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# FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-542

*pPL657* rDNA CONSTRUCT IN DOMESTIC PIGS

*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-alpha-1,3-galactose sugar residues on biological derivatives of the homozygous GalSafe® lineage that are intended to be used as sources of food or human therapeutics including excipients, devices, drugs, or biological products.

Sponsored by:

Revivicor, Inc., a wholly owned subsidiary of United Therapeutics Corporation

## Executive Summary

The *pPL657* rDNA construct in the glycoprotein galactosyltransferase alpha-1,3 gene in a line of domestic pigs (referred to as GalSafe® pigs) is approved for the claim of “undetectable endogenous galactose-alpha-1,3-galactose sugar residues on biological derivatives of the homozygous GalSafe® lineage that are intended to be used as sources of food or human therapeutics including excipients, devices, drugs, or biological products.”

Proprietary Name	Application Type and Number	Sponsor
<i>pPL657</i> rDNA CONSTRUCT IN DOMESTIC PIGS	New Animal Drug Application #141-542	Revivicor, Inc., a wholly owned subsidiary of United Therapeutics Corporation

GalSafe® pigs contain an intentional genomic alteration (IGA) that was accomplished by inserting an additional piece of genetic material, known as recombinant DNA (rDNA), into their genome. The *pPL657* rDNA construct disrupts the *glycoprotein galactosyltransferase alpha-1,3 (GGTA1)* gene. This gene normally codes for an enzyme that is responsible for production of the alpha-gal sugar (galactose-alpha-1,3-galactose) found on biological surfaces, such as cells, tissues, and organs in all mammals except humans and certain non-human primates. In homozygous GalSafe® pigs, the *GGTA1* gene is knocked out on both alleles, resulting in the intended trait of no detectable alpha-gal sugar on their cells, tissues, or organs. Pigs containing the disrupted *GGTA1* gene pass the trait to their offspring through conventional animal breeding.

The lack of detectable alpha-gal sugar on the cell surfaces of GalSafe® pigs has implications for people who suffer from alpha-gal syndrome (AGS), an allergy to red meat from food producing mammals (beef, pork, and lamb, for example) and other products containing mammalian based materials, including cosmetics and medicines (for a more detailed discussion, see review of Platts-Mills et al., 2020). This allergy, which was first discovered in the U.S. in the mid-2000s, occurs in some people after they are bitten by a Lone Star tick (*Amblyomma americanum*). The tick bite transmits alpha-gal sugar molecules into the person’s body, and in some people, triggers an IgE-mediated immune response that later produces an allergic reaction after consuming red meat or other products containing mammalian-based materials. Some people bitten by a Lone Star tick may display mild allergic reactions, but not develop or be diagnosed with AGS. Other people, however, experience more severe reactions, including anaphylaxis requiring immediate medical care. People experiencing more severe reactions represent a small but expanding segment of the population, likely because the ticks’ range is expanding. Food products made from GalSafe® pigs contain undetectable alpha-gal sugar and may provide a red meat option for people with AGS.

GalSafe® pigs may provide a source of porcine-based materials with no detectable levels of alpha-gal to produce human medical products. Sponsors of such human medical products must first submit an application to, and obtain approval from, the appropriate FDA Center that evaluates the safety and effectiveness of these products before they can be used to treat people. The presence of the alpha-gal sugar in transplanted animal cells, tissues, or

organs causes hyperacute IgG, IgM, or IgE-mediated immune responses in people and subsequent rejection of the transplant. Therefore, the lack of the alpha-gal sugar in human medical products made from GalSafe<sup>®</sup> pigs may reduce immune reactions and rejection after xenotransplantation (transplanting live cells, tissues, or organs from a pig into a person) or xenografting (transplanting decellularized pig tissues such as heart valves, ligaments, or dermis into a person). Rejection of a pig-to-human xenotransplant/xenograft can be a problem in all xenotransplant/xenograft recipients, not just those with AGS. Porcine-based materials from GalSafe<sup>®</sup> pigs could also be used as components of other human therapeutics (for example, heparin as a blood thinner or gelatin as an excipient). The use of such materials in human therapeutics would be primarily intended for people who have AGS, and therefore, may have an allergic reaction to standard porcine derived products.

### **Safety and Effectiveness**

GalSafe<sup>®</sup> pigs themselves are not a drug and are not subject to FDA approval; rather, the intentional genomic alteration (the *pPL657* rDNA construct), which is contained in GalSafe<sup>®</sup> pigs, is the regulated article subject to FDA approval. For approval, the sponsor must show that the IGA is safe and effective for its intended use.

#### *Molecular Characterization of the Altered Genomic DNA*

A targeting vector was generated that was comprised of the *pPL657* rDNA construct and a vector backbone. The *pPL657* rDNA construct which integrated into the genome consisted of the DNA sequence used to disrupt the *GGTA1* gene and the sequence of the neomycin phosphotransferase (*nptII*) gene, an antimicrobial resistance marker. The presence of the *nptII* in the genome required a more thorough hazard analysis. (See the Human Food Safety discussion below for more information about the characterization of the potential hazard posed by the *nptII* gene and the basis for the determination that it is unlikely to represent a human food safety hazard.) DNA sequence analysis of the *pPL657* rDNA construct showed that it was consistent with the intended design. The vector backbone consists of DNA sequences that were used to propagate the rDNA construct in the laboratory during the assembly process but which were not integrated into the animal's genome, as confirmed by DNA sequence analysis.

#### *Molecular Characterization of the Lineage of Animals Whose Genomes Have Been Intentionally Altered*

The *pPL657* rDNA construct was stably integrated at the targeted location in the *GGTA1* gene and was inherited consistently across multiple generations of GalSafe<sup>®</sup> pigs. The data demonstrated that the GalSafe<sup>®</sup> pigs' genotype did not change over the lifespan of an individual pig or across generations.

