

Date of Approval: December 27, 2018

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-511

Bc2371 rDNA construct in R69 New Zealand white rabbits

Heritable Construct

Domesticated Rabbits

Bc2371 rDNA construct integrated at a single site on chromosome 3p1.1-2 in a specific, diploid line (R69) of hemizygous and homozygous New Zealand white rabbits (*Oryctolagus cuniculus*) derived from the founder animal R69 directing the expression of the human gene for Factor VII in the mammary gland such that recombinant human Factor VII (rhFVII) zymogen is present in the rabbit milk to enable purification and activation of rhFVIIa intended for the treatment of hemophilia A or B patients with inhibitors to Factors VIII and IX.

Sponsored by:

LFB USA, Inc.

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I. GENERAL INFORMATION

A. File Number

NADA 141-511

B. Sponsor

LFB USA, Inc.
175 Crossing Blvd.
Framingham, MA 01702

Drug Labeler Code: 086047

C. Proprietary Name

Bc2371 rDNA construct in R69 New Zealand white rabbits

D. Species/Class

Domestic Rabbits (*Oryctolagus cuniculus*)

E. Indication

Production of recombinant hFVII zymogen in the milk of R69 New Zealand white rabbits, to enable purification and activation of recombinant hFVIIa intended for the treatment of hemophilia A or B patients with inhibitors to Factors VIII and IX.

II. SUMMARY

Blood contains proteins called clotting factors that can help stop bleeding. Hemophilia, an inherited bleeding disorder in humans, is characterized by a lack or decrease in clotting factors VIII (hemophilia A) or IX (hemophilia B). A proportion of hemophilia patients produce antibodies or inhibitors to the clotting factors that are administered to them, which may further complicate bleeding episodes related to hemophilia. Human Factor VII (hFVII) is a protein that when activated to human Factor VIIa (hFVIIa) causes blood to clot as part of the coagulation cascade comprised of multiple clotting factors.^{1,2} In the United States, hemophilia occurs in approximately 1 in 5000 male births, and about 20,000 males in the United States currently have this disorder. Incidence of hemophilia A is approximately four times that of hemophilia B. Given hemophilia is associated with a recessive gene linked to the X chromosome (females are XX, males are XY), hemophilia is generally considered to be much more prevalent in males and is therefore more consistently reported in the male population. In addition, approximately 20% of hemophilia A patients, and 3% of hemophilia B patients, have inhibitors to clotting factors.

As a possible means of addressing complications with inhibitors to clotting factors VIII and IX in humans, the sponsor is pursuing an approval, through FDA's Center for Biologics Evaluation and Research (CBER) of a human biologic containing purified and activated hFVIIa. The sponsor has produced genetically engineered (GE³) rabbits that express recombinant hFVII (rhFVII) in their milk for downstream processing to produce the human biologic rhFVIIa.

As part of the requirement for the approval of the human biologic, the sponsor obtained approval of the current new animal drug application (NADA) for the recombinant DNA (rDNA) construct for human Factor VII (hFVII), inserted into the genome of GE New Zealand white rabbits. The rDNA construct, designated "Bc2371," is expressed in the rabbit's mammary gland such that rhFVII protein is produced in the milk of the GE rabbits. The milk collected from the GE rabbits then serves as bulk source material for downstream production of the human biologic. The human biologic is subject to a separate approval, a biologics license application (BLA), through CBER. It is not until the sponsor obtains BLA approval (or other authorization) from CBER that they may legally distribute the biologic for treating human disease.

FDA/CVM's review of the sponsor's safety and effectiveness data supporting NADA approval followed the multi-step, risk-based, and hierarchical approach to submitting and assessing data, as captured in Guidance for Industry 187.⁴ A summary of the applicable steps in this approach to agency review are presented in Sections IV

¹ Centers for Disease Control and Prevention: <https://www.cdc.gov/ncbddd/hemophilia>. Accessed 12/04/2018.

² National Institutes of Health: <https://www.nhlbi.nih.gov/health-topics/hemophilia>. Accessed 12/04/2018.

³ Intentionally altered genomic DNA in animals reflects current description of what has been traditionally referred to as genetically engineered animals.

