**Finding of No Significant Impact (FONSI)**

**In support of an approval of an original application**

**for**

**A heritable intentional genomic alteration (deletion of exon 7 of CD163 gene) in diploid, heterozygous and homozygous founder animals and offspring of domestic pigs (Sus scrofa domesticus). The deletion is intended to confer resistance to porcine reproductive and respiratory syndrome virus (PRRSV) in the homozygous animals.**

**Genus plc**
**DeForest, WI**

The Center for Veterinary Medicine (CVM) has considered the potential environmental impact of this action and has concluded that this action will not have a significant impact on the quality of the human environment and, therefore, an environmental impact statement will not be prepared.

Genus plc (Genus) is requesting the approval of an original application for the use of a heritable intentional genomic alteration (IGA) (deletion of exon 7 of *CD163* gene) in diploid, heterozygous and homozygous founder animals and offspring of domestic pigs (*Sus scrofa* domesticus). The deletion is intended to confer resistance to porcine reproductive and respiratory syndrome virus (PRRSV) in the homozygous animals known as "PRRSV-Resistant Pigs." These animals will be maintained by Genus to provide elite maternal and paternal genetics to pork producers in the form of semen, oocytes, live males, and live females that are both heterozygous and homozygous for the IGA. Only pigs homozygous for the IGA are resistant to PRRSV; heterozygous pigs are not.

In support of the application, Genus has provided an Environmental Assessment (EA). A copy of the EA is attached. CVM provided guidance to and assisted Genus by outlining the types of environmental information required for inclusion in the EA. CVM independently evaluated the EA to ensure the scope and content were adequate and to ensure that the information presented in the EA was accurate. CVM determined that the EA provides sufficient evidence to support a finding of no significant impact (FONSI).

Approach to Risk Assessment

Risk to the environment resulting from the approval of the IGA contained in pigs was assessed in the EA following the recommendations in Guidance for Industry #187A and #187B and the principles on risk analysis outlined by the National Research Council (NRC)[1] where risk is equal to the joint probability of exposure (establishment and/or presence), and the conditional probability of harm given that exposure has occurred. The EA assessed the risk of environmental effects from the approval of the IGA by determining if pigs containing the IGA pose any more risk to the environment than their comparator, domestic pigs without the IGA.

[1] NRC. (2002). Animal Biotechnology: Science-based Concerns. Board on Agriculture and Natural Resources, Board on Life Sciences, The National Academies Press, Washington, DC, pp. 200. https://doi.org/10.17226/10418

This comparison was made by considering how the IGA affects, if at all, the likelihood of exposure and the likelihood and severity of environmental harms of an escaped domestic pig.

The primary factor that could lead to a difference in environmental risk between pigs with and without the IGA is if, under natural conditions, the IGA imparted a fitness advantage to the pig (i.e., greater survival and/or reproductive success) through the alteration of non-target phenotypic traits and/or resistance to PRRSV. Based on observations during monitoring of multiple generations of pigs with the IGA, the IGA is not expected to alter non-target phenotypic traits (e.g., mating behavior, aggression) that could affect the likelihood of exposure or the likelihood and severity of harms to the environment. Genus also provided information to demonstrate that there are no hazards associated with the IGA (e.g., presence of foreign DNA, off-target alterations, new expression products), that the IGA and PRRSV-resistant phenotype were inherited stably across multiple generations, and that pigs with the IGA did not differ phenotypically from their comparator (e.g., reproduction, growth, mortality). Therefore, the risk evaluation focused on whether the IGA could impart a potential fitness advantage to pigs with the IGA through the resistance to PRRSV resulting in a greater likelihood of exposure and/or harms in the natural environment compared to domestic pigs without the IGA.

It is important to note that due to the proposed conditions of use, the EA does not evaluate risk specific to facilities or locations within the United States (US) and pigs with the IGA could be held on any farm in the US with no required conditions of containment or carcass and waste disposal. In addition, the production scheme and animal management procedures (e.g., containment, stocking density, biosecurity plans) of pigs with the IGA are not expected to differ from domestic pigs without the IGA.