#### *Phenotypic Characterization of GalSafe<sup>®</sup> Pigs*

No animal safety concerns were noted for GalSafe<sup>®</sup> pigs beyond those that would be expected in well-managed, commercial swine operations. Swine management considerations (including animal health, nutrition, housing, and reproduction) were similar to commercial swine operations.

### *Genotypic and Phenotypic Durability of GalSafe® Pigs*

Both the genotype and phenotype of GalSafe® pigs were conserved across multiple generations. The genotype and phenotype were durable, the *pPL657* rDNA construct was stably inherited, and the phenotype was consistent and predictable. The sponsor also provided a plan to ensure that future GalSafe® pigs will continue to be equivalent to the GalSafe® pigs evaluated for this approval. The sponsor will perform genotyping of all animals to ensure that only homozygous GalSafe® pigs will be sourced for human food or medical uses.

### *Claim Validation*

Data from three complementary studies [enzyme-linked immunosorbent assay (ELISA), tissue histology, and flow cytometry] validated the claim that the *pPL657* rDNA construct in the *GGTA1* gene results in undetectable endogenous alpha-gal sugar residues on food and biological derivatives from homozygous GalSafe® pigs. The studies demonstrated the lack of detectable alpha-gal sugar on the cells, tissues, and organs of these pigs as well as demonstrated the expression of anti-alpha-gal IgG and IgM antibodies. The flow cytometry study also confirmed that the intended trait of no detectable alpha-gal sugar on cell surfaces is durable in GalSafe® pigs and passed on to their offspring. The sponsor will continue to use flow cytometry to monitor the durability of the intended trait in the pigs after approval.

### **Human Food Safety**

FDA evaluated human food safety for the general population (not specifically for people with AGS) and concluded there is reasonable certainty of no harm to human consumers of food products made from GalSafe® pigs. FDA also concluded that the safety of food products made from GalSafe® pigs is no different than the safety of food products made from commercial pigs that do not contain the IGA (the *pPL657* rDNA construct). These conclusions were based on toxicology and microbial food safety evaluations.

### *Toxicology*

FDA identified the *nptII* gene in the *pPL657* rDNA construct as a potential human food safety hazard. This gene codes for aminoglycoside-3'-phosphotransferase, an enzyme that catalyzes the phosphorylation of aminoglycoside antimicrobial drugs, including neomycin, and confers resistance to these drugs in bacteria that contain the gene. Because the *nptII* gene is a known antimicrobial resistance marker, it was used as a molecular biology tool during the development of the IGA, allowing researchers to identify and select cells for successful integration of the *pPL657* rDNA construct in the GalSafe® pigs' genomes.

Based on evidence from the scientific literature, information in databases of the DNA and amino acid sequences of known food allergens and toxins, and the permitted use of the *nptII* gene in genetically engineered plants intended for human food, FDA concluded that it is unlikely that the protein encoded by the *nptII* gene is a food allergen or other human food safety hazard.

The sponsor conducted a study of the compositional and nutritional components of the edible muscle tissue from GalSafe® pigs compared to pigs that do not contain the IGA. The

study did not identify any toxicological or nutritional hazards to human consumers of food products made from GalSafe® pigs.

FDA did not identify any direct or indirect toxicological effects from disrupting the *GGTA1* gene or integrating the *pPL657* rDNA construct in GalSafe® pigs on the safety of human food products made from these pigs.

#### *Microbial Food Safety*

The evaluation of microbial food safety was limited to the potential human food safety hazard posed by the inserted *nptII* gene and its expressed protein (aminoglycoside-3'-phosphotransferase). FDA assessed the risk for the inserted *nptII* gene and its expressed protein to promote the emergence or selection of antimicrobial-resistant bacteria of human health concern in or on GalSafe® pigs. Although the presence of the *nptII* gene represented a potential hazard, FDA concluded that the microbial food safety risk is low and mitigated by the following:

- **The low number of GalSafe® pigs entering the food supply each year:** The approval covers a single swine farm that can produce a maximum of 1,000 GalSafe® pigs annually. This number is adequate for purposes of breeding, food production, and tissue procurement for human medical products; however, it represents < 0.0014% of the annual U.S. market hog production. Further, the pigs will be processed at a single United States Department of Agriculture (USDA)-inspected slaughterhouse and will not be commingled with other pigs that do not contain the IGA.
- **Focused labeling to exclude aminoglycoside use:** GalSafe® pigs have and will be raised without the use of aminoglycosides, including neomycin. Not using this class of antimicrobial drugs will minimize selective pressure that could contribute to potential antimicrobial resistance in bacteria in or on the pigs. The following statement is in the product labeling (related to the *pPL657* rDNA construct in GalSafe® pigs) and in the facility's standard operating procedures: *"To mitigate the potential development of bacterial resistance to aminoglycoside antimicrobial drugs, GalSafe® pigs should not be treated with an aminoglycoside."*
- **Ongoing surveillance for antimicrobial resistance:** The sponsor will continue to isolate and test bacteria collected during different life stages of GalSafe® pigs (with samples to be made available to the National Antimicrobial Resistance Monitoring System) to monitor for the development of resistance to aminoglycosides as well as resistance to other classes of antimicrobial drugs important in human medicine.

#### *Labeling of Food Products*

The U.S. Department of Agriculture has regulatory oversight over the labeling and processing of food products made from GalSafe® pigs.

#### **Conclusions**

Based on the data submitted by the sponsor for the approval of the *pPL657* rDNA construct in GalSafe® pigs, FDA determined that the IGA is safe and effective.

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## **I. GENERAL INFORMATION**

### **A. File Number**

NADA 141-542

### **B. Sponsor**

Revivacor, Inc., a wholly owned subsidiary of United Therapeutics Corporation  
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Blacksburg, VA 24060

Drug Labeler Code: 086134

### **C. Proprietary Name**

*pPL657* rDNA CONSTRUCT IN DOMESTIC PIGS

### **D. Species/Class**

Domestic Pigs (*Sus scrofa domestica*)

### **E. Indication**

Undetectable endogenous galactose-alpha-1,3-galactose sugar residues on biological derivatives of the homozygous GalSafe® lineage that are intended to be used as sources of food or human therapeutics including excipients, devices, drugs, or biological products.

