New substances: risk assessment, New Substances Notifications 22051, 22196, 22197 and 22198; Schedule 5 of the *New Substances Notification Regulations (Organisms)*

Notified organisms:

- A gene edited *Sus scrofa domesticus* (Landrace), descendent of Elite Line L02, with deletion of the SRCR5 domain of the CD163 protein conferring resistance to porcine reproductive and respiratory virus (NSN-22051, hereinafter referred to as L02-0)
- A gene edited *Sus scrofa domesticus* (Large White), descendent of Elite Line L03, with deletion of the SRCR5 domain of the CD163 protein conferring resistance to porcine reproductive and respiratory virus (NSN-22196, hereinafter referred to as L03-0)
- A gene edited *Sus scrofa domesticus* (mix breed of Pietrain, Large White, Hampshire and Durocs), descendent of Elite Line L65, with deletion of the SRCR5 domain of the CD163 protein conferring resistance to porcine reproductive and respiratory virus (NSN-22197, hereinafter referred to as L65-0)
- A gene edited *Sus scrofa domesticus* (Duroc), descendent of Elite Line L800, with deletion of the SRCR5 domain of the CD163 protein conferring resistance to porcine reproductive and respiratory virus (NSN-22198, hereinafter referred to as L800-0)

Schedule of the NSNR(O): Schedule 5 - Information Required in Respect of Organisms Other than Micro-organisms

First day of assessment period: April 23, 2025

Last day of assessment period: December 18, 2025 (due to extension of the assessment period)

Organism type: Mammals

Use: Import of porcine reproductive and respiratory virus (PRRSV) resistant pigs for conventional breeding in commercial pig production systems

Anticipated quantity: confidential and not for disclosure.

Assessment level of concern:

- Human health hazard: Low
- Human exposure: Low
- Environmental hazard: Medium
- Environmental exposure: Low

Assessment conclusion under section 64 of the *Canadian Environmental Protection Act, 1999*: Low risk, not suspected to be toxic

Recommended action: No further action recommended.

Waiver: None

Synopsis

S. s. domesticus lines L02-0, L03-0, L65-0 and L800-0 (hereafter L02-0, L03-0, L65-0 and L800-0) were notified for import and subsequent use for conventional breeding in commercial pig production systems. Other potential uses of L02-0, L03-0, L65-0 and L800-0 could include its display in agricultural fairs and petting zoos, as companion animals, artificial insemination as well as in scientific research.

There is no evidence to suggest a potential risk of adverse environmental effects at the exposure levels predicted for the environment, from the notified use in conventional breeding in commercial pig production systems or from identified potential uses. The gene edit is not expected to increase environmental hazard for L02-0, L03-0, L65-0 or L800-0 pigs compared to unedited domestic pigs. Despite the medium potential for hazard, the risk to the environment associated with L02-0, L03-0, L65-0 and L800-0 is not suspected to meet the criteria in paragraphs 64(a) or (b) of *Canadian Environmental Protection Act, 1999* (CEPA) owing to the low potential for exposure.

Similarly, there is no evidence to suggest a risk of adverse human health effects at the exposure levels predicted for people living in Canada from use of the notified lines in conventional breeding in commercial pig production systems as well as other identified potential uses. Owing to the low potential for hazard and the low potential for exposure, L02-0, L03-0, L65-0 and L800-0 are not suspected to meet the criteria in paragraph 64(c) of CEPA.

No risk management is recommended.

This assessment was subject to scientific experts peer review external to the New Substances program.

Background information

The notified lines L02-0, L03-0 and L800-0 are gene edited pigs of the Landrace, Large White and Duroc breeds, respectively, while L65-0 is a gene edited pig of mixed breeds Pietrain, Large White, Hampshire and Durocs. In all four lines, a deletion of exon 7 of the *CD163* gene confers a homozygous recessive resistance to porcine reproductive and respiratory syndrome virus (PRRSV) by removing the virus binding site. PRRSV binds to the scavenger receptor cysteine-rich domain 5 (SRCR5) of the CD163 protein which leads to infection. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas 9 was used to delete the DNA coding for this protein domain, leaving the rest of the gene functional while preventing the virus from binding and infecting the notified lines. L02-0, L03-0 and L800-0 will be imported from the United States for conventional breeding in commercial pig production systems. L02-0, L03-0 and L800-0 could be potentially used as display in agricultural fairs and petting zoos, as companion animals, artificial insemination and in scientific research.

