Risk Assessment Summary Conducted Pursuant to the *New Substances Notification Regulations (Organisms)* of the *Canadian Environmental Protection Act, 1999*
NSN 15676: Cassie line of genetically modified *Sus scrofa domestica*

This document has been prepared to explain the regulatory decision taken under Part 6 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999) regarding the manufacture or import of the Cassie line of genetically modified *Sus scrofa domestica* (domesticated pig), hereinafter the EnviroPig™, by the University of Guelph that is intended for introduction into the environment.

The EnviroPig™ was notified pursuant to subsection 3(1) of the CEPA 1999 *New Substances Notification Regulations (Organisms)* (NSNR [Organisms]).

Environment Canada and Health Canada have assessed the information submitted by the University of Guelph and other available scientific information in order to determine if the EnviroPig™ meets the criteria set out in section 64 of CEPA 1999[1].

Regulatory Decision

Based on hazard and exposure considerations related to the proposed use of the notified organism, the risk assessment conducted by Environment Canada and Health Canada concluded that the EnviroPig™ does not cause harm to the Canadian environment or human health. Therefore, manufacture or import of the EnviroPig™ may proceed as of the assessment period end date (November 26, 2009).

However, a significant new activity (SNAc) provision was recommended based on the uncertainties regarding possible environmental impacts of the notified organism in activities outside the scope of this assessment. These SNAc provisions outline information requirements for those activities. Details describing the SNAc provisions for the notified organism were published in the Canada Gazette, Part I on 20 February 2010 and can be found at the following URL: http://canadagazette.gc.ca/rp-pr/p1/2010/2010-02-20/html/notice-avis-eng.html#d103.

This evaluation does not include an assessment of health risk in the occupational environment, nor does it include an assessment of the potential human exposure and health risks associated with the use of the notified organism in products derived from it in or as an item that falls under the purview of the *Food and Drugs Act* (such as a food) or any other federal legislation (such as use of by-products in livestock feed).

[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.

Summary Assessment

NSNR(O) Schedule: 5 (Information Required in Respect of Organisms Other than Micro-Organisms).
Organism Identity: the Cassie line of transgenic *S. scrofa domestica* of the breed "Yorkshire" or "Landrace" that, as a result of genetic modifications, has had the phytase gene from *Escherichia coli* strain K12, under the control of a mouse promoter, introduced into chromosome 4 of its genome (EnviroPig™).
Notifier: University of Guelph
Date of decision: November 26, 2009
Proposed use: Production of pigs able to secrete phytase in the saliva, with the capability to use phytate-bound phosphorus in cereal.

History/Background

S. scrofa are even-toed ungulates of the Order Artiodactyla, Suborder Suiformes, Subfamily Suidae, and Tribe Suini[1].They are found throughout Europe and continental Asia as far south and east as Peninsular Malaysia, as well as to the islands of Sumatra and Java [2]. Members of the species include all domesticated *S. scrofa* breeds as well as the ancestral Eurasian Wild Boar from which all domesticated breeds descend. Feral populations of the Eurasian Wild Boar have been established in many parts of the world such as Australia, Brazil, Argentina, the United States [3] and Canada [4] as a result of intentional release for hunting purposes or escape from game farms.

Major centers of domesticated pig production are mainly found in temperate climates with approximately 61.8% of production taking place in Asia, 20.0% in Europe, 9.2% in North America, 5.4% in South America, 2.4% in Africa and 0.5% in Oceania [5].

In Canada, *S. scrofa* is an introduced species and does not have a natural, broad geographic distribution outside of production facilities. Most pigs are produced in Manitoba, Ontario, and Quebec although there is a trend to increased production in the Western provinces [4].

Phytate, also known as "myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate" accounts for up to 80% of phosphorus in common cereal grains, oil seed meals, and by-products [6,7]. The high phosphorus concentrations in pigs' manure are a result of their inability to hydrolyze this phytate. In turn, the phytate-bound phosphorus becomes a major environmental pollutant by leaching into nearby surface and groundwater, eventually ending up in lakes, streams or ponds where it causes algal booms and excessive weed growth.

The purpose of developing the EnviroPig™ was to produce phytase in their salivary glands, an enzyme capable of hydrolyzing phytate which is not naturally occurring in the animal. The notifier claims that the production of phytase in the salivary glands results in the following benefits:

- reduction in the cost of producing pigs by removing the need to supplement the diet with phosphate and/or phytase without affecting growth of the pigs;
- digestion of phytate eliminates the chelating capacity of this molecule, increasing the bioavailability of minerals (e.g., phosphorus, calcium, magnesium) and other nutrients [8,9,10]; and
- reduction of phosphate load in the manure, thereby reducing the phosphate level in runoff and its consequent pollution potential to the aquatic environment.

The present assessment does not evaluate the validity of the above claims.

Since the EnviroPig™ was derived from an oocyte harvested from a Yorkshire-Landrace cross gilt and the semen from a Yorkshire boar, both these breeds were considered in this risk assessment.

Genetic Construct/Modification

The EnviroPig™ was produced by pronuclei injection of a fertilized zygote derived from a Yorkshire-Landrace cross-breed gilt with semen from a boar of the Yorkshire herd. The transgene expression cassette from which the sequences were excised and purified was isolated from a plasmid containing the following genetic elements: 1) Mouse parotid secretory protein (PSP) 5' Flanking Sequence and promoter; 2) Phytase gene derived from *Escherichia coli* K12; 3) Mouse PSP terminator and 3' Flanking Sequence; 4) Plasmid backbone sequences including the origin of replication, and 5) β-lactamase gene encoding ampicillin resistance in order to allow selective growth of *E. coli* during plasmid production.