## **II. PRODUCT DEFINITION**

*pPL657* rDNA construct in the glycoprotein galactosyltransferase alpha-1,3 gene (*GGTA1*) in the hemizygous and homozygous GalSafe® lineage<sup>1</sup> of domestic pigs (*Sus scrofa domestica*) resulting in undetectable endogenous galactose-alpha-1,3-galactose sugar residues on biological derivatives of the homozygous GalSafe® lineage that are intended to be used as sources of food or human therapeutics including excipients, devices, drugs, or biological products.

## **III. MOLECULAR CHARACTERIZATION OF THE ALTERED GENOMIC DNA**

When referring to the *pPL657* rDNA construct throughout this document, reference is made to the rDNA integrated into the animal's genome, or the IGA. The targeting vector used to introduce the *pPL657* rDNA construct into the genome consists of a vector backbone and the *pPL657* rDNA construct prior to insertion. The vector backbone consists of the rDNA sequences required for propagation of the construct in the laboratory during the assembly process. The vector backbone comes from a commercially available standard vector commonly used in molecular biology and genetics laboratories and is not intended to integrate into animal's genome as

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<sup>1</sup> GalSafe® is Revivacor's registered tradename for their lineage of pigs and is not itself subject to FDA approval. Rather, the regulated article subject to FDA approval is the intentional genomic alteration (*pPL657* rDNA construct), which is contained in the GalSafe® lineage of pigs.

confirmed by DNA sequencing. The *pPL657* rDNA construct is comprised of DNA sequences homologous to the targeted region of the porcine *GGTA1* gene flanking an *nptII* selectable antimicrobial resistance marker and DNA regulatory elements needed for the expression of the *nptII* selectable marker. The identity, sequence, and orientation of each element in the *pPL657* rDNA construct prior to insertion were verified using DNA sequence analysis software. DNA sequence analysis showed that the sequence was consistent with the intended design. It should be noted that the presence of *nptII* was identified as a potential hazard (see discussion under Section VI). However, FDA concluded there are no identifiable direct or indirect effects associated with the targeting vector including its elements and the *nptII* gene in the GalSafe® lineage of pigs that impact safety.

The information the sponsor submitted in support of the Molecular Characterization of the Altered Genomic DNA is sufficient and consistent with the intended design.

#### **IV. MOLECULAR CHARACTERIZATION OF THE LINEAGE OF ANIMALS WHOSE GENOMES HAVE BEEN INTENTIONALLY ALTERED**

The sponsor provided a detailed description of the methods and breeding strategy used for the production of the pigs with the IGA, also referred to as the GalSafe® lineage. The IGA (the *pPL657* rDNA construct) was introduced into the genome of GalSafe® pigs through rDNA-mediated homologous recombination using porcine fetal fibroblasts followed by somatic cell nuclear transfer (SCNT) into enucleated oocytes. Resulting embryos were transferred to surrogate dams. Five hemizygous GalSafe® lineage progenitors were identified and bred to produce homozygous GalSafe® animals.

To demonstrate successful integration of the *pPL657* rDNA construct in the targeted region, the sponsor provided data from polymerase chain reaction (PCR) assays, long-range PCR (LR-PCR), Southern blotting assays, and DNA sequence analyses of the insertion site.

PCR and LR-PCR assays of the entire insert and flanking genomic regions carried out using genomic DNA isolated from porcine fetal fibroblast cells, hemizygous GalSafe® lineage progenitor animals, and homozygous GalSafe® animals of subsequent generations demonstrated successful integration of the *pPL657* rDNA construct in the targeted region and transmission to subsequent generations of animals.

Southern blotting assay data using various probes further confirmed successful integration of the *pPL657* rDNA construct in the targeted region and demonstrated that no vector backbone was integrated in the GalSafe® pig's genome during that process. Southern blotting assays also confirmed that both *GGTA1* alleles in homozygous GalSafe® pigs carried the integrated *pPL657* rDNA construct.

DNA sequencing of the integrated construct and flanking genomic regions in hemizygous GalSafe® lineage progenitor and homozygous GalSafe® animals demonstrated that the structure of the integrated DNA construct was consistent with the intended design and showed the absence of the vector backbone in the flanking genomic regions. No major differences were identified between the integrated sequences in hemizygous and homozygous animals.



Based on the review of these data and information, FDA concluded that the *pPL657* rDNA construct was stably integrated at the targeted location in the *GGTA1* gene and was inherited with no alterations across multiple generations. The data support the conclusion that the genotype is not changing over the lifespan of an individual animal or across generations.

FDA concluded that the data and information the sponsor provided in support of the Molecular Characterization of the Lineage of Animals Whose Genomes Have Been Intentionally Altered is consistent and in agreement with the Molecular Characterization of the Altered Genomic DNA. FDA's review of the submitted data did not identify any specific hazards intrinsic to the integration of the rDNA construct into the animal. It should be noted that the presence of the selectable antimicrobial resistance marker, *nptII*, was identified as a potential hazard (see discussion under Section VI), however, FDA concluded there are no identifiable direct or indirect effects associated with *pPL657* rDNA construct or its elements, including the *nptII* gene in the GalSafe® lineage of pigs that impact safety.

