Finding of No Significant Impact (FONSI)

In support of an approval of a New Animal Drug Application (NADA) for the pPL657 rDNA construct in the glycoprotein galactosyltransferase alpha 1,3 gene (GGTA1) in the hemizygous and homozygous GalSafe® lineage of domestic pigs (Sus scrofa domesticus) resulting in undetectable endogenous galactose-α1,3-galactose sugar residues on biological derivatives of the homozygous GalSafe® pigs that are intended to be used as sources of food or human therapeutics including excipients, devices, drugs or biological products

Revivicor, Inc.
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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.

Revivicor, Inc. (Revivicor) is requesting the approval of a new animal drug application (NADA) for the *pPL657* rDNA construct in the genome of the GalSafe® line of pigs, which includes both hemizygous and homozygous pigs[1], that are produced and grown only under the conditions specified in the approved application. The intentional genomic alteration (IGA) results in undetectable endogenous galactose-α1,3-galactose (alpha-gal) sugar residues on biological tissues in these pigs. Homozygous GalSafe® pigs are intended to be used as sources of food or human therapeutics including excipients, devices, drugs or biological products.[2] No more than 1,000 GalSafe® pigs per year will be produced at a single facility in northern Iowa and slaughtered at a single abattoir (slaughterhouse) in southern South Dakota that is inspected by the United States Department of Agriculture.

The United States Food and Drug Administration's (FDA) approval of this NADA is for the specific set of conditions described in the NADA [including the applicant's environmental assessment (EA)], and as enumerated in FDA's approval letter. Any production outside the scope of the approval would be unapproved and will result in the article, in this case the *pPL657* rDNA construct in the genome of the GalSafe® pig line, being considered an unsafe new animal drug and, therefore, adulterated within the meaning of section 501(a)(5) of the Federal Food, Drug, and Cosmetic Act. Revivicor must continue to notify FDA about proposed changes in any conditions established in the approved application and obtain FDA approval of a supplemental application for the change where necessary (21 CFR 514.8). Major and moderate changes, including any additional production or slaughter facilities and an increase in the number of pigs raised per year, would require the filing and review of a supplemental NADA. Approvals of any such supplemental applications would constitute a major agency action and trigger additional environmental analysis under the National Environmental Policy Act (NEPA), unless otherwise excluded.

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[1] Homozygous is defined as having the construct on two alleles; whereas, hemizygous has the construct on only one allele.

[2] This NADA approval is for the *pPL657* rDNA construct in the genome of the GalSafe® pig line. The use of the human therapeutics sourced from the homozygous GalSafe® pigs will require additional FDA approval(s) and a separate environmental analysis under NEPA will be conducted.

In support of the current application, Revivicor has provided an EA dated October 19, 2020. A copy of the EA is attached. We have reviewed the EA and find that it supports a FONSI.

The EA focused on the risk of environmental impacts resulting from two exposure pathways:

1. The escape[3] of GalSafe® pigs from a single production facility in northern Iowa and a single abattoir in southern South Dakota into the affected environment, and
2. The introduction of the *neomycin phosphotransferase II* gene (referred to herein as the *nptII* gene) that confers neomycin resistance and its expression product, aminoglycoside 3'-phosphotransferase II (referred to herein as the NPTII protein), resulting from the IGA, into the natural environment via manure or other wastes (including carcasses and any remnants) from GalSafe® pigs at the single production facility in northern Iowa and single abattoir in southern South Dakota.

These pathways and associated risks were evaluated in the EA using several risk-related questions, which are discussed below.

Escape of GalSafe® pigs

The risk to the affected environment from the exposure of GalSafe® pigs is defined in the EA by a series of risk-related questions that focus on the likelihood of escape, survival, reproduction and establishment, as well as the potential consequences of this exposure.