⁴ CVM Guidance For Industry Website:

<https://www.fda.gov/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/default.htm>. Accessed 12/04/2018.

through IX below. CVM's review took into consideration potential hazards and risks to animals, humans, and the environment associated with insertion and expression of the rDNA construct in multiple generations of the GE animal lineage. To confirm the safety of the rDNA construct to GE rabbits, CVM evaluated animal management/husbandry (e.g., animal identification, housing, containment, physical and biological security, health surveillance and management, nutrition, reproduction, milking). Based on data and information that LFB provided, CVM confirmed safety of the rDNA construct to the GE rabbits, and the durability or stability of the intended genomic change (inserted rDNA construct) and intended phenotype/claim (presence of rhFVII in the milk of GE does) over multiple generations of GE rabbits. CVM's review also found that the sponsor's post-approval plan for continued monitoring of the intended genotype and phenotype (including a remediation plan for addressing genotypic or phenotypic durability failures should they occur), routine and adverse drug experience reporting, and contingency/recovery should unplanned, catastrophic events at the facility occur (e.g., natural disaster, serious disease outbreaks, etc.) is acceptable. Relative to food safety, LFB provided a written commitment that all rabbits or their derivatives would be withheld from the food supply, and CVM's review verified that LFB's procedures assured that the likelihood of this occurring to be remote.

III. PRODUCT DEFINITION

Below is the description of the product subject to CVM's regulatory oversight. The product definition identifies, among other things, the genetic alteration and its characteristics (in this case, an inserted rDNA construct that directs the production of rhFVII in the mammary gland of the lineage of GE animals), the animal description, ploidy, zygosity, and the intended use or claim made for the lineage of GE animals.

"*Bc2371* rDNA construct integrated at a single site on chromosome 3p1.1-2 in a specific, diploid line (R69) of hemizygous and homozygous New Zealand white rabbits (*Oryctolagus cuniculus*) derived from the founder animal R69 directing the expression of the human gene for Factor VII in the mammary gland such that recombinant hFVII zymogen is present in the rabbit milk to enable purification and activation of recombinant hFVIIa intended for the treatment of hemophilia A or B patients with inhibitors to Factors VIII and IX."

IV. MOLECULAR CHARACTERIZATION OF THE CONSTRUCT

The purpose of this particular step is to describe the components and composition of the regulated article, and is intended, among other things, to identify potential hazards to animals, humans or the environment. The Agency's evaluation of the submitted data and information did not identify any specific hazards to animals, humans, or the environment that were intrinsic to the *Bc2371* rDNA construct. The Agency considers data and information submitted in support of the Molecular Characterization of the Construct as adequate to support the characterization of the construct used to generate the R69 line of GE rabbits.

Prior to integration in the host genome, the full length *Bc2371* rDNA construct used to develop the R69 line of GE rabbits comprises two major components: the insert region (also referred to as the final fragment for injection) which encodes the rhFVII protein and the flanking vector backbone sequence. The insert region is the portion of the construct that is designed to integrate into the host genome while the replication

vector consists of DNA elements necessary for *in vitro* propagation of the construct in the laboratory during intermediate steps of the generation of the construct. The identity, sequence, and orientation of each element in the *Bc2371* insert as reported by the sponsor were verified using DNA analysis software. Sequence analysis performed on the rhFVII open reading frame (ORF) within the insert region showed no gaps when aligned with the human FVII gene (F7) indicating the ability to produce functional rhFVII protein in lactating mammary gland cells. The replication vector is from a standard vector commonly used in molecular biology and genetics laboratories. It does not contain any sequence elements known to pose a hazard to the GE animal itself, other animals, human handlers, or the environment. *Bc2371* is designed in such a way that the replication vector is completely eliminated upon insert excision and subsequent elution and is not present in the final rDNA fragment eluate used in the microinjection of embryos and therefore integration of the vector into the genome of the embryos is not possible.

The insert region comprises the synthesized rhFVII ORF and regulatory sequences, such as an insulator element from the chicken beta-globin gene and regulatory sequences from the goat beta-casein gene. The rhFVII sequence is identical in protein coding ability to the human FVII gene and optimized for maximal stability and expression in rabbits. The additional regulatory sequences do not express any proteins. Their primary function is to ensure the high level of expression of the rhFVII protein in the mammary gland: the chicken insulator element ensures a high level of expression of the rhFVII protein and the goat beta-casein sequence localizes expression of rhFVII in the mammary gland. These regulatory sequences have been well studied and used in development of other GE animals expressing different proteins and have not been found to contain any hazardous sequence elements or generated adverse events in these animals.

Source and function of the sequence elements within the *Bc2371* rDNA construct along with the stepwise construction process leading to the final insert and the primary nucleotide sequence of the final construct have been analyzed and determined not to contain any hazards to the GE animal, other animals, humans, or the environment. The procedure used to excise and elute the final fragment for injection uses appropriate reagents and protocols to ensure no extraneous or hazardous materials (such as chemicals, live viruses, or bacteria) are inadvertently introduced into the rabbit embryos and genomes.