## **V. PHENOTYPIC CHARACTERIZATION OF ANIMALS WITH THE IGA**

### **A. Production Facility/GalSafe® Animal Operation**

The sponsor's GalSafe® Animal Operation is maintained at a contract production facility in Iowa. Personnel at that facility are responsible for all general animal husbandry procedures, implementing site standard operating procedures (SOP) for animal care, and documentation of observations on the GalSafe® herd. Individual health monitoring of pigs is performed through daily health observations on all pigs in the GalSafe® Animal Operation. GalSafe® pigs are isolated and segregated from other swine at the facility. GalSafe® pigs are housed individually or co-housed and meet or exceed space requirements [American Dairy Science Association (ADSA), American Society of Animal Science (ASAS), and Poultry Science Association (PSA), (ADSA, ASAS, and PSA), 2010]. This facility is a USDA-registered facility and is accredited by AAALAC International. In addition, animal well-being is overseen by the contract facility's Institutional Animal Care and Use Committee (IACUC). The facility is designed and operated with biosecurity, containment, and animal health as major considerations. Physical barriers and SOPs for material and personnel entry and exit are in place to prevent internal/external contamination and entry by unauthorized personnel. Controls are in place to maintain/monitor environmental conditions, food and water, and disposal of waste materials.

### **B. Source Genetics and Reproduction**

Pigs in the GalSafe® Animal Operation at the Iowa facility consist primarily of the Large White crossbred swine. Initial animal sourcing was from North America (Canada and United States). Pigs sourced from Canada were used to produce the first pigs with the *pPL657* construct in their genome, including the lineage progenitors for the GalSafe® herd. The sponsor out-crossed progeny with pigs (live animals or semen) without the IGA purchased from within the United States. The sponsor currently maintains its pig herd closed to further introduction of outside animals, which is self-sustained through use of observed natural breeding or artificial insemination using fresh-extended semen collected on-site from sires

maintained in the GalSafe® Animal Operation. An exception is that a minimal number of pigs or semen without the IGA may be introduced periodically (using established quarantine and screening procedures at the facility) to out-cross for purposes of reducing inbreeding. Females are generally bred two to three times at intervals of 12-24 hours while they remain in estrus.

### **C. Animal Housing**

Animal housing at the facility consists predominantly of a large animal barn design with internal penning and feeding areas. All pigs are contained in a facility that has at least three barriers from the pigs to the outside environment. The perimeter of the facility is surrounded by an approximately 6-foot-high perimeter fence (tertiary environmental barrier) that is topped with barbed wire. There is a lockable gate on this perimeter fence line to allow controlled entrance and exit of the facility. The pigs are housed inside fully enclosed buildings (secondary environmental barrier) that is partitioned into various sized pens (primary environmental barrier). The barriers are designed to ensure that animals cannot escape or break from containment. The buildings do not allow the entrance of birds or other small animals. All buildings, including gates, doors, and pens, are checked at least twice daily by farm personnel. Indoor lighting is provided for a 12-hour period every day. Fresh air is provided to the building via mechanical ventilation.

Pigs are segregated before sexual maturity into age and size cohorts, and where applicable, physiological status (estrus, farrowing, nursing, etc.). Young piglets up to approximately two months of age are housed in dedicated nurseries.

### **D. Animal Identification**

At farrowing, each piglet is identified with a unique animal identification (ID) that is maintained throughout the life of the pig and ensures traceability of any pig or derivatives through all operational areas. The unique ID is recorded on written documents and placed directly on the pig via ear notch and ear tag. After completion of a genotypic assessment on an individual pig, an additional identifier is placed on the pig that contains pre-defined symbols that correspond to the pig's genotypic identity. In short, each pig is identified with "G0" (without the IGA), "G1" (hemizygous for the IGA), or "G2" (homozygous for the IGA). Applicable records reference the unique ID through all operational areas in order to determine the complete production history of the pig including the ultimate disposition for any pig in the GalSafe® herd. Specific procedures for identifying and maintaining pig identification are described in applicable SOPs that are maintained at the facility.

### **E. Feeding and Nutrition**

Piglets are allowed to nurse from their sows until weaning. If needed, piglets are fed a pre-starter feed as needed 2-3 weeks after farrowing.

All pigs are maintained on a vegetarian diet, documented free of all rendered mammalian-derived protein sources. Ingredients in the feed vary and are specifically formulated by the supplier for the nutrient requirement of pigs of the National Research Council (NRC, 1998) throughout each life stage. All feed bins

are inspected daily, and pigs are fed to allow *ad libitum* consumption until approximately 200 days. All feeds are obtained from a single feed manufacturing source and stored in designated feed locations that are protected from pests. Feed suppliers are qualified based on ability to provide non-rendered mammalian derived protein sources as well as provide specific feed ingredients in each lot of feed.

## **F. Health Management Procedures and Observations**

The primary goals of the healthcare program for the GalSafe® pigs are to reduce the prevalence of disease, prevent disease epidemics, and maintain pig health and well-being. The sponsor's herd health program at their farm represents an integrated approach that encompasses procedures related to animal husbandry, nutrition, preventative health, routine daily health observations, veterinary medical evaluation, and therapy.

### **1. Preventative Health Procedures**

A prophylactic health program for GalSafe® pigs is directed by the attending veterinarian. The program is typical of vaccination and parasitic programs for pigs and includes standard medications administered to pigs reared in commercial production systems. The attending veterinarian for the GalSafe® Animal Operation reviews the program annually and makes adjustments as necessary.

The sponsor employs a disease surveillance program, with at a minimum quarterly testing of representative sentinel animals for specific disease pathogens to detect subclinical diseases at an early stage in order to maintain optimal performance of the herd and to evaluate biosecurity. If the presence of certain pathogens is detected via serological testing, necropsy, or other methods, appropriate corrective action may be instituted facility-wide to eradicate or reduce the prevalence of the disease, including a review of biosecurity procedures. The health assurance program extends to pigs that are found dead or died with unknown etiology after weaning. An investigation is performed to identify the cause of death. Where appropriate, this consists of a necropsy by qualified staff followed by a written summary of findings. Tissue or other samples may be further analyzed by histology or other methods by an appropriately qualified laboratory. The evidence is reviewed by the veterinary staff to identify the most appropriate corrective action to institute for the GalSafe® herd.

Additionally, a rodent control program is designed for the facility and is an active program delegated to a third party. Each building unit is surrounded by gravel or concrete as a buffer zone to prevent the access of rodents to the facility. Additionally, a qualified third party installs and maintains rodent traps, bait stations (poison), and insecticides.