Hazard

The environmental hazard potential L02-0, L03-0, L65-0 is determined to be **medium** for the following reasons:

1) *S. scrofa* as a species, which includes wild pigs, domestic pigs, and the notified lines L02-0, L03-0, L65-0 and L800-0, have invasive properties. Domestic pigs reproduce quickly, with sows producing 2-3 litters a year and about 12 piglets per litter. Both wild, domestic and the notified lines can survive harsh climates and habitats and eat many different types of food.

2) PRRSV resistance provides a benefit for the notified lines L02-0, L03-0, L65-0 and L800-0 on farms where pigs live in high density and therefore high rates of viral transmission occur. In the wild, any advantage of PRRSV resistance is low because pig populations are sparse and therefore the transmission of the virus is low. Since resistance only appears in pigs with two copies of the edited SRCR5 gene, the resistance trait is not expected to spread into wild populations. There is also no evidence of transmission of PRRSV to other species of animals.

3) *S. scrofa* as a species, including both wild, domestic pigs, and the notified lines L02-0, L03-0, L65-0 and L800-0, have the potential to damage ecosystems through rooting, trampling, wallowing, bioturbation, and fecal contamination, and by disturbing ground nests to eat eggs. These behaviours harm biodiversity and the environment both immediately and long-term.

4) No physiological or behavioural differences have been found between domestic pigs, the parental line and the notified lines L02-0, L03-0, L65-0 and L800-0, except that the notified lines have a new trait which confers the resistance to PRRSV.

5) No foreign DNA was inserted into the notified lines. The CRISPR-Cas 9 gene editing methods used to develop lines L02-0, L03-0, L65-0 and L800-0 are targeted, specific and well understood. The resistance to PRRSV is only present in homozygous progeny bearing the edited gene; heterozygous pigs are not resistant to PRRSV. The gene edit is not expected to increase environmental hazard for L02-0, L03-0, L65-0 and L800-0 pigs compared to unedited domestic pigs. That is, the gene edited pigs do not pose an environmental hazard above and beyond what is already identified for *S. scrofa* as a species (see paragraph 3).

The indirect human hazard potential of L02-0, L03-0, L65-0 and L800-0 is determined to be **low** for the following reasons:

1) The CRISPR-cas9 method used to produce the notified lines does not introduce foreign genetic material that could raise any indirect human health concerns.

2) Imported animals are quarantined before entering commercial breeding facilities to prevent introduction of exotic pathogens as described in the import permit.

3) The genetic changes in L02-0, L03-0, L65-0 and L800-0 are well described, stably integrated and no off-target or non-target genotypic differences were detected. No other phenotypic differences (other than PRRSV resistance) were observed between the notified lines and their unedited parental lines.

4) While there are reported cases of zoonotic infections attributed to domestic pigs, the gene edit in L02-0, L03-0, L65-0 and L800-0 only confers resistance to the PRRS virus and does not alter susceptibility to other pathogens. Therefore, the zoonotic potential of the notified lines is not expected to be any different than the unedited parental lines L02, L03, L65 and L800.

5) Sequence identity of the edited CD163 protein did not match any known allergens or toxins. As there are no new proteins introduced from the gene editing process, L02-0, L03-0, L65-0 and L800-0 are not expected to lead to allergenicity or toxicity that is different from L02, L03, L65 and L800.

6) While there is no history of commercial breeding and/or production for the notified lines, the parental and related lines are widely used in the pork industry in Canada without any reported adverse effects.

Hazards related to organisms used in the workplace should be classified accordingly under the Workplace Hazardous Materials Information System (WHMIS)[1]. Living organisms used in food and feed are not covered in this assessment[2].

Exposure

The environmental exposure potential L02-0, L03-0, L65-0 and L800-0 is determined to be **low** for the following reasons:

1) Females and boars from each notified line of L02-0, L03-0, L65-0 and L800-00 pigs will be imported into Genus PLC's breeding facilities in Saskatchewan. Each facility has three levels of confinement in place as well as strict biosecurity protocols to prevent the release of the notified lines.

[1] 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.

[2] In Canada, food and feed approvals of gene-edited pigs are subject to the *Food and Drugs Act* (administered by the Food and Nutrition Directorate of Health Canada) and the *Feeds Act (*administered by the Canadian Food Inspection Agency*),* respectively.

2) Live PRRSV-resistant pigs from the notified lines may be sold to any Canadian farm. Large pig farms have strict confinement measures to control disease, weather exposure, and limit outside entrance. Although small farms generally have lower confinement measures and often allow some outside access, they are also subject to provincial laws and regulations prohibiting their release to the environment.