The general information submitted by the sponsor in support of molecular characterization of the construct including stepwise construct synthesis details, primary sequence of the final rDNA construct, and standard operating procedures used, is sufficient and consistent with the theoretical design. No mobilizable elements, sequences encoding toxins, allergens, or any other bioactive molecules were identified in the vector or its intermediates. The Agency's evaluation of information and data did not identify any specific hazards to animals, humans, or the environment that are intrinsic to the *Bc2371* rDNA construct. The Agency considers as complete the data and information submitted in support of the Molecular Characterization of the Construct to address the characterization of the construct used to generate the R69 line of GE rabbits.

V. MOLECULAR CHARACTERIZATION OF THE GE ANIMAL LINEAGE

The sponsor provided data and information describing the molecular characterization of the R69 animal lineage of GE New Zealand white rabbits producing rhFVII in their milk. This was necessary to establish that the integrated *Bc2371* rDNA construct was stably transmitted across multiple generations of the R69 rabbit lineage.

Based on the Agency's review of the methods, data, and information submitted by the sponsor, the Agency confirmed the integration, composition, sequence, stability and heritability of the integrated rDNA construct in the founder animal and subsequent generations. Agency's review of these data established that the integrated rDNA construct does not pose any significant risks to the animals, humans, or the environment.

Standard molecular biology methods and protocols were used to introduce the final targeting construct into the animal's genome. Based on the Agency's review, neither the methods nor the final suspension buffer for the injection fragment used to produce the GE animal contain any identifiable hazards worthy of concern for the health of animals, humans, or the environment.

A combination of Southern analysis, DNA sequencing, and FISH, was used to determine the genotype over multiple generations of the R69 lineage. The data supported that the genotype of the R69 line of GE rabbits is stable over eight generations.

VI. PHENOTYPIC CHARACTERIZATION OF THE GE ANIMAL LINEAGE

In support of phenotypic characterization of the GE animal lineage, the sponsor provided their comprehensive procedures for facility and animal management, including data and information on the health of animals (e.g. records and data summaries related to veterinary oversight, daily health monitoring, treatments, and periodic pathogen screening). Based on the Agency's review of the data and information submitted in support of phenotypic characterization, no animal health hazards/risks for health events (type or frequency) were observed for the R69 rabbits when compared to the non-GE/wild-type (WT) rabbits. In conclusion, there were no identified hazards to the animals, humans, or the environment with respect to the *Bc2371* rDNA construct used in R69 rabbits producing rhFVII in their milk.

A. Production Facility

The LFB USA facilities in Massachusetts encompass 380 acres of land in total. The area is comprised of two species-specific areas physically separated by a public road, with dedicated operations, facilities, and personnel. The Charlton Rabbit Facility (CRF) is located on a 212 acre specific parcel of that land and is dedicated for the production and use of R69 rabbits and milk collection within the Sevenfact (rhFVIIa) program. The GE R69 rabbits are restricted to the CRF which was designed and is maintained as a species-specific, specific pathogen free (SPF), barrier facility with dedicated staff. Separate from CRF, LFB USA facilities in Charlton include areas for milk storage, testing, and intermediate processing along with company related support activities for the program as appropriate. Access to CRF property is restricted with appropriate perimeter fencing controlled by security access to serve as an initial barrier. Procedural controls and security access limits are also employed for personnel entry into the building. Gowning and

passage through an "air shower" is required. Security access controls prevent unauthorized area access and further promotes species segregation.

B. Source Genetics and Reproduction

To produce the original founder GE rabbits (in the U.S.), fertilized one-cell embryos from New Zealand white (NZW) rabbits were microinjected with the purified *Bc2371* rDNA construct. Following a brief period of *in vitro* culture, the microinjected embryos were transferred into hormonally synchronized wild-type (WT) female NZW recipients. Offspring (F0) from these females were then screened by PCR analysis for the presence of the *Bc2371* rDNA construct using hFVII-specific primer sequences.

To establish the initial colony of production rabbits, fresh semen from an F1 R69 male was exported from the U.S. to France to establish colonies of rabbits for milk production used for subsequent purification/activation of rhFVII to rhFVIIa for use in non-clinical animal and human clinical trials. In 2015, the Charlton, MA, U.S. facility was established by importing from France non-GE and R69 rabbits (F3-F4 generations) for mating (artificial insemination) of R69 males to non-GE females, and multiple generations of GE females have been produced for source material collection. Does in the production herd are not used to produce future breeding animals.