Due to multiple levels of physical (e.g., pens, building, perimeter fence) and procedural (e.g., training, security, daily observations) containment at both the production facility in Iowa and the abattoir in South Dakota, it was concluded in the EA that the likelihood of GalSafe® pigs escaping from containment is extremely low. This includes unintentional release such as could occur through malicious activities or natural disasters. In addition, if the GalSafe® pigs did escape, the likelihood that any would survive and disperse is very low for several reasons. First, all animals at both facilities are adequately identified to ensure they can be recovered in case of escape. In addition, daily containment checks and weekly animal inventories would result in rapid identification of missing animals, and subsequently, a quick search and recovery effort of escaped animals. Second, Iowa and South Dakota's harsh winters and freezing temperatures would likely make it difficult for escaped GalSafe® pigs to find food and survive for part of the year. Third, state regulations allow the hunting of feral pigs with few restrictions and citizens are encouraged to report sightings. Iowa and South Dakota are also home to several predators that could prey on escaped GalSafe® pigs, such as coyotes, foxes and birds of prey. Finally, the IGA is not expected to confer any selective advantage with regard to survival that would increase the fitness of these pigs. The low likelihood for these animals to survive and disperse is supported by a lack of feral pig populations in Iowa and South Dakota, even though Iowa is by far the highest pig producing state in the United States.

Reproduction and establishment of pigs with the IGA in the affected environment is extremely unlikely to occur due to a lack of potential mates. Iowa and South Dakota do not have established feral pig populations with which escaped GalSafe® pigs could breed. In addition, the likelihood of a breeding pair of GalSafe® pigs escaping together and reproducing in the affected environment is extremely low. There are multiple containment measures in place and only a small number of intact male boars at the Iowa facility. This likelihood is even lower at the abattoir because intact male boars are not expected to be sent to slaughter; therefore, breeding pairs would not be present at the abattoir. Finally,

[3] Escape includes the potential for unintentional release; e.g., by malicious activities or natural disaster.

the IGA is not expected to confer any selective advantage with regard to reproduction that would increase the fitness of these pigs. Therefore, it is concluded that, even though some of the GalSafe® pigs with the IGA are physically able to reproduce, it is extremely unlikely. Subsequently, based on the low likelihood for GalSafe® pigs to escape, survive and reproduce as describe above, it is concluded that the likelihood of establishment of a feral pig population from the escape of GalSafe® pigs in Iowa or South Dakota is extremely low.

Lastly, the likely effects on the environment should GalSafe® pigs escape and ultimately establish are no different than if domesticated pigs without the IGA escaped and became feral; they would likely alter local habitats and cause destruction of crops, pasture land and wilderness areas. Based on all the information presented above, the EA concludes that the likelihood that pigs with the IGA could escape, survive, disperse, reproduce and establish is extremely low and the risk to the affected environment is minimal. Therefore, no significant environmental impacts to the human environment resulting from escape of the GalSafe® pigs (i.e., exposure to the affected environment) would be expected.

Introduction of the nptII gene and NPTII protein into the natural environment

The risk to the affected environment from exposure to the *nptII* gene contained in the *pPL657* rDNA construct that is inserted in the animal's genome, and its expression product, the NPTII protein, was evaluated in the EA using two risk-related questions. These questions focused on the risk of toxicity to the environment resulting from the presence of the *nptII* gene and the NPTII protein and the likelihood of increased antimicrobial resistance in the affected environment through application of manure from GalSafe® pigs to crop land, composted carcasses and remnants of GalSafe® pigs, and/or disposal of rendered products resulting from the slaughter of homozygous GalSafe® pigs.

It was concluded in the EA that the risk of toxicity to the environment due to the presence of the *nptII* gene and the NPTII protein is extremely low. DNA, such as the *nptII* gene, is ubiquitous in the environment and does not pose a significant toxicological risk to the environment. The *nptII* gene is not composed of anything unique from any other DNA and dietary DNA has no direct toxic effect. Proteins, including the NPTII protein, are also widespread in the environment and the NPTII protein is not expected to result in toxicity. Furthermore, the NPTII protein is expected to degrade rapidly due to the presence of proteases and peptidases in the soil environment, limiting its potential exposure. Therefore, it was concluded in the EA that no significant environmental impacts to the human environment resulting from this exposure would be expected.