Exposure Assessment

The difference in the likelihood of exposure of pigs with and without the IGA was determined by assessing the difference in the likelihood of escape, survival, dispersal, reproduction and establishment in the natural environment.

The NRC has determined that the likelihood of pigs escaping captivity is moderate and, as domestic pigs are known to occasionally escape from US farms and during transport, the risk assessment conservatively assumes that the pigs with the IGA will escape. The likelihood of escape would be the same for pigs with and without the IGA because resistance to PRRSV does not provide any advantages to improve the likelihood of pigs escaping (e.g., does not increase aggression, speed or endurance). However, resistance to PRRSV could, in theory, provide a fitness advantage to pigs homozygous for the IGA through improved survival and reproduction in the natural environment compared to domestic pigs without the IGA.

If the pigs homozygous for the IGA could survive and/or reproduce better in the US environment due to resistance to PRRSV, there could be introgression of the IGA into wild pig populations. This could, theoretically, cause an increase in invasive wild pig populations and exacerbate the environmental harms caused by wild pigs. However, this is unlikely to occur for two reasons. First, this fitness advantage would only be conferred to homozygous pigs that are infected with PRRSV. If the prevalence of PRRSV in wild populations is zero or close to zero, then the IGA confers no fitness advantage. The prevalence of PRRSV in wild pig populations in the US has been found to be extremely low based on historical data, ranging from 0-3.4%, indicating that PRRSV is not considered to be a primary cause of mortality of wild pigs and, therefore, the IGA likely does not impart a fitness advantage to wild pigs. Second, the IGA is homozygous

recessive, meaning that only pigs homozygous for the IGA would be resistant to PRRSV. It would be difficult to establish a population of pigs homozygous for the IGA in the US environment for a number of reasons. The most direct way to achieve this would be for two pigs homozygous for the IGA to escape and breed together. This is unlikely to occur because most farms do not hold intact boars and sows/gilts at the same facility. In fact, most farms do not hold intact boars at all. More likely, an escaped pig homozygous for the IGA would mate with a wild pig. All the offspring would be heterozygous for the IGA and not resistant to PRRSV. Those offspring would then have to mate together, or mate with another homozygous individual (e.g., backcross with their homozygous parent) to result in a 25 or 50% chance, respectively, of producing a PRRSV-resistant animal (see Figure 6-1 of the EA). Combined with there being no selective advantage to being resistant to PRRSV due to the low prevalence and limited spread of the virus in wild populations, the homozygous genotype would be expected to be bred out of the wild pig population after a few generations. Thus, under natural conditions, the IGA is expected to impart little to no fitness advantage to domestic and wild pigs.

In addition, the prevalence of PRRSV in wild pig populations is not expected to substantially increase from historic levels. PRRSV transmission is thought to be unidirectional from domesticated farm pigs to wild pigs. Therefore, an increase in the prevalence of PRRSV in wild pigs would likely only occur if there were an increased prevalence in domesticated pigs. However, the prevalence of PRRSV in domestic pigs is likely to decrease with the presence of pigs homozygous for the IGA on farms throughout the US. Even if PRRSV prevalence was higher in wild pig populations than the historical data suggests, the presence of pigs with the IGA is unlikely to affect the wild pig population rate long-term. The main factors limiting the population growth rate of wild pigs in the US are hunting, access to food, and climate. The IGA does not impart any additional advantages to survival and reproduction under natural conditions; therefore, the pigs with the IGA are still subject to the main factors that will limit their survival and reproduction just as it does for domestic and wild pigs without the IGA. If, in a rare circumstance, pigs homozygous for the IGA are present in the wild and the prevalence of PRRSV substantially increases, introgression of the IGA would likely occur, and the majority of the surviving population would likely be resistant to certain strains of PRRSV. This would result in a negative feedback loop where the prevalence of PRRSV in that wild pig population would then decrease to zero due to the resistance, but the surviving wild pigs would still be subjected to the main factors limiting their population (hunting, food and climate). Thus, the population growth in the natural environment is not expected to be different between pigs with the IGA and domestic pigs without the IGA, regardless of the prevalence of PRRSV.