The genealogy of the R69 rabbits is recorded for each animal and may be used to aid selection of optimal milk producing rabbits. To ensure the stability of the production colony, semen from qualified breeding males (i.e., males that have sired females of the production group and that have passed the genetic characterization testing) is collected for artificial insemination of R69 or non-GE females to maintain the breeding colony and to produce hemizygous females for milk production. At the facilities in France, semen from qualified breeding males that are as close as possible to the genetic founder in terms of generation number (F3 males) is collected as part of a Master GE Bank. The semen from this bank is stored at two different sites in France to prevent loss due to a catastrophic event. The rest of the breeding male (hemizygous and homozygous alike) semen as well as the living males are considered part of the Working Transgenic Bank. Additional semen will be collected, as needed, from future qualified offspring of those males to ensure the ability to continuously propagate the rhFVIIa production group. Currently, semen collected from males is used to breed does in the colony; the CRF does not currently cryopreserve semen for the Master GE Bank. Genetic backup for CRF rests in semen and/or live GE and non-GE rabbits at the facilities in France.

C. Animal Identification, Segregation, and Caging

Animals are segregated within cages, racks and rooms according to GE status (e.g., non-GE vs. GE). Each rabbit within the colony is given a unique identification number that is applied redundantly by tattooing, implantation with electronic transponders, and use of identification cards at the cage level. Ear tags are used and typically placed within the first few days after birth. Ear tags provide temporary identification of kits until microchipped at approximately 11 days of age, and ears are tattooed at approximately 35 days of age. A master list of all assigned numbers is maintained to facilitate individual rabbit identification and

traceability. Confirmation of GE status via PCR is conducted and documented for new/colony replacement animals according to established procedures. The PCR is performed on the first 7 offspring from any homozygous male to confirm the rDNA construct is heritably passed from sire to offspring.

The campaign groups (i.e., cohorts of does subject to breeding, gestation, and lactation for a specific time period) are color-coded with stickers on the cage card for rapid identification of what group a doe belongs to. Therefore, the groups correspond to does lactating and milked, does resting and breeding, and does pregnant. The timing is planned to minimize the gap between milk campaigns so that milking occurs on a relatively continuous basis.

D. Feeding and Nutrition

Kits are allowed to nurse directly from the doe until the time of weaning when kits are approximately three weeks of age. Kits retained as part of the breeding program are transitioned to solid feed.

LFB USA feeds two pelleted feeds. Commercially prepared and irradiated pelleted feeds are specifically manufactured for LFB USA and provided to the rabbits via feeders within the cage. In accordance with established procedures, all feed undergoes a decontamination and inspection process (i.e., confirmation of bag integrity, and irradiation per coloration of an indicator/sticker) prior to entry into the barrier facility. Before filling the feed receptacles, the quality of the concentrate is visually inspected for any signs of gross contamination such as mold, using established procedures. Feed is used within established manufacturer expiration periods. The rabbits receive a nutritionally balanced ration with respect to the rabbits' age, size, and condition (NRC, 1977).⁵ Two diets are currently in use at CRF:

- One diet is provided to kits and weanlings up to 13 weeks of age to allow ad libitum consumption. Animals over 13 weeks of age are provided a daily ration of 150 grams (includes males and young non-lactating females).
- Another diet is provided to females approximately 6 days prior to anticipated breeding and/or kindling date and during lactation to allow ad libitum consumption. Females will return to a daily ration of 150 grams until the next kindling/lactation cycle.

Rabbits have free access to water via automatic watering devices.

E. Milking Procedures and Nursing Kits

Breeding activity typically begins around 17 weeks of age and GE females are designated into 1 of 3 groups (lactating/milked, resting/breeding, and pregnant) that rotate and cycle through a total of 17 milk collection campaigns a year. Each group is bred and milked approximately 5-6 times a year.

⁵ National Academy of Sciences, National Research Council, Nutrient Requirements of Rabbits, Second Revised Edition, 1977.

For replenishment of GE females (milk production colony), non-GE females are mated to GE males every 6 weeks for a total of 8 breedings per year. The offspring are brought into the milking rotation upon reaching breeding age. The WT colony is replenished by non-GE males and females mating every 6 weeks for 8 breedings per year. GE males are replenished by mating GE males to GE females 3-4 times per year. Animals are not typically culled based on age but rather a variety of factors including health and breeding performance.

Throughout the entire milking campaign, does are kept with their kits/litter to ensure the necessary physiological signal for milk production. The does are separated from the kits at set times daily to ensure that adequate milk is produced and available for collection. Separately, does are afforded adequate time daily (currently once a day) to feed her kits.

Standardized procedures have been developed for collecting milk from the R69 rabbits and associated record keeping/documentation. This milk collection procedure follows standard dairy milking practices and has only been slightly modified to best fit the rabbit with its unique anatomy and physiology. Milk is collected utilizing a bovine dairy milking machine modified for use on the rabbit. Appropriate vacuum settings and pulsation cycles have been set for use in the rabbit to maximize milk collection while affording the optimal safety and animal health and welfare to the rabbit. Procedures are in place for care, cleaning and maintenance of the milking unit/equipment following standard and universally recognized dairy equipment cleaning practices.

