, 2001). The notifier characterized the antigenic specificity of the HA protein, determined the virus titre, and sequenced the notified strain to confirm that the genome segments are nearly identical to those of its donor organisms.

[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.

The genetic modifications consist of rearranging six gene segments from an attenuated master donor virus with two surface proteins from the wild-type virus. The end result of the 6:2 reassortment is an attenuated vaccine that elicits protective immune responses in humans against the virulent influenza strains circulating at the time of notification. The data provided by the notifier showed genetic phenotypic and genetic stability in the modifications.

HAZARD CONSIDERATIONS:

Environmental Hazard

Pre-clinical studies using previous FluMist® vaccine strains with a dose of 10^7 FFU (same dose used in human vaccination) have shown no adverse effects in various animals such as: mouse, rabbit, dog, cat, horse, sheep, goat, cattle, pig, canary, quail, pigeon, etc. Further studies on ocular irritancy and reproductive effects have showed no irritation/ocular damage at any point during or after treatment and no adverse developmental effects resulted from exposure of the embryos and fetuses to the active vaccine. Although a different influenza B vaccine strain was used in these studies, it is considered to be an acceptable surrogate for the *ca* B/Massachusetts strain because the genes implicated in the viral replication and attenuation are the same in both strains. Information provided by the notifier and an updated search of the scientific literature showed no evidence of pathogenic potential of *ca* B/Massachusetts or wild-type influenza B in aquatic and terrestrial plants, vertebrates and invertebrates and this was considered adequate for these organisms considering the characteristics of the *ca* B/Massachusetts strain. As such, waivers for the requirement to test pathogenicity and toxicity of the notified strain on aquatic plants, invertebrates and vertebrates as well as terrestrial plants and invertebrates were accepted.

Based on the results described above from pre-clinical studies, its limited host range and specific tissue tropism, the notified strain is unlikely to cause adverse effects in non-human species. The potential for the notified micro-organism to cause adverse effects on the environment, or its biological diversity was, therefore, considered low**.**

Human Health Hazard

An important factor in the pathogenesis of influenza viruses is tissue tropism. Tissue tropism is mainly determined by the ability of the virus to attach to specific receptors of the host cell. The binding specificity of the HA protein differs between virus. Human influenza viruses bind specifically to human tracheal epithelial tissue containing the SA-αa2,6 motif and as such have a limited host range (Suzuki, 2005).

Since the 1970's, most influenza B outbreaks have been linked to either B/Yamagata-like or B/Victoria-like strains (Rota et al., 1990). Symptoms usually associated with influenza infection are headache, cough, chills, fever, myalgia, malaise and anorexia. Nevertheless, infection is usually self-resolving and symptoms are usually gone by the sixth day (Wright and Webster, 2001).

Given that the notified strain contains the same viral segments as previous FluMist® vaccine formulations, it is expected that the *ca* B/Massachusetts strain will have an equivalent safety profile. Various quadrivalent and trivalent formulations of the FluMist® vaccine (containing the same master donor viruses and similar attenuated subtypes) have been administered to over 30,000 subjects in controlled clinical studies over multiple years and in various countries and the vaccine has demonstrated an acceptable safety and tolerability profile. Results from a clinical trial in 2011, which enrolled 1,800 adults 18-49 years of age, have shown that the most common solicited symptom during 0-14 days after the vaccination was runny/stuffy nose with rare, unfavorable and unintended signs such as oropharyngeal pain, upper respiratory tract infection, and headache. Nevertheless, the safety profile of FluMist® has been consistent across multiple years regardless of the strains included in the vaccine.

The safety of similar FluMist® vaccine formulations has also been investigated in immunocompromised individuals. No serious adverse events were attributed to the vaccine during a randomized, double-blind, placebo controlled trial with HIV-infected and HIV-negative adults 18-58 years of age.

A waiver for antibiotic susceptibility has also been granted since antibiotics are not effective against viruses. Furthermore, since the notified strain is attenuated, any accidental exposure to this strain is not expected to cause severe infection requiring treatment. Antiviral agents, such as Oseltamivir and Zanamivir, could be used to treat upper respiratory tract symptoms (PHAC, 2013).

As such, the use of the notified micro-organism is not expected to cause adverse effects to the general population. Its potential hazard to human health was considered low.

EXPOSURE CONSIDERATIONS

The notified strain *ca* B/Massachusetts will be imported from USA as part of the FluMist® vaccine formulation by AstraZeneca Canada Inc. The vaccines will then be shipped as FluMist® to flu vaccination clinics and pharmacies across Canada where they will be administered to human patients. Approximately 500,000 doses containing a total of approximately 5×10^{13} FFU of the notified strain will be imported in Canada during the 2013-2014 flu seasons.

Potential routes of introduction of *ca* B/Massachusetts into the environment which could result in indirect human exposure are through nasal shedding from immunized patients and through the disposal of unused portions of the vaccine.

