Summary Report of Risk Assessment Conducted Pursuant to subsection 108(1) of the *Canadian Environmental Protection Act, 1999*

New Substances Notification 20497: Coxsackievirus A21 (Kuykendall strain)

Regulatory decision

Under the provisions for Animate Products of Biotechnology in Part 6 of the *Canadian Environmental Protection Act, 1999* (CEPA), and pursuant to section 108 of the Act, the Minister of the Environment and the Minister of Health have assessed information in respect of the substance, Coxsackievirus A21 (Kuykendall strain), that is a living organism. It was determined that Coxsackievirus A21 (Kuykendall strain) is suspected to be toxic since unrestricted releases may result in it entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity, constitute or may constitute a danger to the environment on which life depends, or constitute or may constitute a danger to human life or health in Canada.

In order to ensure that the living organism does not cause harm to the Canadian environment or human health, its manufacture and import has been authorized subject to conditions as described in Ministerial Condition No. 20497, published in the Canada Gazette Part I, Vol. 154, No. 42, October 17, 2020. These conditions in effect restrict its uses to those proposed by the notifier.

Identity

The notified organism, Coxsackievirus A21 (Kuykendall strain), is a naturally-occurring enterovirus. Upon administration to patients, Coxsackievirus A21 (Kuykendall strain) will target the cellular receptor (named intercellular adhesion molecule 1, ICAM-1/DAF), which is found in abundance on the surface of cancer cells compared to normal cells. Coxsackievirus A21 (Kuykendall strain) is expected to elicit a T-cell mediated tumour-specific antigen response resulting in selective cell destruction within the tumour.

Notified and potential uses

Coxsackievirus A21 (Kuykendall strain) was notified as part of an investigational oncolytic virus therapy targeting ICAM-1/DAF receptors on cancer cells, in patients with advanced solid tumours. Other potential uses included its use in clinical trials for other malignancies or use in a commercial cancer therapy drug product.

Hazard assessment

The environmental hazard potential of Coxsackievirus A21 (Kuykendall strain) is assessed to be low because:

1. Coxsackievirus A21 (Kuykendall strain) is part of the *Picornaviridae* family of viruses that are only reported to cause respiratory illness in terrestrial vertebrates including: bovine, equine, simian,

porcine, ovine, canine, murine and avian species (Stott, 1975). However, humans are the only known natural host of the Coxsackievirus A21 (Kuykendall strain).

2. Coxsackievirus A21 (Kuykendall strain) is a naturally occurring virus that is designated a risk group (RG) 1 animal pathogen by the Public Health Agency of Canada (PHAC, 2023) and it is not considered a terrestrial animal pathogen by the Canadian Food Inspection Agency (CFIA).
3. No genetic modifications have been made to the notified organism, Coxsackievirus A21 (Kuykendall strain), and so its host specificity and pathogenesis are unaltered.
4. Data from published literature indicated that in an experimental setting, newborn and young mice inoculated with the virus were permanently paralysed, but these effects were not observed in adult mice (Sickles et al. 1959). No other negative effects were reported in adult mice. In addition, no other naturally occurring infections were reported in aquatic and terrestrial plants, invertebrates and vertebrates.
5. Data from animal studies conducted by the notifier in mice indicated that Coxsackievirus A21 (Kuykendall strain) was well-tolerated when administered via subcutaneous, intravenous or intrahepatic injection. Histomorphological responses related to Coxsackievirus A21 (Kuykendall strain) administration were limited to inflammatory lesions at injection sites, which is not unexpected following injection of a live virus.

The human health hazard potential[1] of Coxsackievirus A21 (Kuykendall strain) is assessed to be medium because:

1. Coxsackievirus A21 (Kuykendall strain) is a Risk Group 2 human pathogen (as classified by PHAC) as it causes infection in humans resulting in a moderate individual risk, but a low community risk.
2. Data from published literature suggested that Coxsackievirus A21 (Kuykendall strain) causes mild upper respiratory infections in otherwise healthy individuals, has been the causative agent of several outbreaks and circulates naturally within the human population.
3. Data from published literature did not yield any concerns specific to vulnerable populations. However, PHAC's Pathogen Safety Data Sheet for Coxsackievirus (PHAC, 2014) indicates that young children are more susceptible to infection.
4. Symptoms resulting from respiratory infection are similar to those of a common cold, such as low-grade fever, cough, and increased nasal secretions, and resolve naturally without medical intervention (Bloom et al., 1962; Oie and Van Der Veen, 1967).
5. Historically, Coxsackievirus A21 (Kuykendall strain) has been used in experimental studies to track the spread of illness where it was administered to healthy participants via intranasal inhalation causing upper respiratory infections (Buckland et al. 1964; Buckland et al. 1965).
6. Clinical trials in adult patients investigating its use as a therapeutic oncolytic virus have been completed or are ongoing in Australia, the United States and the United Kingdom. At the time of the assessment, no severe adverse effects had been reported amongst study participants regardless of