Rabbits are brought into the milking parlor and their general health evaluated and noted. The mammary gland/teats are prepared for collection by application of an appropriate sanitizer and wiped with a paper towel to remove any potential dirt/feces/bedding debris. Just prior to milk collection, oxytocin is administered intravenously (IV) to each doe to facilitate milk "let down". Milk is collected under vacuum utilizing "teat cups" that are attached through milk lines to a receiver containing a collection bottle. Once milking is completed, the doe is returned to her cage and has access to her kits/litter. It is during these processes that GE does in the milk production colony are subject to frequent handling, observation for health status, and documentation of such observations (observed up to nine times a day, see discussions related to morbidity below).

Does from a cohort that begin lactation at the same time are milked as a single group (or "campaign"). Milk collected from does on Days 4-22 of a given cohort contribute to source material for that campaign. Does are presented for milking in groups of 6 and collected as a single "mini-pool" for each day; mini-pools across the lactation are pooled for that campaign. Multiple campaigns may be pooled into a single "starting material pool" for entry into the manufacturing process for the human biologic.

F. Health Management Procedures and Observations

Animal care:

LFB USA established a Rabbit Oversight Committee (ROC) to oversee rabbit operations, and utilizes standards set forth by the National Research Council (2011) as a basis for evaluating and maintaining best practices as well as LFB

USA's own policies for animal health, welfare and care.⁶ The ROC composition includes two veterinarians, one scientist, one non-affiliated member and two LFB rabbit operations personnel, and operates according to established procedures. The rabbit health program was developed from an in-depth knowledge of the relevant rabbit diseases of concern and in conjunction with the recommendations from an international expert veterinary and virology panel the sponsor convened, representing veterinary, viral, academic, industry, and research disciplines. Operational procedures govern animal handling and standardized practices are employed to maintain the health and well-being of the colony.

Veterinary and on-call coverage is provided at all times. Dedicated CRF staff performs all routine animal husbandry activities, including but not limited to daily feeding, milking, watering, and breeding of the rabbits. Execution of daily tasks and associated training is conducted in accordance with documented procedures. Personnel performing daily husbandry tasks are trained to notice and report any changes in a rabbit's appearance and/or behavior (to include pain and/or distress) to the veterinary team. If a rabbit's condition is of concern, a member of the veterinary team is contacted and an evaluation initiated and appropriate actions taken as appropriate.

Following daily animal health observations and handling procedures, if there are any observations of concern, an animal observation/treatment record is opened and maintained for any rabbit presenting with a potential clinical/health abnormality.

Mastitis:

The management practices used to minimize risk of mastitis include: proper hygiene and preparation of the mammary gland for milk collection, proper positioning of milking equipment during milking operations, and avoidance of over-milking the rabbit. Additional practices include: proper maintenance of the milking equipment, proper cleaning of the milking equipment, documentation of mastitis and subsequent removal of animals from the milking campaign, and provision of a clean, dry housing environment.

Therapeutic Treatment

Treatment of a clinical entity or health related matter of an animal is based on the severity of the clinical presentation and all such treatments are recorded. All conditions, no matter how minor, are fully documented. Animals receiving medical intervention (with potential impact to source material) are removed from the active milking campaign according to established procedures. Standard operating procedures (SOPs) exist to prevent treated/medicated animals from contributing to source material collection for downstream processing to produce the human biomedical product.

⁶ National Academy of Sciences, National Research Council, Guide for the Care and Use of Laboratory Animals, 8th edition, 2011.

Morbidity

GE does in the milk production colony are routinely handled upwards of nine times per day with an increased opportunity to make health related observations on these does. As a result, all reported observations, regardless of severity or whether follow-up therapy is needed, are subsequently documented and incorporated/counted in morbidity totals. Observations related to daily health monitoring, veterinary care, and therapies administered for any condition, regardless of the severity, that personnel have identified for a particular animal's health (i.e. dry skin or hair loss due to nesting behaviors, etc.) are included in morbidity tabulations. Rabbits may have more than one observation recorded as a new health case in the same period of time. The resulting morbidity data tabulations (represented by new health cases) are consistent with the heightened attention afforded to a highly monitored population of GE does in the milk production colony. Morbidity (Table1) represents the prevalence of new treatment/health cases initiated by the Rabbit Operations staff for a given period (quarterly, 2017) for R69 and non-GE rabbits at CRF. Indeed, the increase in overall morbidity prevalence in GE vs. non-GE rabbits was predominantly associated with integument/hair observations, likely due to signs related to nesting behavior during frequent observation and handling. When new cases of skin/hair/integument morbidity were removed from consideration, morbidity was generally comparable between GE and non-GE rabbits (see Table 1).