3) Within Canada, provincial and territorial laws like Ontario's O. Reg. 703/21: Invasive Species Control Areas prohibit releasing wild or domestic pigs into the environment therefore limiting environmental exposure. Wild pig control programs such as Canada's Invasive Wild Pig Strategy are in place at the provincial/territorial and national levels. Movement of all domestic pigs, including the notified lines, must be reported as either a group movement or individual movement depending on the situation, by both the shipper and receiver through PigTRACE, a Canadian Pork Council program (CPC). CFIA authorizes CPC to be a responsible administrator of the pig identification and reporting requirements as laid out in the *Health of Animals Act* and regulations.

4) Other potential uses identified for the notified line L02-0, L03-0, L65-0 and L800-0 pigs include for artificial insemination, research purposes, as a livestock exhibit, or as a companion animal. Research would be conducted under containment, and other potential uses would be subject to the above-mentioned laws and regulations prohibiting the release of pigs to the environment.

5) PRRSV is rare in wild pig populations, so there is little selection pressure for the resistance; thus, the PRRSV resistance trait is unlikely to persist if the gene edited pigs escape from farms into the environment. Interbreeding of the notified animals with wild animals will not spread resistance because two copies of the gene deletion are required for any resistance, and interbreeding will only generate heterozygous animals with no disease resistance.

6) All pigs, including the notified lines, can reproduce rapidly and survive in diverse environments, enabling hybridization with wild boars. However, simulation model results from the Finding of No Significant Impact published by the United States Food and Drugs Administration indicated that the proportion of homozygous individuals in the wild population remains low over time, suggesting minimal introgression of PRRSV resistance into wild populations.

7) It is expected that the environmental impact of the notified lines L02-0, L03-0, L65-0 and L800-0 will be no different than non-gene edited domesticated pigs that are currently produced in Canada.

The indirect human exposure potential of L02-0, L03-0, L65-0 and L800-0 is determined to be **low** for the following reasons:

1) Females and boars of each notified elite line will be imported into Genus PLC's breeding facilities in Saskatchewan. Each facility has three levels of confinement in place as well as strict biosecurity protocols to prevent the release of the notified line.

2) The notified lines are not intentionally released outside their rearing facility and adequate procedures are in place to limit their escape during production and transport. In the unlikely event of accidental release, animals will be identified through their unique identification tag, quarantined, and safely returned into the facility at the end of 30 days of monitoring for potential health issues. Any release must be reported to the relevant provincial and federal authorities, as required by the systems in place.

3) Movement of L02-0, L03-0, L65-0 and L800-0 pigs between farms is reported by both the shipper and receiver, and in most cases as a group movement, in PigTRACE, a Canadian Pork Council program responsible for traceability requirements under the *Health of Animals Regulations*. Semen is collected onsite, recorded in boar stud management software, and shipped the same day via boar stud staff or couriers. These control measures would likely reduce the likelihood of human exposure.

4) Other potential uses for L02-0, L03-0, L65-0 and L800-0, including in agricultural fairs and petting zoos, as a companion animal and in scientific research, are not expected to significantly impact human exposure.

5) The unedited elite lines (L02, L03, L65 and L800) have been in commerce in Canada for decades and people in Canada would have been exposed to them without any reported adverse effect. Potential exposure to genetically edited pigs will not result in a different outcome from that of their unedited counterparts.

Risk characterization

The gene edit is not expected to increase environmental hazard for L02-0, L03-0, L65-0 or L800-0 pigs compared to unedited domestic pigs. Despite the **medium** potential environmental hazard, the environmental risk associated with the use of L02-0, L03-0, L65-0 and L800-0 for conventional breeding in commercial pig production systems or for other identified potential uses is determined to be **low** owing to the **low** potential for environmental exposure.

Owing to the **low** potential hazard and the **low** potential exposure, the human health risk associated with the use of L02-0, L03-0, L65-0 and L800-0 for conventional breeding in commercial pig production systems or for other potential uses is assessed to be **low**.

Risk assessment conclusion

There is no evidence to suggest a potential risk of adverse environmental effects at the exposure levels predicted for the environment from the intended and identified potential uses of the notified lines L02-0, L03-0, L65-0 and L800-0. The risk to the environment associated with L02-0, L03-0, L65-0 and L800-0 is not suspected to meet the criteria in paragraphs 64(a) or (b) of CEPA. No further action is recommended.

Similarly, there is no evidence to suggest a potential risk of adverse human health effects at the exposure levels predicted for people in Canada from the intended and identified potential uses of L02-0, L03-0, L65-0 and L800-0. The risk to human health associated with L02-0, L03-0, L65-0 and L800-0 is not suspected to meet the criteria in paragraph 64(c) of CEPA. No further action is recommended.