### **2. General Health**

All pigs and their respective housing are inspected and documented daily for the following: building and environmental conditions (temperature, humidity,

and air quality), food and water consumption, wounds, illness, behavior, or other health conditions. If an animal appears to have a health care concern, the course of treatment is determined by written protocol. If the symptoms of the pig do not appear on the protocol or if the animal does not improve after the first treatment, supervisors or veterinarians are alerted to evaluate the animal and identify appropriate treatment. This treatment may include administration of an animal drug(s) as required.

To discuss these results relative to historical values, various swine reports from the National Animal Health Monitoring System (NAHMS) from USDA-Animal and Plant Health Inspection Service (APHIS) were cited (see Section X, References).

### 3. Observations on Morbidity, Mortality, and Reproduction

Occurrence of morbidity and mortality post-weaning (growing and breeding populations) of GalSafe® pigs is provided in Tables V.1 and V.2 below.

**Table V.1. Summary statistics for overall mortality of post-weaned (growing and breeding populations) GalSafe® pigs (2012-2018).**

Observation	Mean	Standard Deviation	Minimum	Maximum
Number of Pigs/Quarter	0.7	1.1	0.0	4.0
Percentage	1.4	2.0	0.0	6.7

**Table V.2. Summary statistics for overall morbidity<sup>a</sup> of post-weaned (growing and breeding populations) GalSafe® pigs (2012-2018).**

Observation	Mean	Standard Deviation	Minimum	Maximum
Number of Pigs/Quarter	6.6	4.1	1.0	19.0
Percentage	15.3	9.8	2.6	42.9

<sup>a</sup>Overall morbidity is based on recorded therapy (Rx) events for health observations in systems/categories such as the following: reproductive, lameness (hoof, limbs), respiratory, gastrointestinal, skin/integument, or central nervous system.

Post-weaning mortality percentage (Table V.1) for the GalSafe® pigs (1.4%) compared favorably to the ranges in mortality reported for commercial swine operations (1.7-4.3%, USDA, 2015). Likewise, incidence of overall morbidity (15.3%, Table V.2) compared favorably to morbidity rates reported for commercial swine production (USDA, 2020), where prevalence of specific diseases post-weaning (e.g., nursery, grow to finish, wean to finish) ranged from approximately 1-50% (USDA, 1992, 2015, 2020). While morbidity was

attributed to a number of underlying observations/systems, the predominant observations related to those of the respiratory and gastrointestinal systems; incidence for specific categories/systems was  $\leq 5\%$ .

Data summarized in Table V.3 below were generally comparable to those seen in commercial swine production (USDA, 2015). There were two notable exceptions. While piglet mortality at birth was similar to that of the USDA report (10% for GalSafe® pigs, 8.5% from USDA, 2015), pre-weaning mortality tended to be higher in the GalSafe® piglets (25%) compared to values USDA reported (12.6%). Litter sizes (numbers born, born alive, and weaned) also tended to be reduced vs. USDA-reported values (e.g., total live born piglets per litter, 7.4 - GalSafe® and 9.4 - USDA, 2015). This was likely due, at least in part, to inbreeding in the limited GalSafe® population, limited numbers of dams/litters evaluated, and high variability among dams/litters as demonstrated by the large standard deviations. When Revivacor, Inc. periodically performed outcrossing of GalSafe® pigs on those without the IGA, reproductive performance generally improved. Notably, the litter sizes for the GalSafe® herd were comparable to those reported for small swine enterprises (USDA, 2009 & 2014, 8.0 and 7.0 total live born piglets per litter, respectively).

**Table V.3. Summary statistics for litter characteristics of pigs in the GalSafe® herd, 2012-2018 (101 litters and 656 piglets born).**

<b>Observation</b>	<b>Mean</b>	<b>Standard Deviation</b>	<b>Minimum</b>	<b>Maximum</b>
Total Piglets Born/Litter (n)	8.1	2.8	3.0	16.0
Live Piglets at Birth/Litter (n)	7.4	2.9	2.0	16.0
Mortality at Birth/Litter (n)	0.8	0.7	0.0	3.0
Pre-Weaning Mortality/Litter (n)	1.9	1.1	0.0	4.0
Weaned Piglets/Litter (n)	5.4	2.8	2.0	13.0
Birth Weight/Piglet (lb.)	2.5	0.4	1.9	3.6
Mortality at Birth (%)	10.0	10.0	0.0	35.0
Pre-Weaning Mortality (%)	25.0	14.0	0.0	56.0

Given the GalSafe® herd is of limited size and genetic basis (derived from a limited set of lineage progenitor(s), see Section IV above), it is likely that inbreeding experienced in the GalSafe® herd contributed to some of the effects on litter characteristics. During breeding seasons where the sponsor implemented outcrossing using swine genetics external to the GalSafe® herd, there was a general improvement in reproductive performance.

#### 4. Other Phenotypic Observations

The sponsor evaluated live animal growth pre-weaning (Generations G3, G4, and G5), and post-weaning (G3 and G4). They found that average weaning weight at 21 days of age for the three generations evaluated ranged from approximately 12-15 lb./piglet, consistent with weaning weights noted in commercial operations (USDA, 1992). For the two generations in which post-weaning growth rates (average daily gain (ADG) through approximately six months of age) was monitored, ADG was approximately 1.6 lb./day, somewhat lower than growth rates noted in commercial swine production (Flohr et al., 2016, Hines et al., 2019, O'Meara et al., 2020). Given the GalSafe® herd is of limited size and genetic basis (derived from a limited set of lineage progenitor(s), see Section IV above), it is likely that inbreeding experienced in the GalSafe® herd contributed to some of the effects on post-weaning growth. Thus, the somewhat reduced growth rates of GalSafe® pigs may be expected, and do not represent a concern with respect to potential hazards for animal safety.