Table 1. Quarterly morbidity summary for Q1-Q4 2017 (Prevalence¹) in genetically engineered (GE) and non-GE rabbits.

	2017 Q1		2017 Q2		2017 Q3		2017 Q4	
	GE	Non-GE	GE	Non-GE	GE	Non-GE	GE	Non-GE
GE status of rabbits								
Rolling Population	680	106	654	100	687	100	753	111
Morbidity Cases (Integument) ²	113	6	81	2	90	6	117	5
Morbidity Cases (less integument) ³	140	33	134	24	160	22	212	19
% Morbidity (less Integument) ⁴	20.6%	31.1%	20.5%	24.0%	23.3%	22.0%	28.2%	17.1%

¹Prevalence (% Morbidity) is the number new treatment/health cases in rabbits expressed as a percentage of rolling population for that quarter. Note that a rabbit may have more than one new case during a quarter.

²Total number of new morbidity cases attributed to hair/skin/integument only.

³Total number of new morbidity cases that include observations related to the following systems/categories: musculoskeletal, respiratory, reproductive, digestive, nervous, mammary, ophthalmic, urinary, behavior, and unthrifty/failure to thrive (integument excluded).

⁴% morbidity (less integumentary) represents the number of new morbidity cases minus those cases attributed to hair/skin/integument expressed as a % of the rolling population for that quarter.

Mortality

Mortality rates for R69 and non-GE rabbits are determined from multiple subcategories reported as health and non-health related causes. Underlying causes of mortality (Table 2) include categories as varied as

sudden death syndrome (SDS), euthanasia for non-treatable issues (e.g. musculoskeletal irregularities, birthing complications, etc.). Routine colony population management/husbandry procedures are not included in this summary. Tabular mortality summaries are based on detailed quarterly reports from LFB that contain the applicable data broken down by specific category/ body system. Examples of health-related categories/systems are: musculoskeletal, reproduction, urogenital, ophthalmic, respiratory, central nervous system, cardiovascular, failure to thrive, and spontaneous demise. Categories that are not indicative of physical health concerns (examples are behavior, colony management, and animals submitted to diagnostic laboratories for colony health surveillance) are not included in the table. Rabbits within these categories are counted as part of standard culling/colony management procedures, and are generally, otherwise healthy animals. Mortality (Table2) represents the prevalence of new death cases for a given period (quarterly, 2017) for GE and non-GE rabbits at CRF.

Table 2. Quarterly mortality summary for Q1-Q4 2017 (Prevalence¹) in genetically engineered (GE) and non-GE rabbits.

	2017 Q1		2017 Q2		2017 Q3		2017 Q4	
	GE	Non-GE	GE	Non-GE	GE	Non-GE	GE	Non-GE
GE status of rabbits								
Rolling Population	680	106	654	100	687	100	753	111
Mortality Cases ²	17	1	36	10	27	7	32	3
% Mortality	2.5%	0.9%	5.5%	10.0%	3.9%	7.0%	4.2%	2.7%

¹Prevalence (% Mortality) is the number of animals that died or were euthanized for cause in a period expressed as a percentage of rolling population for that quarter.

²Table captures mortalities due to spontaneous death and euthanasia for untreatable and serious health conditions (musculoskeletal, central nervous, ophthalmic, respiratory, urinary, and unthrifty/failure to thrive). Mortality in the table do not include euthanasia for purposes of routine colony population management purposes.

Morbidity and Mortality Determinations for Reporting Purposes Defined

The rolling population is the average of the monthly census for the facility for that quarter. The end of the month census is the total animal count the last day of the month but does not include kits who are still nursing. Therefore prevalence (% Morbidity and Mortality) is the number of animals with new health cases (morbidity) or deaths (mortality) in a period, expressed as a percentage of the rolling population for a particular quarter.

Summary

Based on the evaluation of morbidity and mortality data, there are no biologically significant differences in health status between GE and non-GE rabbits attributable to the presence of the rDNA construct in GE rabbits. Apparent increases in

morbidity of GE vs. non-GE rabbits are attributable to the increased frequency of handling and observation of GE rabbits (hair/skin/integument). In addition, prevalence of mortality rates is not increased in GE vs. non-GE rabbits. The health status of the rabbits at CRF is further supported by the lack of positive results in pathogenicity testing (see further discussion below).

Pathogen screening:

LFB contracts rabbit disease testing to various local/regional veterinary diagnostic laboratories based upon the availability of field experts with a track record of diagnostic quality. Routine SPF health screening, through a health monitoring (modified sentinel testing) program is performed on an established schedule to include animals that are statistically representative of the entire colony and population (gender, age, genetics [e.g., R69 vs. non-GE]). Established procedures govern action and follow-up response (up to and including product recall) in the event of confirmed positive results for agents on the SPF exclusion list.