These conclusions were further evaluated using population modeling to illustrate and quantitatively describe whether the escape of PRRSV-resistant pigs from a farm in the US could result in introgression of the IGA into invasive wild pig populations, and subsequently, result in increased survival and population size of wild pigs. Simulations were performed using a population model (SLiM version 4.0.1) to evaluate the frequency of the IGA in wild pig populations and any resulting increase in wild pig population size under a variety of conditions. These scenarios were developed to reflect conservative projections of conditions and reasonable worst-case scenarios, including a higher prevalence of PRRSV in wild populations (5%) and a scenario covering natural disasters (600 pigs escaping every 5 years). When escaped pigs with the IGA were introduced into a large initial wild population, the frequency of the IGA was quickly diluted by the many wild-type alleles. When pigs with the IGA were introduced into a small initial wild pig population, an increase in the frequency of the IGA was noted. However, this increase in frequency did not cause a resulting increase in the wild pig

population size. These results corroborate the conclusions in the EA that there would be no difference in exposure or harms between pigs with the IGA and domestic pigs without the IGA.

Thus, as described above, because there is little to no fitness advantage to being resistant to PRRSV under natural conditions, the likelihood of survival, dispersal, reproduction and establishment in the environment is no different between pigs with the IGA and domestic pigs without the IGA. Therefore, the likelihood of exposure of pigs with the IGA is the same as domesticated pigs without the IGA.

Effects Assessment

The likelihood and severity of harms occurring to the US environment would also not differ between pigs with the IGA and domestic pigs without the IGA. Environmental harms resulting from exposure to pigs include habitat destruction, predation of other species, competition for resources, and disease transmission. The potential harms to the environment could be impacted by resistance to PRRSV in several ways. The first, and most likely, is indirectly through an increase in wild pig populations and a subsequent increase in the environmental harms caused by pigs. Second, the likelihood or severity of harms could be impacted through an increase of the introgression of other domestic traits (e.g., litter size, growth rate) to wild populations that could then cause increased survival or reproduction. Third, the reduction of PRRSV on farms could further reduce the prevalence of PRRSV in the wild and lead to increased wild pig populations, even without escape of pigs with the IGA. However, the prevalence of PRRSV in wild pig populations is extremely low and does not limit survival or growth of wild pig populations. Importantly, in order for these scenarios to occur, there again must be a fitness advantage to being resistant and a resulting increase in survival and reproduction. As discussed above, this is unlikely to occur.

Finally, the risk of harms to the environment resulting from the IGA itself was evaluated. These harms include toxicity resulting from the presence of the IGA in waste from pigs containing the IGA and toxicity to non-target organisms that consume the tissues of pigs with the IGA. No hazards with the IGA itself have been identified (e.g., new expression products, presence of antimicrobial resistance markers). Therefore, no specific requirements are necessary for the disposal of carcasses and waste (e.g., manure, blood). In addition, human food and animal feed safety were evaluated by CVM and no concerns were identified. Therefore, the risk to the environment from the IGA itself is low.

Conclusion

Based on FDA's review of information and analyses presented in the applicant's EA, the IGA is not expected to alter any non-target phenotypic traits and is expected to impart little to no fitness advantage to the pigs under natural conditions. Therefore, there would be no difference in the likelihood of escape, survival, dispersal, reproduction and establishment between pigs that contain the IGA and domestic pigs without the IGA. In addition, the severity and likelihood of harms occurring in the US environment would be no different between the two groups. Therefore, FDA has determined that the risk to the environment resulting from exposure to pigs with the IGA is no different from the risk to the environment resulting from exposure to domestic pigs without the IGA. Based on this determination, we conclude that no significant impacts on the quality of the human environment in the US would be expected, and therefore, an EIS is not required.

*{see appended electronic signature page}*

Matthew Lucia, DVM
Director
Office of New Animal Product Evaluation
Center for Veterinary Medicine
U.S. Food and Drug Administration

# Electronic Signature
# Addendum for Submission ID

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