Blood samples for hematology and serum chemistry determinations were collected from GalSafe® pigs to assess physiological status. Values from GalSafe® pigs were compared to reference ranges (from testing laboratory and other veterinary references) and to values of comparator pigs without the IGA housed off-site (Large White crossbred pigs without the IGA not routinely housed at the Iowa facility). Hematology and serum chemistry values of GalSafe® pigs were similar to those of comparators without the IGA; values from GalSafe® and comparator pigs were also within normal physiological ranges. Therefore, these data do not represent a concern with respect to potential hazards for animal safety.

Thirteen healthy GalSafe® pigs (ten females, three males; Generations G3, G4, or G5) being culled for herd management purposes were selected for necropsy by the attending veterinarian. Necropsies included an anatomical and pathological evaluation of the skin, respiratory, cardiovascular, hematopoietic, digestive, musculoskeletal, urogenital, and nervous systems, and normal necropsy procedures to look for obvious signs of clinical disease. Gross evaluation revealed no evidence of pathology or anatomical abnormalities that would identify potential animal safety hazards in GalSafe® pigs.

#### **G. Conclusions on Phenotypic Characterization**

It is concluded that no animal safety concerns were noted in GalSafe® pigs beyond those that would be expected in comparators without the IGA under conventional swine management practices. This conclusion was based upon the evaluation of data provided by the sponsor, and our facility inspections that included animal health observations, growth, reproduction, animal nutrition, and general animal husbandry conditions.

## VI. HUMAN FOOD SAFETY

FDA evaluated human food safety for the general population (not specifically for the alpha-gal sensitive population) and concluded that there is reasonable certainty of no harm for human consumers of food from the GalSafe® pig. The basis for the conclusion is described below.

### A. Toxicology

The primary food safety question is whether there is any difference between the food from the GalSafe® pig and food from a comparator conventional animal without the IGA.

To determine if the food is as safe as food from pigs commonly consumed by the public, FDA evaluated the product to identify and characterize any potential food safety hazards of the GalSafe® pig associated with consumption of the edible tissues by the general population in the United States.

#### 1. Hazard Identification

For the hazard identification, the following information was examined:

- the proposed IGA (including the process to generate the alteration)
- the molecular characterization of the altered genomic DNA
- the phenotypic characterization of animals with the IGA
- the safety (health status) of the animals
- any unintended effects, especially those associated with indirect toxicity due to alteration of physiological processes, and
- any changes in the composition of the edible tissues due to the IGA

##### a. Molecular and Phenotypic Characterization of the IGA and animals with the IGA

As described in Sections III and IV, the *pPL657* rDNA construct was stably integrated at the targeted location in the *GGTA1* gene and inherited across multiple generations. The molecular characterization indicated that the *pPL657* rDNA construct contains a selectable antimicrobial resistance gene marker (neomycin phosphotransferase, *nptII*), a tool commonly used in molecular biology to identify and select fibroblast cells that had successfully incorporated the IGA *in vitro*. Once identified, these cells were used as nuclear donors for somatic cell nuclear transfer (cloning) to produce founder animals with the IGA for the animal lineage. As the *nptII* gene is potentially expressed in the pigs, FDA has identified *nptII* in the *pPL657* rDNA construct as a potential food safety hazard. However, the phenotypic characterization described in Section V.F., revealed no indication of direct or indirect toxicity to the animals with the IGA that may cause a potential food safety concern.

b. Animal Health Status

FDA did not identify any animal safety or welfare concerns noted in GalSafe® pigs beyond those that would be expected in comparator pigs without the IGA under conventional swine management practices (Section V). These assessments provide a first screen for animals exhibiting unintended traits. Food derived from an animal of known and acceptable health status is an indication of suitability for human consumption. Based on the consistent information from the molecular characterization of the IGA, the animal health records, and the compositional analysis study, FDA has not identified any indirect effects resulting from the IGA.

c. Compositional and Nutritional Analysis of Edible Tissues

A study of the compositional and nutritional components of edible muscle tissue from the GalSafe® pig was conducted by the sponsor. The study included a comparison of the muscle among homozygous and hemizygous GalSafe® pigs and comparator pigs without the IGA. Large White female pigs (*Sus scrofa domesticus*) were genotyped and phenotyped for identity. Homozygous or hemizygous pigs and pigs without the IGA (control) from at least three separate litters were raised until appropriate slaughter age ( $268 \pm 16.1$  days). After weighing and processing the pigs, samples of muscle (from tenderloin) were frozen and shipped to a contract research laboratory for analysis. Samples were pooled by genotype and analyzed for 93 different analytes including moisture, protein, fat, ash, calories, carbohydrates, dietary fiber, fatty acid profile, mineral profile, selenium, cholesterol, sugar profile, vitamin profile, and amino acid profile.

Most values fell within 20% of reference values for raw pork tenderloin from the National Nutrient Database for Standard Reference available from the USDA (USDA, FoodData Central). Of the 19 values reported to be > 20% different from the USDA database, only three values (Total Trans Fat %, Saturated Fatty Acid (C12:0), and Niacin) had a > 20% difference between GalSafe® pigs and the comparator pigs without the IGA. These values were 23.8%, 22.4%, and 23.3% different from the comparator control pigs, respectively, suggesting that the differences were due to either to the small sample size, husbandry/feeding practices, or the processing of the samples. Additionally, the live weight for three of the five GalSafe® pigs at slaughter was greater than the average live weight of typically marketed pigs, which may explain the higher fat/fatty acid content. The analysis, although conducted with a very limited number of samples, did not identify any toxicological or nutritional hazards to humans consuming the GalSafe® edible tissues.

Based on the review of the information, FDA identified the *nptII* gene in the *pPL657* rDNA construct as a potential food safety hazard, however, FDA concluded there are no identifiable direct or indirect effects associated with *pPL657* rDNA construct or its elements, including the *nptII* gene in the GalSafe® lineage of pigs (see conclusions below). No other hazards were identified that required further characterization.