It was also concluded in the EA that the likelihood of increased antimicrobial resistance in the affected environment occurring due to the presence of the *nptII* gene and the NPTII protein in manure and waste products of GalSafe® pigs is limited. Although not yet shown to occur, it is possible for the *nptII* gene from GalSafe® pigs to be transferred to soil bacteria when introduced into the environment, which could theoretically cause an increase in soil bacteria resistant to aminoglycoside antibiotics. However, antimicrobial resistance genes, including the *nptII* gene, and antimicrobial resistance occur naturally in the environment. And, the likelihood of an increase in antimicrobial resistance in the environment to occur due to application or disposal of manure, carcasses, or any other remnants of GalSafe® pigs to the environment is expected to be limited for the following reasons. First, the likelihood of the spread of antimicrobial resistance to additional bacteria by conjugation in the soil environment is expected to be lower than in the gut of the GalSafe® pigs. Second, the

likelihood of horizontal gene transfer[4] by natural transformation is limited because it requires several coexisting conditions to be met concurrently (e.g., the DNA needs to be present in the extracellular environment, the recipient bacteria must be in a state of competence, and the translocated DNA must be stabilized). Third, the GalSafe® pigs will not be exposed to aminoglycoside antibiotics, reducing the possibility of selective pressure from aminoglycoside antibiotic challenge.

Even if antimicrobial resistance did increase in the environment due to the presence of the *nptII* gene, it was concluded in the EA that this increase would be limited to the local environment (e.g., the soil environment where manure, carcasses, or other animal remnants are applied). The GalSafe® pigs are produced at a single facility in northern Iowa and slaughtered at a single abattoir in southern South Dakota. The production facility will hold no more than 1,000 GalSafe® pigs per year. Therefore, the amount of manure, carcasses, and any other remnants of the GalSafe® pigs, and thus the amount of *nptII* gene, entering the environment will be limited. Thus, it was concluded in the EA that the risk from increased antimicrobial resistance to the wider environment is minimal and that no significant environmental impacts to the human environment resulting from exposure to the *nptII* gene and NPTII protein would be expected.

Conclusion

Based on FDA's review of information and analyses presented in the applicant's EA, the FDA has determined that the likelihood that pigs with the IGA could escape, survive, disperse, reproduce and establish in the affected environment is extremely low and the risk to the affected environment resulting from exposure to the GalSafe® pigs is minimal. In addition, it is concluded that the risk of toxicity and increased antimicrobial resistance in the affected environment, which would include soil and water environment (e.g., via runoff of the *nptII* gene, NPTII protein, or antimicrobial resistant bacteria containing the *nptII* gene)[5] due to the presence of the *nptII* gene and NPTII protein is minimal. Exposure is expected to be limited due to production of no more than 1,000 GalSafe® pigs per year at a single facility in northern Iowa and slaughter at a single abattoir in southern South Dakota. Therefore, no significant impacts on the quality of the human environment in the United States would be expected.

{see appended electronic signature page}

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[4] Horizontal gene transfer is the movement of genetic material between unicellular and/or multicellular organisms other than by transmission of DNA from parent to offspring.
[5] The risk of increased antimicrobial resistance in the aquatic environments is expected to be even less than compared to the local soil environment. Only a fraction of the *nptII* gene, NPTII protein, or antimicrobial resistant bacteria containing the *nptII* gene would be expected to be transported from the local soil environment to the aquatic environment. And, this fraction would also undergo considerable dilution further minimizing potential exposure and risk.

Electronic Signature
Addendum for Submission ID

Signing Authority (Role)	Letter Date
Matthew Lucia (Office Director)	12/14/2020

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