Prior to and during establishment of the rabbit colony at CRF, LFB convened an international rabbit expert viral panel (representing veterinary, viral, academic, industry, and research disciplines) to recommend pathogens for one-time and ongoing/periodic surveillance sampling screening. The panel recommended a list of pathogens for one-time screening at the time of colony establishment:

- Rabbit Adenovirus
- Endogenous Retrovirus
- Rabbit Coronavirus
- Rabbit Hepatitis E Virus
- Rabbit Parvovirus
- Rabbit Picobirnavirus

Recommended periodic and ongoing screening was as follows:

Table 3. Health Monitoring Profile for the Charlton Rabbit Facility.

Disease Agent	Frequency
Rabbit Hemorrhagic Disease Virus	Monthly
Rabbit Rotavirus	Monthly
Myxomatosis Virus	Monthly
Endogenous Retrovirus	Annually
Rabbit Coronavirus	Annually
Picobirnavirus	Annually
Rabbit Adenovirus	Annually
Rabbit Parvovirus	Annually
Rabbit Hepatitis E Virus	Annually
<i>Bordetella bronchiseptica</i>	Monthly

Disease Agent	Frequency
<i>Clostridium piliforme</i>	Monthly
Encephalitozoon cuniculi	Monthly
<i>Pasteurella multocida</i>	Monthly
<i>Salmonella spp.</i>	Monthly
<i>Escherichia coli</i> (O103) with enteropathogenic strains	Monthly
<i>Streptococcus b-haemolytic B</i>	Monthly
Ectoparasite: <i>psoroptes cuniculi</i>	Monthly
Endoparasites: helminths	Monthly
Pathological lesions	Quarterly
Mastitis screen	Each milking

Up to the time of NADA approval, LFB obtained no positive screening tests for any of the listed agents.

Overall and in support of phenotypic characterization of the GE animal lineage, the sponsor provided their comprehensive procedures for facility and animal management, including data and information on the health of animals (including records and data summaries related to veterinary oversight, daily health monitoring, treatments, and periodic pathogen screening). Based on the Agency's review of the data and information submitted in support of phenotypic characterization, it was concluded there were no identified hazards to the animals, humans, or the environment with respect to the *Bc2371* rDNA construct used in R69 rabbits producing rhFVII in their milk. This extends to our assessment of the safety of the rDNA construct to the GE rabbits in that we observed no additional animal health hazards/risks for health events (type or frequency) in the R69 rabbits vs. the non-GE comparators.

VII. FOOD SAFETY

A. Food Safety

The sponsor indicated that they do not intend for the *Bc2371* rDNA construct in the R69 rabbits, nor any materials derived from this GE line of rabbits, to enter the human or animal food supply. This statement was confirmed during the Agency's inspection of animal and waste disposal practices at the sponsor's facilities. The sponsor has SOPs in place for the appropriate disposition of animals, waste, and any milk that does not meet quality standards for use in producing the human biologic. Thus, the likelihood of edible products from R69 rabbits inadvertently being diverted into the food supply is negligible.

Control measures include the following:

- Secure facility, with gated and fenced perimeter and electronic personnel entry and exit monitoring;
- Active on-site security supplemented with video surveillance;
- Specific pathogen free housing within the facility with environmental control and strict animal containment;
- SOPs describing and controlling personnel, materials, equipment, and rabbit, rabbit by-product, and waste flow;
- SOPs for animal identification and disposal that include procedures to ensure that:
 - all R69 animals are uniquely identified with ear tags at birth, and with tattoos and RFID transponders at weaning;
 - all animals are traceable based on their unique animal ID;
 - all rabbits and rabbit by-products are disposed *via* a contractor for off-site incineration.

Based on the high degree of containment at the sponsor's facilities, it is unlikely that any R69 rabbits would escape the facilities in which they are raised and housed. In the extremely unlikely event of escape, each rabbit has a unique form of identification (ear tag, tattoo, RFID), and even if any of those forms of identification were lost, and a rabbit of unknown provenance were discovered near the facility, there is a regulatory analytical method for identity in place at the Agency (see Section B) to determine whether it is an R69 GE rabbit. Additionally, New Zealand white rabbits are visually distinct (size and color) from the native rabbits in the habitat surrounding the sponsor's facilities.

