Risk Assessment Summary Conducted Pursuant to the New Substances Notification Regulations (Organisms) (NSNR[O]) of the *Canadian Environmental Protection Act, 1999*
EAU-308, 309, 310, 311, 312: *Rotavirus strains* W179-9 (G1), SC2-9 (G2), 178-9 (G3), BrB-9 (G4), 179-4 (P1)

This document has been prepared to explain the regulatory decisions taken under Part 6 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999) regarding the import of five *Rotavirus strains* by Merck Frosst Canada Ltd. for introduction anywhere in Canada. All five *Rotavirus strains* were notified pursuant to subsection 3(1) of the CEPA 1999 New Substances Notification Regulations (Organisms).
Although present in the product formulation as a mixture, each rotavirus strain within the mixture was considered a substance as defined in Section 3 of CEPA 1999, and was therefore assessed individually. However, due to their similarities this document will discuss all five *Rotavirus strains W179-9 (G1), SC2-9 (G2), 178-9 (G3), BrB-9 (G4), 179-4 (P1)* while noting any particular differences.

The New Substances Assessment and Control Bureau of Health Canada has assessed information submitted by Merck Frosst Canada Ltd. and other available scientific information in order to determine whether the five *Rotavirus strains* are *toxic*[1] or capable of becoming *toxic* as defined by section 64 of CEPA 1999.

Regulatory Decision:

Based on the hazard and exposure considerations, the risk assessments conducted by Health Canada concluded that the *Rotavirus strains W179-9 (G1), SC2-9 (G2), 178-9 (G3), BrB-9 (G4), 179-4 (P1)* are not considered to be *toxic* to the Canadian environment or human health as described in section 64 of the CEPA 1999. Therefore, import of these *Rotavirus strains* for introduction anywhere in Canada may proceed after April 19, 2006.

The evaluations do not include an assessment of human health risk in the occupational environment nor do they include an assessment of the potential exposure and risk to humans associated with the use of the organisms in or as an item that falls under the purview of the *Food and Drugs Act*.

NSNR(O) Schedule: 1 (import of micro-organism for introduction anywhere in Canada).
Organism Identity:*Rotavirus strains*: W179-9 (G1), SC2-9 (G2), W178-9 (G3), BrB-9 (G4), W179-4 (P1)
Notifier: Merck Frosst Canada Ltd., 16711 TransCanada Highway, Kirkland, Quebec, H9H 3L1
Date of decision: April 19, 2006
Proposed use: Components of a live attenuated oral vaccine (mixture) for the prevention of rotavirus gastroenteritis in infants and children

[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 Re-assortment:

The five notified microorganisms of Merck Frosst Canada Ltd., *Rotavirus strains W179-9 (G1), SC2-9 (G2), 178-9 (G3), BrB-9 (G4), 179-4 (P1)*, are used to formulate a pentavalent live-attenuated oral vaccine known as RotaTeq® as shown in Table 1. The rotavirus parent strains of the reassortants were isolated from human and bovine hosts. Four reassortant rotaviruses express one of the outer capsid proteins (G1, G2, G3, or G4) from the human rotavirus parent strain and the attachment protein (serotype P7) from the bovine rotavirus parent strain. The fifth reassortant virus expresses the outer capsid protein of serotype G6 of the bovine rotavirus parent strain and the attachment protein, P1A[8], from the human rotavirus parent strain. The reassortants are propagated in Vero cells using standard cell culture techniques in the absence of antifungal agents.

Table 1: Rotavirus strains used to generate RotaTeq® Vaccine

Name of Reassortant	**Human Rotavirus Parent Strains and Outer Surface Protein Compositions**	**Bovine Rotavirus Parent Strain and Outer Surface Protein Composition**	**Reassortant Outer Surface Protein Composition (Human Rotavirus Component in Bold)**
G1	WI79 – G1, P1A[8]	WC3 - G6, P7[5]	**G1**, P7[5]
G2	SC2 – G2, P2A[6]		**G2**, P7[5]
G3	WI78 – G3, P1A[8]		**G3**, P7[5]
G4	BrB – G4, P2A[6]		**G4**, P7[5]
P1	WI79 – G1, P1A[8]		G6, **P1A[8]**

The attenuation of the notified strains was achieved through standard microbiological laboratory methods with specific mammalian cell cultures. The genetic composition of each progeny clone of the respective reassortant strain was compared to the parental strains using polyacrylamide gel electropherotyping and nucleotide sequencing.