The notifier provided results from Southern blot analysis demonstrating that two intact copies (as tandem repeats) and one truncated copy of the transgene were inserted into the pig genome at a single site 98bp apart. As well, the notifier provided experimental results from polymerase chain reaction (PCR) analysis demonstrating the absence of the antibiotic resistance gene portion of the plasmid backbone sequences in the Cassie genome.

Stability

The notifier has speculated that the EnviroPig™ possibly originated from a germ line gonadal mosaic transgenic founder gilt (based on the birth of only two trangenic piglets out of twenty). In G_2 and subsequent generations of the EnviroPig™, the ratio of transgenic to non-transgenic offspring was more consistent with Mendelian genetics for non-sex linked genes. This pattern of inheritance further supports the finding that inserted

copies of the transgene were on an autosome (chromosome 4 in this case) and were closely linked.

Genetic stability was tested over six generations using standard PCR techniques targeting the 5 junctions located between the pig chromosome and the transgenes or between transgenes. Restriction digest analyses of the diagnostic PCR products suggested that they likely represent the target regions, which had remained stable over the generations tested.

Phenotypic stability was tested over six generations by measuring phytase activity in saliva collected from all piglets at eleven days of age. A gradual but significant increase in phytase activity was observed from generation two to six, likely as a result of selective breeding.

Hazard Considerations

Environmental Hazard

Potential environmental hazards associated with large scale non-transgenic swine production were not considered within the scope of this assessment. Included within the scope of this risk assessment, and presented in the following paragraphs, were those key factors that were considered to be linked to the living organism, and which may be influenced, directly or indirectly, by the genetic modification.

Given that the founder transgenic pig (Cassie) was produced from the egg of a Yorkshire-Landrace sow and semen from a Yorkshire boar, the biology and history of use of these two breeds were evaluated in determining all potential hazards associated with the living organism. These two breeds are considered to have a safe history of use in Canada (primarily in indoor production facilities; Brian Sullivan, Canadian Swine Breeders Association, personal communication). When considering the genetic modification itself, the phytase gene has not been altered at the sequence level and the functional assays of saliva samples showed no altered functionality of the produced enzyme. Furthermore, phytase itself has a history of safe use in swine production as a feed supplement (American Association of Swine Veterinarians; http://www.aasv.org/shap/issues/v18n2/v18n2p90.html).

All evidence suggested that, other than for the production of the phytase enzyme (primarily in the salivary gland), the EnviroPig™ was physiologically comparable to non-transgenic pigs.

The impact of the transgene on the survivability of these transgenic pigs in the Canadian environment and the potential for invasiveness outside of normal contained production facilities remains unknown. There are no documented reports suggesting that domestic pigs can or can not survive a Canadian winter without human intervention. However, it is theoretically possible that the genetic modification, which is claimed to reduce dietary requirements for phosphorus supplements, could increase the chance of survival of

escaped pigs and lead to subsequent establishment of feral populations. Given that there are reports of feral wild-boar populations in a few regions in Canada [4,11], and the potential for interbreeding between domesticated pigs and wild boars, it is conceivable that the escape of transgenic pigs in those regions where wild boar populations exist may result in the introduction of the transgene into the feral wild boar gene pool with unknown consequences. Because of this uncertainty, the use of the Significant New Activity powers in CEPA 1999 were recommended.

Human Health Hazard

Information provided by the notifier and an in-house search of the scientific literature yielded no indication of an increase in pathogenic/zoonotic or allergenic potential of the EnviroPig™ compared to unmodified conventional counterparts as there was no significant physiological differences observed in the health status between the two groups.

Studies done in 2004 on fecal cultures showed that the Enviropig™ harbors no more pathogenic organisms than regular pigs found on Ontario farms. Given the extensive use of phytase as a feed additive, it is unlikely that the phytase from the EnviroPig™ would act any differently on the gastrointestinal tract microbial flora than *E. coli*-derived phytase used in feed. The probability is low that the inserted genetic materials or the loss of endogenous genomic sequences will allow the notified organism to acquire new traits that can cause adverse effects to human health.

Exposure Considerations

The environmental exposure potential of the EnviroPig™ is considered to be low since production is only intended for secure, indoor facilities, with industry-standard control procedures and management practices.

Intentional release of live EnviroPig™ into the environment is not anticipated for the reason given above. Within these secure, indoor facilities, management practices will be required to prevent mixing of transgenic and non-transgenic animals. These include physical separation, as well as tagging and maintaining a traceability system for the transgenic animals. In the event that an accidental release or mixing occurs, the same tagging and traceability system will allow for identification of transgenic animals.

Risk Characterization

Taking into account the history of use of the non-modified organism, the genetic elements introduced, the phenotypic outcome, the intended use of the Cassie line of the EnviroPig™ and the measures in place at production facilities, no significant adverse effects to the environment or human health are expected. Therefore, Environment Canada and Health Canada have concluded that the EnviroPig™ does not cause harm to the Canadian environment or human health and thus does not meet the criteria outlined in section 64 of CEPA, 1999. However, Significant New Activity (SNAc) provisions have

been applied in response to the identified uncertainties and to ensure any new activity, beyond that which has been assessed, will be notified for further risk assessment. The link to these provisions has been provided at the beginning of this report.

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