## 2. Hazard Characterization

To characterize any potential food safety hazards due to the incorporation of the neomycin resistance gene (*nptII* gene) into the genome of the GalSafe® pigs, FDA considered the following:

1. potential toxicity or allergenicity to humans from consumption of the expressed protein
2. potential for development of antimicrobial resistant bacteria (see Section VI.B)

The *pPL657* rDNA construct contains a selectable antimicrobial resistance gene marker used to identify porcine cells that successfully incorporated the IGA as part of the production strategy. The *nptII* gene encodes for the aminoglycoside-3'-phosphotransferase protein (NPTII protein), an enzyme that catalyzes the phosphorylation of aminoglycoside antimicrobials, which confers bacterial resistance to the antimicrobial neomycin. The neomycin resistance gene and expressed NPTII protein have been well-characterized in the literature and the gene is permitted in genetically engineered plants for certain uses in food for human and animal consumption as published in the FEDERAL REGISTER (59 FR 26700) on May 23, 1994.

Information in the literature provided evidence that the NPTII protein is heat labile, not modified by glycosylation, not resistant to degradation by pepsin, and is rapidly degraded by digestive enzymes (Fuchs et al., 1993). Thus, NPTII does not possess any of the characteristics associated with allergenic proteins. The protein sequence homology of NPTII protein with known human allergenic proteins was also analyzed by the sponsor using predictive databases. Based on the analyses, no biologically meaningful homologs of known human allergens for both linear and conformational IgE epitopes are expected to occur. Therefore, FDA concludes that there is minimal concern for an immunogenic or allergenic response to consumers for the NPTII protein in the edible tissues.

Oral toxicity studies conducted in rodents have demonstrated no adverse effects when fed either purified NPTII protein or diets containing flour from transgenic potato plants expressing NPTII protein (Fuchs et al., 1993; Rahnama et al., 2017; Sattarzadeh et al., 2018). A search of the *nptII* sequence against several databases also demonstrated no homology to known toxins. Considering the weight of evidence from the literature, predictive databases, and its use in genetically engineered plants, FDA concludes that it is not likely the NPTII protein is a food allergen or a toxicant.

### Toxicology Conclusion

FDA concludes there are no identifiable direct or indirect effects of the inactivation of the *GGTA1* gene or the *pPL657* rDNA construct and components on the safety of food from the GalSafe® lineage of pigs. There are no substances of toxicological concern resulting from the *pPL657* rDNA construct in the genome of GalSafe® pigs. Therefore, a tolerance for

substances resulting from insertion of the *pPL657* rDNA construct in the genome of GalSafe® pigs is not needed.

## **B. Microbial Food Safety**

FDA considered microbial food safety relevant to GalSafe® pigs with the IGA; specifically, the hazard that the inserted *nptII* gene, or its expression product, might present a risk with respect to promoting emergence or selection of antimicrobial-resistant bacteria of human health concern in or on GalSafe® pigs.

FDA's goal was to assess whether continual exposure to the *nptII* gene and/or its expression product presents a risk to humans. This might occur through consumption of edible tissues from GalSafe® pigs with the potential for emergence and/or selection of antimicrobial-resistant bacteria of human health concern in or on pigs. This may result in an adverse health consequence in humans consuming edible products from these pigs.

Based on 1) information submitted by the sponsor for the purpose of demonstrating that food from GalSafe® pigs is as safe to humans as any comparable food product from pigs without the IGA, 2) information and data available in the public domain, and 3) information from research/testing efforts within FDA, FDA concludes that the impact to human health from entry of edible tissues from GalSafe® pigs into the food supply with respect to microbial food safety is low, and is mitigated by:

- **The low number of animals generated and entering the food supply:** No more than 1,000 GalSafe® pigs will be produced and available for processing for human consumption each year. The inventory of market hogs in the United States in 2020 is approximately 73 million [Quarterly Hogs and Pigs (September 24, 2020), National Agricultural Statistics Service, USDA]; therefore, the number of GalSafe® pigs going to market likely represent < 0.0014% of the total United States market hog population. This low percentage would not present a significant hazard to the overall food supply. Further, the pigs will be processed at a single USDA-inspected abattoir, and not commingled with other swine without the IGA, further decreasing the chance for the spread of bacteria among multiple locations and multiple food chains.
- **Focused labeling to exclude aminoglycoside use:** GalSafe® pigs will be grown to market weight and age and sent to slaughter without the use of aminoglycoside antimicrobial drugs, including neomycin. This specific prohibition will be an expressed part of the health management and veterinary intervention portions of the SOPs for management of these pigs. The statement:

*To mitigate the potential development of bacterial resistance to aminoglycoside antimicrobial drugs, GalSafe® pigs should not be treated with an aminoglycoside.*

has been incorporated into the product labeling and the animal management procedures.

- **Ongoing surveillance for antimicrobial resistance:** The sponsor intends to engage in collection, isolation, and testing of bacteria collected during different life stages of the GalSafe® pigs to monitor for development of resistance to aminoglycosides, or resistance to other classes of antimicrobial drugs important in human medicine. As part of the post-approval efforts, fecal samples and retail meats will be made available to the National Antimicrobial Resistance Monitoring System (NARMS) and qualified contract laboratories for testing and reporting of susceptibility to medically important human antimicrobial drugs.

A potential concern about the presence of the *nptII* gene and/or its expression product in GalSafe® pigs is that complete genes like *nptII* might be transferred from tissues into bacteria and other organisms. To evaluate this further, an in-depth search of publicly available published literature, unpublished data from FDA researchers, and the NARMS program was performed. Based on the literature search and findings of submitted proprietary information, FDA concludes that risks associated with one or more copies of the *nptII* gene in or on GalSafe® pigs at the time of slaughter (or their edible products), are expected to be mitigated by the measures discussed above.

### C. Analytical Method

Because a tolerance has not been assigned, a validated analytical method is not necessary. Although not required for food safety, the sponsor's durability plan does include a validated method of identity.

