

**Risk Assessment Summary Conducted Pursuant to the New
Substances Notification Regulations (Organisms) of the
Canadian Environmental Protection Act, 1999
EAU-425: Rotavirus strain RIX4414**

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 import of *Rotavirus* strain RIX4414 by GlaxoSmithKline Inc. that is intended to be used as a component of a live attenuated oral vaccine for the prevention of rotavirus gastroenteritis in infants. However, under the above regulations, this intended use also allows for this strain to be introduced anywhere in Canada.

Rotavirus strain RIX4414 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 GlaxoSmithKline Inc. and other available scientific information in order to determine if *Rotavirus* strain RIX4414 meets the criteria set out in section 64 of CEPA 1999¹.

Regulatory Decision

Based on the hazard and exposure considerations, the risk assessment conducted by Health Canada concluded that the *Rotavirus* strain RIX4414 does not cause harm to the Canadian environment or human health as described in section 64 of the CEPA 1999. Therefore, the import of *Rotavirus* strain RIX4414 for introduction anywhere in Canada may proceed after August 4, 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 (import of a micro-organism that will be introduced anywhere in Canada)

Organism Identity: *Rotavirus* strain RIX4414

Notifier: GlaxoSmithKline Inc., 7333 Mississauga Rd., Mississauga, ON, L5N 6L4

Date of decision: August 4, 2008

Proposed use: Component of the live attenuated oral vaccine Rotarix™ used for the prevention of rotavirus gastroenteritis in infants 6-24 weeks old.

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

Rotavirus is a genus within the family *Reoviridae*. There are seven species of rotavirus, referred to as groups A, B, C, D, E, F and G. Each is classified into G and P-types, which are determined by the two outer layer viral proteins, VP7 and VP4, respectively. Globally, Group A rotaviruses carrying genotypes G1 to G4 and [P4] or [P8] have consistently been found to be the most common cause of rotavirus disease in humans, with G1[P8] as the most prevalent and ubiquitous (Santos & Hoshino, 2005; Gentsch *et al.*, 1996).

Rotavirus strain RIX4414 originates from a human G1P[8] rotavirus strain isolated from the feces of an infant with a symptomatic rotavirus infection in 1988. It was initially cultured in primary African Green Monkey kidney cells for 26 passages (P26) at J. Gamble Institute of Medical Research in Cincinnati, USA. P26 was then transferred to Dyn Corporation where it was further attenuated to develop passage 33 (P33) referred to as strain 89-12. AVANT Therapeutics, Inc. initiated the development of strain 89-12 as a vaccine through pre-clinical and clinical trials. Attenuated *Rotavirus* strain RIX4414 was developed from the vaccine candidate strain 89-12.

The G1[P8] identity of the notified strain RIX4414 was confirmed by RT-PCR using primers specific to different G and P genotypes (Gouvea *et al.*, 1990; Gentsch *et al.*, 1992), and by sequencing the VP4 and VP7 genes.

HAZARD CONSIDERATIONS

Environmental Hazard

Information was found to demonstrate that some rotaviruses, belonging to other rotavirus groups and possessing different genotypes, are capable of infecting terrestrial vertebrates, including, minks, racoons, skunks, calves, horses, dogs, antelopes, addax, impala, deer, gazelle, sheep, rabbits, cats, chickens, turkeys, and quail (Bohl *et al.*, 1978; Jarplid & Mejerland, 1998; Evans, 1984; Browning & Begg, 1996; Baumeister *et al.*, 1983; Eugster *et al.*, 1978; Thouless *et al.*, 1988; Yason & Schat, 1987). Although several genotypes, primarily G3, G6, and G8, are shared between humans and animals (Desselberger *et al.*, 2001), only a few G1 genotype strains have been detected in animals (Blackhall *et al.*, 1992; Ciarlet & Liprandi, 1994) and these are not commonly diarrhea-inducing pathogens in animals (Steyer *et al.*, 2006). Pre-clinical studies in 5- and 21-day old Fischer F344 rats inoculated orally with four doses of 1.0×10^6 CCID₅₀ rotavirus vaccine (G1[P8] specificity) at two-week intervals showed no toxic effects. The notifier's request for waiver on the pathogenicity data requirements for aquatic and terrestrial plants, invertebrates and vertebrates was granted based on evidence from the scientific literature which indicates that human rotaviruses have a limited host range and very specific tissue tropism.

In the unlikely event that the notified strain is transmitted to non-human hosts, it is expected that *Rotavirus* strain RIX4414 will have a significantly reduced ability to cause

pathogenic effects compared to wild-type human rotavirus due to its attenuation. Its potential hazard to the environment and on the conservation and on biological diversity is, therefore, considered low.

Human Health Hazard

Different groups of Rotavirus exist and although cases of rotavirus B and C have been reported, Group A rotaviruses are the most important from a public health standpoint (WHO, 2007) 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 (Health Canada, 2004). Previous exposure confers protective immunity to all subsequent rotavirus infection in both adults and children. Where fatalities occur, they are associated with severe dehydration secondary to diarrhea.

The Rotarix™ vaccine, containing the notified strain, was developed to protect humans (infants 6-24 weeks old) for the prevention of gastroenteritis caused by rotavirus strains G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8], by stimulating the body to mimic the immune response to natural rotavirus infection. The vaccine was granted approval from the US Food and Drug Administration in April 2008 (FDA, 2008) and is currently licensed in over 100 countries worldwide. The notifier submitted Phase III clinical findings from a total of 80,705 vaccinated infants in different geographical regions globally which show that the vaccine was efficacious against moderate to severe rotavirus gastroenteritis. In adults, Zibrik et al. (2007) reported that volunteers administered with one oral dose of the Rotarix™ vaccine, the same dose recommended for infant vaccinees, did not display any symptoms of gastroenteritis.