[1] Hazards related to micro-organisms used in the workplace should be classified accordingly under the Workplace Hazardous Materials Information System (WHMIS).

dose or method of administration. Some adverse effects were reported but were transient and easily managed.[2]

7. As part of other clinical trials, some patients injected with Coxsackievirus A21 (Kuykendall strain) were observed to have infectious virus post-administration, but in almost all cases neutralizing anti-Coxsackievirus A21 (Kuykendall strain) antibodies were detected.
8. No antivirals are approved for treatment for respiratory illness associated with Coxsackievirus A21 (Kuykendall strain), as it is generally mild and self-resolving. For participants of the clinical trial, Coxsackievirus A21 (Kuykendall strain) is expected to be eliminated from the body because of the innate immune response and no other intervention is required.

Hazards related to micro-organisms used in the workplace should be classified accordingly under the Workplace Hazardous Materials Information System (WHMIS)[3].

Exposure assessment

The environmental and indirect human exposure potential of Coxsackievirus A21 (Kuykendall strain) is assessed to be low because:

1. Coxsackievirus A21 (Kuykendall strain) will be imported to Canada in a frozen state and securely transported to two clinical trial sites. Only planned target doses of Coxsackievirus A21 (Kuykendall strain) will be administered to a maximum of 30 clinical trial participants under controlled conditions in two healthcare centres by properly trained healthcare professionals. General biosafety measures in healthcare settings and contingency plans for accidental spills are expected to be in place to minimize the spread of the virus.

2. Coxsackievirus A21 (Kuykendall strain) can survive outside the human body, either on objects or materials which can carry infection, (such as clothes, utensils, and furniture) or in the natural environment (PHAC, 2014). Although Coxsackievirus A21 (Kuykendall strain) is not expected to replicate or proliferate outside of a human host, Coxsackieviruses can remain viable in the environment (e.g. air, water, soil) for an extended period of time, upwards of 60 days (Rzezutka and Cook, 2004). The survival of Coxsackievirus in the environment is highly variable and dependent on physical parameters such as temperature, humidity, exposure to UV radiation, and presence of organic material (Wyn-Jones and Sellwood, 2001).

3. As indicated by the notifier, Coxsackievirus A21 (Kuykendall strain) is expected to be shed via saliva and feces from participants in the clinical trial. This may possibly lead to humans and environmental species being exposed to Coxsackievirus A21 (Kuykendall strain). However, this exposure, based on

[2] In Canada, biologic drugs are assessed for safety, quality and efficacy under the Food and Drugs Act and Regulations, administered by the Biologic and Radiopharmaceutical Drugs Directorate of Health Canada.

[3] A determination of whether one or more of the criteria of section 64 of CEPA are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposure in the general environment. For humans, this includes, but is not limited to, exposure from air, water and the use of products containing the substances. A conclusion under CEPA is not relevant to, nor does it preclude, an assessment against the criteria in the *Hazardous Products Regulations,* which is part of the regulatory framework for the Workplace Hazardous Materials Information System (WHMIS) for products intended for workplace use.

the number of participants in the clinical trial, is likely comparable to exposure from the virus naturally circulating in the environment. Other potential uses, such as use as a commercialized oncolytic drug product, could increase exposure.

Risk characterization

Risk is typically described as the probability of an adverse effect occurring based on hazards and a particular scenario of exposure. Different exposure scenarios can be described based on the intended and/or potential uses (if any) involved. In the present case, the organism will be imported and used as part of an investigational oncolytic virus therapy or for potential commercial use as a cancer therapeutic.

Owing to the low potential for environmental hazard and the low potential for environmental exposure, the environmental risk associated with the use of Coxsackievirus A21 (Kuykendall strain) as an investigational or a commercial cancer therapy product is assessed to be low.

Owing to the medium potential for human health hazard and the potential for higher exposure if this living organism were to be listed on the Domestic Substances List, the human health risk associated with the use of Coxsackievirus A21 (Kuykendall strain) as an investigational or a commercial cancer therapy product is assessed to be medium.

Risk assessment conclusion

There is no evidence to suggest a potential risk of adverse environmental effects at the exposure levels predicted for the Canadian environment from the notified uses although commercialized uses may lead to increased release/exposure in the environment. The risk to the environment associated with Coxsackievirus A21 (Kuykendall strain) is not suspected to meet the criteria in paragraphs 64(a) or (b) of CEPA. No further action is recommended.

There is no evidence to suggest a potential risk of adverse human health effects at the exposure levels predicted for the Canadian environment from the notified use. However, potential uses outside of a clinical trial setting could pose a risk to the general population. On this basis, the risk to human health associated with Coxsackievirus A21 (Kuykendall strain) is suspected to meet the criteria in paragraph 64(c) of CEPA. A Ministerial Condition is recommended to limit uses outside of clinical trial settings, which can include potential commercialization.

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