## VII. GENOTYPIC AND PHENOTYPIC DURABILITY

Data were provided that demonstrate that both the genotype and phenotype of the GalSafe® lineage are conserved over multiple generations. The LR-PCR procedure cited in Section IV above was used to evaluate genotypic durability, and the flow cytometry procedure cited in Section VIII below was used to evaluate phenotypic durability. The sponsor will use these assays to monitor genotypic and phenotypic durability after NADA approval. These data adequately demonstrate that the genotype and phenotype of these pigs are durable, the *pPL657* rDNA construct is stably inherited, and the phenotype is consistent and predictable. Based on these data, the sponsor also provided a plan to ensure that future animals in the GalSafe® lineage will continue to meet the product definition. This included the sponsor's: (1) plan for monitoring genotypic and phenotypic durability after NADA approval, (2) plan for addressing genotypic and phenotypic durability failures, (3) recordkeeping and reporting plans as a means of documenting and communication (to FDA) observations related to durability and animal health/safety, and (4) contingency/disaster preparedness and recovery procedures for maintenance and/or re-derivation of the GalSafe® lineage of pigs. Together, the data and information the sponsor provided assure that the GalSafe® lineage will continue to be equivalent to those pigs evaluated prior to NADA approval.

## VIII. CLAIM VALIDATION

To demonstrate the claim that the *pPL657* rDNA construct in the *GGTA1* gene results in undetectable endogenous alpha-gal sugar residues on biological derivatives of the homozygous GalSafe® pigs, the sponsor provided data from appropriately controlled (e.g., biological derivatives from GalSafe® vs. comparator pigs without the IGA, internal assay controls, both positive and negative), ELISA, tissue histology, and flow cytometry studies. The bases for the determination that endogenous alpha-gal was undetectable on biological derivatives were as follows:

ELISA: Mammals with endogenous alpha-gal on cells do not produce anti-alpha-gal antibodies, whereas those that do not have endogenous alpha-gal on cells produce alpha-gal antibodies. The ELISA assay determined the presence of IgG and IgM antibodies to alpha-gal in the blood of GalSafe® pigs and absence of the antibodies in comparator pigs without the IGA.

Histology and Flow Cytometry: The sponsor used a carbohydrate binding protein (a lectin) isolated from *Griffonia simplicifolia* plants (*G. simplicifolia* lectin B4; GS-I-B4) to detect alpha-gal sugar residues on cells for both histology (tendon and dermis) and flow cytometry (blood lymphocytes). GS-I-B4 specifically binds to alpha-gal sugar residues in samples. Biotinylated GS-I-B4 lectin or fluorescein isothiocyanate (FITC)-conjugated GS-I-B4 lectin was used to visualize the presence of alpha-gal in histological tissue sections; for flow cytometry, FITC-conjugated GS-I-B4 lectin was used to differentiate between blood lymphocytes negative or positive for alpha-gal.

FDA's review of the ELISA study confirmed that GalSafe® pigs produce IgG and IgM antibodies against alpha-gal sugar residues, whereas the comparator pigs without the IGA did not, thereby supporting the sponsor's proposed claim relative to the lack of detectable alpha-gal sugar residues on the organs, tissues, or cells of GalSafe® pigs.

FDA's review of the histology study established no morphological differences between tissues (tendon and dermis) from GalSafe® and comparator pigs without the IGA. Tissue and cell morphology were normal with no evidence of inflammation or degeneration. These findings were consistent with the macroscopic anatomical and physiological data as well as the information evaluated under Phenotypic Characterization, and further supported the conclusion that GalSafe® pigs are as healthy as comparator pigs (without the IGA) of similar breeds. In addition, alpha-gal was not detected on tissue sections from GalSafe® pigs, but was detected on tissue sections from comparators, further supporting the sponsor's claim.

FDA's review of the flow cytometry study established a clear difference between GalSafe® and comparator pig samples attributable to the absence of detectable alpha-gal sugar residues on the surface of the GalSafe® pig lymphocytes regardless of the sex, age, and generation of GalSafe® pigs evaluated. This conclusion was also consistent with, and complementary to, the histology study conclusions. The flow cytometry procedures were used to establish phenotypic durability over multiple of GalSafe® pigs (see Section VII above).

Taken together these three complementary approaches demonstrated that there is no evidence that alpha-gal is expressed in GalSafe® pigs within the limits of

detection of these assays. Therefore, based on the review of data and information submitted in support of the claim, FDA concluded that the studies demonstrate undetectable endogenous alpha-gal sugar residues in GalSafe® pigs.

## **IX. AGENCY CONCLUSIONS**

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR part 514, and reflect the recommendations in Guidance for Industry 187. Data demonstrate that the *pPL657* rDNA CONSTRUCT IN DOMESTIC PIGS is safe and effective in the disruption of the *GGTA1* gene, resulting in undetectable endogenous alpha-gal sugar residues on biological derivatives of the homozygous GalSafe® line. Additionally, data demonstrate that consumption of food from the GalSafe® lineage will not represent a public health concern.

### **A. Exclusivity**

The exclusivity provisions of section 512(c)(2)(F) of the Federal Food, Drug, and Cosmetic Act do not apply to the *pPL657* rDNA CONSTRUCT IN DOMESTIC PIGS because under section 106 of the Generic Animal Drug and Patent Term Restoration Act (Pub.L. 100-670), FDA cannot approve an abbreviated new animal drug application (ANADA) for a new animal drug that is primarily manufactured using recombinant DNA, recombinant RNA, hybridoma technology, or other processes involving site specific gene manipulation techniques. Therefore, a sponsor cannot submit an ANADA to market a generic version of the *pPL657* rDNA CONSTRUCT IN DOMESTIC PIGS.

### **B. Patent Information**

For current information on patents, see the Green Book Reports in the Animal Drugs @ FDA database.

## **X. REFERENCES**

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