The hazard likely to be posed by inadvertently consumed and digested rhFVII zymogen also is negligible: rhFVII (*via* its activated form, rhFVIIa) is one of several blood clotting factors that comprise the normal coagulation cascade and is naturally produced in humans. The purpose of producing rhFVII zymogen in the R69 GE rabbits is for the treatment, with further purified/activated rhFVIIa human biologic, of hemophilia A and B patients with inhibitors to Clotting Factors VIII and IX. In addition, rhFVII is highly-conserved, with an approximately 74% amino acid identity between rabbits and humans. The Agency expects that rhFVII would be digested, as would rabbit FVII in milk or edible tissues from R69 rabbits.

It is concluded that there is a reasonable certainty that R69 GE rabbits will not be introduced into the human or animal food supply. Based on Agency evaluations there is a low⁷ level of concern should edible products of R69 GE rabbits inadvertently enter the food supply.

B. Analytical Method for Identity

1. Description of Analytical Method for Identity

The regulatory analytical method for the presence of the *Bc2371* rDNA construct in rabbit muscle tissue is a polymerase chain reaction (PCR) method

⁷ The agency has previously determined that a food safety assessment for biopharm animals that relies primarily on stringent animal identification and disposal protocols, and the probability of introduction of food products from those highly contained GE animals was negligible, that the agency's conclusions with regard to level of concern regarding highly unlikely food consumption risk would be categorized as "low", "medium", or "high".

which provides acceptable sensitivity for routine monitoring to identify and confirm whether the tissue is from R69 GE rabbits. The method detects the presence of the *Bc2371* rDNA construct at a single integration site on chromosome 3p1.1-2 in the genome of R69 GE rabbits.

2. Availability of the Method

The validated regulatory method for detection of the *Bc2371* rDNA construct is on file at the Center for Veterinary Medicine, 7500 Standish Place, Rockville, MD 20855. To obtain a copy of the analytical method, please submit a Freedom of Information Summary request to:

<https://www.accessdata.fda.gov/scripts/foi/FOIRequest/requestinfo.cfm>.

VIII. GENOTYPIC AND PHENOTYPIC DURABILITY

The data provided demonstrate that both the genotype and phenotype of the R69 lineage are conserved over multiple generations (Generations F1-F9 for genotype; see Section V above; Generations F5-F7 for phenotype, see Table 4, Section IX below). These data adequately demonstrate that the genotype and phenotype of these rabbits are durable, the rDNA construct for rhFVII 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 R69 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 procedures for maintenance and/or re-derivation of the R69 lineage of GE rabbits. Together, the data and information the sponsor provided assure that the R69 lineage will continue to be equivalent to those rabbits evaluated prior to NADA approval.

IX. CLAIM VALIDATION

The sponsor's enzyme-linked immunosorbent assay (ELISA) method is used to confirm the presence of rhFVII zymogen in the milk of R69 does (per their claim in the product definition; see Section III above). The sponsor's description of their ELISA method, which is based on a modified commercially-available kit, was reviewed and the method was found to be appropriate for the purposes of validating their claim. The sponsor's validation data of the ELISA method was also reviewed and the method was shown to be suitably validated in all applicable parameters.

LFB's phenotypic durability plan (see Section VII above) provides for continued monitoring of phenotype to assure continued qualification of production does, and for phenotypic failure with remediation procedures implemented based upon follow-up investigation/risk assessment.

In Generations F5, F6, and F7, approximately 96% of does qualified to enter production for Lactation 1. At Lactation 2, approximately 80% of the retested does met the qualification criterion; the remaining unqualified does (~20% of those retested) were removed from the production group. Unqualified does met the criteria for genotypic durability prior to their entry into the production. Rather, reduced

concentrations of rhFVII in milk of these does reflect normal biological variation such that a very small number of rabbits might not qualify to remain in the production colony. Table 4 provides a summary of the qualified does and milk sample values (average and range) from these generations.

Table 4. Qualification results from ELISA method for hFVII in milk samples in R69 lineage production rabbits (Generations F5, F6, and F7).

Number of CRF Qualified Females	918
Average Day 4 rhFVII Concentration (mg/mL)	0.3757
Range of Day 4 rhFVII Concentrations (mg/mL)	0.1005 – 0.8652

Based on the review of data and information submitted by the sponsor, the Agency concluded that the data support the claim established in the product definition (Section III above).

X. 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. The data demonstrate that the *Bc2371* rDNA construct in R69 New Zealand white rabbits, when used according to the labeling, is safe and effective for the expression of a rhFVII encoding gene in the mammary gland, such that rhFVII zymogen is present in the rabbit milk to enable purification and activation of rhFVIIa intended for the treatment of hemophilia A or B patients with inhibitors to Factors VIII and IX. Food or feed from R69 New Zealand white rabbits is not permitted in the food or feed supply.

EXCLUSIVITY

The exclusivity provisions of section 512(c)(2)(F) of the Federal Food, Drug, and Cosmetic Act do not apply to this drug 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 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.