The results of sequence analysis for some of the notified strains showed only a one nucleotide difference between the nucleotide sequences of the respective strain to the sequenced reference parent rotavirus strains resulting in each case in one amino acid substitution. However, the protein profiles from all of the notified strains indicate that the same viral proteins are present between three process validation and two production lot samples. This indicates that the potential for expression of unpredicted novel traits or for introduction of uncharacterized genetic materials appears to be significantly low.

Hazard Considerations:

Genetic Re-assortments

The notified strains have the possibility to reassort with a wild-type animal or human virulent strain, which could give rise to a variety of new genotypes or reversion to the wild-type virulent strains. However, only a few human rotavirus strains have high homology to all 11 genes of animal rotaviruses, suggesting that interspecies reassortment resulting in animal gastroenteritis are rare [1]. Thus, the likelihood is considered low that the excreted notified strains would undergo reassortment that result in strains causing adverse effects in non-human species.

Environmental Hazard

Despite their ubiquity in nature, there have been no reports in the literature indicating that either human or bovine rotaviruses are pathogenic to terrestrial and aquatic plants and other aquatic species. Only a few studies have associated rotavirus with pathogenicity to terrestrial invertebrates. Under laboratory conditions, a rotavirus-like particle was found to be pathogenic to *Microplitis croceipes,* a parasitic wasp species native to Georgia, US [2-3]. However, rotavirus infection is the leading cause of severe, life-threatening viral gastroenteritis in terrestrial mammals and avian species. Considerable scientific evidence demonstrates the nature and degree of pathogenicity, virulence, and infectivity of rotavirus to animals and birds, including minks, racoons, skunks, calves, foals, pigs, dogs, antelopes, addax, impala, deer, gazelle, sheep, rabbits, cats, chickens, turkeys, and quail [4-12].

To substantiate the safety of the vaccine on terrestrial vertebrates, the notifier conducted a 10-week single-dose and repeated-dose oral toxicity study of the vaccine in mice. Results show that oral administration as a single dose or as a 3-dose regimen was well tolerated. There were no treatment-related effects on mortality, physical signs, body weight, food consumption, haematological or biochemical parameters.

The notified strains are unlikely to invade non-human species since they each respectively contain human host-specific proteins. In the event that it is transmitted to non-human host cells, each of the notified strains has a reduced ability to replicate and cause infection, since they are attenuated. Thus, the likelihood is considered low that the excreted notified strains would undergo reassortment that causes adverse effects in non-human species.

Human Health Hazard

Naturally occurring human rotavirus strains are recognized as Risk Group 2 pathogens by the Public Health Agency of Canada. Although several different groups and serotypes of rotaviruses may cause disease, the majority of human pathogenic strains belong to G serotypes 1 to 4. Group A rotaviruses are the most important from a public health standpoint and are the leading cause of viral gastroenteritis among premature infants, children 6 months to 2 years of age, the elderly and individuals with compromised immunity. It is estimated that rotaviruses cause 25% of all mortality due to diarrheal diseases, and 6% of deaths among children that are under 5 years of age [13]. The clinical presentation of the illness is variable, but in general it is self-limited, has an explosive onset, and is manifested by varying combinations of diarrhea, nausea, vomiting, and low-grade fever [14].

Diarrhea caused by rotaviruses involves two main mechanisms: malabsorption and secretion. The malabsorptive component of rotavirus diarrhea appears to be related to the primary infection with the virus. The process leading to diarrhea is triggered by the disruption of Ca^{+}

homeostasis. The intestinal secretion is stimulated by the activation of the enteric nervous system and the action of NSP 4 viral protein. The re-assortment process used to derive the notified vaccine strains results in the disruption of either the mechanism of entry or attachment, thereby preventing the cascade of events leading to gastroenteritis in humans.

The RotaTeq® vaccine is described by the notifier, as having the ability to provide protection in humans against rotavirus gastroenteritis by stimulating an anti-viral immune response similar to the immune response triggered by natural rotavirus infection. The vaccine was granted approval from the US Food and Drug Administration in February 2006 [15]. Phase III clinical findings from more than 70,000 vaccinated infants show that the vaccine was efficacious against moderate to severe rotavirus gastroenteritis with no reported adverse immunological reactions.