Respiratory shedding is usually considered to be the most important transmission mode for influenza viruses. Like other respiratory viruses, *ca* B/Massachusetts may be transmitted either by large droplets (> 100 μm mass median diameter) or by respirable airborne droplets (<5 μm mass median diameter). Larger droplets settle quickly and are more likely to contaminate surfaces. Small droplets remain suspended in air, travel further and are more likely to cause infection in the lower respiratory tract. Transmission via hand contact with contaminated

surfaces is also a possibility; however, this is limited due to the rapid inactivation of the influenza virus on inanimate objects.

Water is unlikely to be a source of transmission of the notified vaccine strain since drinking water treatment plants are expected to inactivate the influenza viruses. Given that the typical levels of free chlorine in drinking water systems in Canada range from 0.04 to 2.0 mg/L (higher than the levels required to kill typical influenza viruses), the notified strain is unlikely to survive the water treatment process (Health Canada, 2009).

Unused portions of the vaccine are disposed of as biological waste at the respective flu vaccination clinics or pharmacies, or steam sterilized for 30 minutes at 121^{o}C. Further procedures are in place to prevent inadvertent release of the notified vaccine strain during transport and vaccination sites.

Taking all these factors into consideration, the indirect human health exposure to the notified organism was considered to be low.

While not established for influenza B viruses, environmental factors, including relative humidity (RH), temperature, and ultraviolet (UV) radiation have been reported to affect the survival of influenza A viruses outside their hosts. According to Harper G.J. (1961), both RH and temperature have strong effects on the inactivation rates of airborne influenza A viruses. Lowen et al. (2007) showed that airborne transmission of influenza A virus was enhanced at low temperatures (5°C) and that high temperatures (30°C) interrupted airborne transmission at all RH values. UV radiation has also shown to efficiently inactivate influenza A viruses (Jensen, 1964).

Human to animal transmissions are also a possibility especially during routine animal husbandry practices, where there is close contact between people and livestock. As such, the same mechanism implicated in person-to-person transmission could also take place between humans and animals. The risk of this is considered minimal as the strain is temperature sensitive and does not replicate well in animals used in animal husbandry. While there is a possibility of human-to-animal transmission of *ca* B/Massachusetts, such event is not expected to contribute to the global influenza B infectious load due to the attenuated phenotype of the notified strain. In case of inadvertent release, *ca* B/Massachusetts cannot persist in the environment outside a host cell.

Taking all these factors into consideration, the environmental exposure to the notified strain was considered to be low.

RISK ASSESSMENT CONCLUSION / REGULATORY OUTCOME

Based on the hazard and exposure considerations described above, the risk assessment conducted by Health Canada concluded that the notified micro-organism, *Influenza virus* cold-adapted B/Massachusetts/2/2012 is not expected to cause harm to the Canadian environment or human health as described in section 64 of the CEPA 1999.

The substance is eligible for addition to the Domestic Substances List on the basis of this risk assessment.

REFERENCES

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

Harper, G.J. (1961). Airborne micro-organisms: survival tests with four viruses. J Hyg (Lond) *59*, 479-486.

Health Canada. (2009). Guidelines for Canadian Drinking Water Quality - Chlorine Guideline Technical Document. ISBN: 978-1-100-13416-1. http://www.hc-sc.gc.ca/ewh-semt/alt_formats/hecs-sesc/pdf/pubs/water-eau/chlorine-chlore/tech_doc_chlor-eng.pdf (Viewed June 2013).

Jensen, M.M. (1964). Inactivation of airborne viruses by ultaviolet irradiation. Appl. Microbiol. *12*, 418-420.

Lamb, R.A., and Krug, R.M. (2001). Orthomyxoviridae: the viruses and their replication. In Fields Virology. 4th ed. Knipe, D. M., and Howley, P. M. eds., (Philadelphia: Lippincott Williams and Wilkins) pp. 1487-1531.

Lowen, A.C., Mubareka, S., Steel, J., and Palese, P. (2007). Influenza virus transmission is dependent on relative humidity and temperature. PLoS Pathog. *3*, 1470-1476.

PHAC. (2013). Public Health Agency of Canada FluWatch Report: April 28 to May 4, 2013. http://www.phac-aspc.gc.ca/fluwatch/12-13/w18_13/index-eng.php (Viewed July 2013).

Rota, P.A., Wallis, T.R., Harmon, M.W., Rota, J.S., Kendal, A.P., and Nerome, K. (1990). Cocirculation of two distinct evolutionary lineages of influenza type B virus since 1983. Virol. *175*, 59–68.

Suzuki, Y. (2005). Sialobiology of influenza molecular mechanism of host range variation of influenza viruses. Biol. Pharm. Bull. *28*, 399-408.

Wright, P.F., and Webster, R.G. (2001). Orthomyxoviruses. In Fields Virology. 4th ed. Volume 1, Knipe, D. M., and Howley, P. M. eds., (Philadelphia: Lippincott Williams and Wilkins) pp. 1533-1580.