Since the vaccine strain was attenuated in the course of multiple passages in primary cell and cell line cultures and subsequently tested in extensive, randomized placebo-controlled safety and efficacy trials worldwide, its use is not expected to cause adverse effects within the general population. Its potential hazard to human health is considered low.

Genetic Stability/Reassortment

No sign of reversion to the virulent phenotype was observed during the clinical trials. The rotavirus vaccine strain has the potential to undergo genetic reassortment with other wild-type human rotaviruses. However, the reassortant is not expected to be more virulent than the wild-type strain or have a survival advantage in the environment. The possibility of interspecies reassortments between felines, canines, equines, porcine, bovine, and human rotaviruses has been extensively studied and only a few human rotavirus strains have high homology to all 11 genes of animal rotaviruses (Gentsch et al., 2005). This suggests that interspecies reassortments could happen, but are rare.

The notified strain has the potential to reassort with wild-type rotaviruses in the environment, however, the likelihood that these reassortments could cause adverse effects to humans and the environment is expected to be low.

EXPOSURE CONSIDERATIONS

The notified micro-organism will be imported from GlaxoSmithKline Biologicals (Belgium) to Ontario. The vaccine will be shipped as liquid formulation. Prior to export to Canada, the notifier will test the bulk vaccine for impurities as part of the vaccine's validation and certification. These include residual Vero cell DNA and transmissible and infectious agents, such as *Mycoplasma* and retroviruses.

To minimize the potential wildlife and human exposure to the vaccine strain, procedures are in place for the treatment and disposal of wastes containing the notified organism. The notified organism will be distributed to health care practitioners and health care facilities. All unused vaccine and waste generated through the normal vaccination process will be discarded in approved biological waste containers and disposed of according to provincial regulations.

Contingency plans are in place in case of accidental release during re-packaging, transport and administration. The notifier described procedures designed to limit human exposure in case of an accidental release including the use of protective clothing and oxidizers to decontaminate surfaces.

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. They have been detected in raw sewage and wastewater at a concentration of 90,700 virus particles per litre and 10 to 218 virus particles per litre, respectively (Abbaszadegan, 2006).

Rotaviruses are transmitted predominantly through the faecal-oral route. It is expected that the notified strain will be present in the environment mainly due to the disposal of feces in sanitary sewer systems, disposal of soiled diapers in solid waste landfill sites, and from accidental fouling of water bodies. Although replication does not occur outside living hosts, enteric viruses may remain stable in terrestrial and aquatic environments for months.

Rotaviruses persist in drinking water for up to 64 days at 4°C (Satar et al., 1984). In protected groundwater with temperatures generally below 10°C, enteric viruses have been reported to persist for nearly 2 years (Health Canada, 2004). Rotaviruses are relatively stable in sewage, with numbers peaking in the autumn and winter. Infectivity is lost more rapidly at 37°C under all level of humidity than at 20°C or 4°C (Moe & Shirley, 1982). It can persist on dry inanimate surfaces for approximately 2 months (Kramer *et al.*, 2006).

Some aquatic and terrestrial organisms, particularly economically important agricultural species, could be exposed to the notified strain through the ingestion of crops grown on sewage sludge-amended soil or contaminated groundwater following the irrigation of

agricultural land with wastewater and/or fertilization with sewage sludge (Mara et al., 2007), or by the percolation of rainwater through landfill sites, the release of septic tank field effluents, or the infiltration of contaminated surface water into groundwater (Bitton, 1999; Hurst et al., 2001). The presence of the notified strain in drinking water could result from lack of treatment, insufficient disinfection or inadequate treatment of surface water containing high concentrations of the notified strain (Gutierrez et al., 2007; Payment & Armon, 1989; Gerba & Rose, 1990; Payment et al., 1997).

Naturally occurring human rotaviruses are excreted at a rate of 10^{10} to 10^{12} infectious units per gram of stool (Abbaszadegan, 2006). Fecal shedding of strain RIX4144 was evaluated during clinical trials among a subset of subjects. The incidence of Rotarix™ post-vaccination shedding in infants was 14.6%, compared to 68.6% shedding rate for control subjects who suffered from wild-type rotavirus-induced gastroenteritis during the clinical trials. Using a model to predict the environmental concentration of the rotavirus, the concentration in surface water at approximately 14.6% shedding and assuming no inactivation before release is calculated to be low (1.87×10^{-5} CCID₅₀/L). Using this predicted value, the daily drinking water contaminant intake is also calculated to be low (1.43×10^{-7} CCID₅₀/kg bw/d for adults and 3.01×10^{-7} CCID₅₀/kg bw/d for children).

The disposal of fecal waste in landfill sites from used diapers is expected to reduce the amount of the notified strain released into surface waters. Although the concentration of the vaccine strain released from landfill sites cannot be accurately calculated due to the complexity of variables, including landfill size and location, disposal rate, and other waste sources, a worst-case concentration was estimated to be 4.2 CCID₅₀/tonne of solid waste.

Given that the vaccine strain is attenuated, is only administered to infants and that there will be some containment in landfills and removal at sewage treatment plants, the potential concentration of the notified rotavirus strain in the environment is expected to be significantly less compared to that of naturally occurring rotaviruses. The potential environmental and human exposure to *Rotavirus* strain RIX4414 (apart from humans exposed directly through the immunization process) is therefore considered to be low.

RISK CHARACTERIZATION

Based on the hazard and exposure considerations, the risk assessment conducted by Health Canada concluded that the *Rotavirus* strain RIX4414 does not cause harm to the Canadian environment or human health as described in section 64 of the CEPA 1999.

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