There have been reported cases of reversion to a virulent phenotype from traditional live attenuated vaccines, particularly the oral polio vaccine [16-18]. However due to the notified strains being derived from both parental bovine and human rotavirus strains, reversion would necessitate multiple cell culture passages in order to acquire the original genetic composition of the pathogenic parental strains and is thus unlikely.

Horizontal transmission of the notified strains were not investigated by the notifier since the reassortant strains are shed in a small proportion of the vaccinated infants, and the sample size required to conduct a meaningful evaluation of transmission would not be feasible to study.

Exposure Considerations:

The notified micro-organisms will be imported as the finished vaccine formulation RotaTeq® from Merck & Co. Inc. in the United States.

The potential of exposure to residual DNA from the Vero cell lines or other adventitious contaminants in the final product is negligible since these will be extensively monitored during vaccine validation and certification. The notifier described procedures that will limit potential worker exposure in case of accidental releases from spills during the storage, handling and transport of the vaccine. These include the use of protective equipment and treatment with sodium hypochlorite.

To minimize the potential environmental and human exposure to the notified strains, procedures are in place for the treatment and disposal of wastes containing the notified organisms. All unused vaccine and waste generated through the normal vaccination process will be discarded in approved biological waste containers and disposed according to local regulations. All recalled, unused, and expired products will be returned to Merck Frosst in their original packaging and treated as pharmaceutical wastes. Accidental release from a single dose at the health care facilities should be decontaminated using either heat or autoclave at 120°C for 45 minutes or 95% ethanol or phenolics such as Vesphene and LpH to rapidly and effectively inactivate the rotaviruses.

Environmental release of the notified strains would most likely occur through accidental faecal discharge to recreational water bodies such as lakes, rivers, and swimming pools from vaccinated infants and children or the disposal of human faeces in landfill sites. Since landfills are often not contained, vertebrate and invertebrate species may come in direct contact with faeces in landfills that are exposed to the open air.

It is expected that some aquatic and terrestrial plants, invertebrates and vertebrates could also be potentially exposed to the reassortant rotavirus strains through the irrigation of agricultural land with wastewater or fertilized with sewage, and untreated surface or ground water. This may

include the common and economically important species such as livestock animals (cattle, poultry, etc.) as well as fish and agricultural crops.

Persistence and Dispersal

Naturally occurring rotaviruses are widely distributed in the environment and have the ability to persist in many ecological niches, including aquatic and estuarine ecosystems, groundwater, soil, and sediments. Human and animal rotaviruses enter the water environment primarily by way of sewage discharges. Rotaviruses in wastewater effluents are discharged into aquatic environments where they can accumulate and persist in the sediments longer than in the water column [19].

Rotaviruses have been shown to be most stable at 4°C, less stable at 20°C, and least stable at 37°C [20]. They have been demonstrated to be stable in drinking water for up to 64 days at 4°C [21]. In protected groundwater with temperatures generally below 10°C, rotaviruses have been reported to survive for nearly 2 years. At pH levels of 5 to 9, viruses can persist for considerable periods of time that may range from hours to months [22].

Given that RotaTeq® has low shedding rates, the likelihood of transmission of the excreted vaccine strains to non-target species is minimal. Several key factors including the number of organisms shed, rotavirus host specificity, and various environmental factors such as temperature, pH, light, soil composition, moisture content, organic matter, particulates, salt concentration, antiviral chemicals and microbial activity are expected to limit the dispersal and persistence of the attenuated vaccine strains in the environment. Compared to the naturally occurring rotaviruses, it appears unlikely that the notified strains will survive for a sufficiently long period for it to cause harm, and possibly establish and disseminate in the environment.

Rotaviruses are transmitted predominantly by the faecal-oral route. Individuals infected with naturally occurring Rotaviruses can excrete between 10^{10} to 10^{12} infectious units per gram of faeces. RotaTeq® was found to have low shedding rates of 8.9%, 0% and 0.3% after doses 1, 2 and 3 respectively, during the pre-licensure Rotavirus Efficacy and Safety Trial. It is expected that some rotaviruses can be inactivated by physical, biological and chemical treatments in wastewater plants. However, assuming that there is no inactivation of the notified strains, the levels of attenuated virus in the environment are not expected to be higher than the levels of naturally occurring rotaviruses based on the low shedding rates observed. Thus, the potential environmental and indirect human exposure to the notified strains through the faecal-oral route is expected to be significantly less, compared to the exposure to virulent rotaviruses in the environment.

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