Date of Approval: December 8, 2015

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-453

hLAL rDNA construct in SBC LAL-C chickens

Heritable Construct

Domesticated Chickens

hLAL rDNA construct integrated at a single site (the SYN LAL-C site in chromosome 6) as a single copy, in a specific, diploid line (SBC LAL-C) of hemizygous and homozygous domestic chickens (*Gallus gallus*) derived from the lineage progenitor XLL 109, expressing a human lysosomal acid lipase (rhLAL) encoding gene such that rhLAL protein (intended for the treatment of humans) is present in their egg whites.

Sponsored by:

Alexion Pharmaceuticals Inc.

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I. GENERAL INFORMATION

A. File Number

NADA 141-453

B. Sponsor

Alexion Pharmaceuticals, Inc.
33 Hayden Avenue,
Lexington, MA 02421

Drug Labeler Code: 69334

C. Proprietary Name

hLAL rDNA construct in SBC LAL-C chickens

D. Species/Class

Domesticated chickens (*Gallus gallus*)

E. Indication

Production of recombinant human lysosomal acid lipase (intended for use in humans) in egg whites of genetically engineered chickens.

II. PRODUCT DEFINITION

hLAL rDNA construct integrated at a single site (the SYN LAL-C site in chromosome 6) as a single copy, in a specific, diploid line (SBC LAL-C) of hemizygous and homozygous domestic chickens (*Gallus gallus*) derived from the lineage progenitor XLL 109, expressing a human lysosomal acid lipase (rhLAL) encoding gene such that rhLAL protein (intended for the treatment of humans) is present in their egg whites.

III. MOLECULAR CHARACTERIZATION OF THE CONSTRUCT

Prior to integration in the host genome, the full length hLAL rDNA construct used to develop the SBC LAL-C line of genetically engineered (GE) chickens comprises two major components: the insert region 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 vector backbone consists of DNA elements necessary to propagate the construct in the laboratory during intermediate steps of the generation of the construct. The identity, sequence, and orientation of each element in the rDNA construct as reported by the sponsor were verified using DNA analysis software. Sequence analysis performed on the hLAL coding region within the insert region showed no gaps when aligned with the human *LAL* gene indicating the ability to produce functional hLAL protein in chicken oviduct cells. The vector backbone is from a standard vector commonly used in molecular biology and genetics laboratories around the world. It does not contain any sequence elements known to pose a hazard to the

GE animal itself, other animals, human handlers, or the environment. The vector backbone is not packaged into the transducing particle and is not present during rDNA construct integration into the genome.

The insert region comprises upstream and downstream regulatory sequences from the chicken ovalbumin gene, the hLAL encoding sequence, and two flanking long terminal repeat (LTR) sequences. The chicken ovalbumin enhancer, promoter, upstream sequences, and downstream 3' untranslated region are identical to the native ovalbumin gene present in the chicken genome and do not contain any hazardous sequence elements. The hLAL sequence is identical in protein coding ability to the native human *LAL* gene and optimized for maximal expression in chickens. Chickens have an orthologous *LAL* gene which is very similar to hLAL and therefore, the hLAL encoding sequence in the insert does not constitute a hazard to the chickens as every cell expresses the native chicken version of this housekeeping gene. The LTRs are self-inactivating (deleted for the U3 region in the 3' LTR), replication-deficient (no functional *gag*, *pol*, or *env* genes), and outside and antisense to the hLAL transcriptional unit. Thus, any hazards that could be posed by the LTRs in terms of replication, virulence, recombination with viral sequences in the chicken genome, expressing its own genes, causing promoter interference with respect to the hLAL expression cassette have been minimized or mitigated by design and engineering features of the final hLAL rDNA construct.

Source and function of the sequence elements within the hLAL rDNA construct along with the stepwise construction process leading to the final hLAL rDNA 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 package the construct into transduction-capable particles uses appropriate reagents and protocols to ensure no extraneous or hazardous materials (such as chemicals, live viruses, or bacteria) are inadvertently introduced into the chicken 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 hLAL 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 SBC LAL-C line of GE chickens.

IV. MOLECULAR CHARACTERIZATION OF THE GE ANIMAL LINEAGE

The intent of transducing chicken embryos with the hLAL rDNA construct containing virus particles is to facilitate integration of the insert region (hLAL coding sequence under the control of chicken ovalbumin regulatory sequences) into the chicken genome such that it results in an integrant that is stable, heritable, and expressed in the hen oviduct with the resultant hLAL protein partitioned to the egg white. The sponsor provided data and information describing the derivation and molecular characterization of the hLAL producing SBC LAL-C GE chicken lineage.

The standardized reagents and methods for transduction of chicken embryos, production of GE chickens, and controlled husbandry conditions in place at the sponsor's facilities addressed any possible hazards arising in the steps taken to produce chimeric G_0 chickens. The Agency's review of the data and information provided found no hazards to the chickens, humans, or the environment arising from the transduction of stage X chicken embryos with hLAL rDNA construct containing particles.

Based on the Agency's review of the sponsor-provided detailed descriptions of methods and results used to verify the integration, location, and composition of the integrated rDNA construct (see Table 1) in the G_0 chimera as well as subsequent generations, the Agency confirmed the integration of the rDNA construct, its site of integration, composition, sequence, stability, and heritability as per Mendelian segregation. Agency review of these data did not indicate that the integrated rDNA construct or its locus of integration posed a hazard to the chickens, humans, or the environment.

Table 1: Summary of the assays performed to verify integration, location, and composition of the integrated rDNA construct in SBC LAL-C chickens. Data were provided for animals from the first three generations.

Assay	**G_1**	**G_2**	**G_3**
TaqMan PCR (blood)	√	√	√
Southern Blot- BlpI	√	x	x
Southern Blot- ApaLI	√	x	x
Southern Blot- HindIII	√	x	x
Southern Blot- BciVI	√	x	x
Insertion site PCR	√	√	√
hLAL ORF sequence	√	√	√
FL rDNA construct sequence	√	x	√
Zygosity PCR	x	x	√

FL = Full length
PCR = Polymerase chain reaction
ORF = Open reading frame
√ = Data provided
X = No data provided

Southern blot analysis data using multiple restriction enzymes and various probes indicate that the rDNA construct was integrated at a single locus that is stably inherited in all G_1 progeny examined. PCR amplification on genomic DNA extracted from multiple generations of GE chickens confirms that the SYN LAL-C integration site in chicken chromosome 6 remains unaltered across seven generations. PCR amplification of the hLAL coding region within the integrated rDNA construct and sequencing of the resulting amplicon provide additional confirmation that the inserted rDNA construct contains hLAL protein coding sequence with no signs of any sequence alterations. Analysis of the sponsor-provided full length rDNA construct sequence from two noncontiguous generations of SBC LAL-C GE chickens (G_1 and G_3, see Table 1 above) shows conclusively that all functional elements of the hLAL rDNA construct insert region are integrated, there are no changes in sequence within the critical functional elements that could affect hLAL function, and that the rDNA construct sequence

remains unaltered as it is passed on from one generation to another. The sequence of the rDNA construct integrated in the genome of SBC LAL-C GE chickens is consistent with the data presented in support of the Molecular Characterization of the Construct. Based on the review of these data and information, the Agency concludes that the full length rDNA construct is stably integrated as a single copy at a single locus in chicken chromosome 6 and is inherited with no alterations across multiple generations tracing their lineage back to a single G_0 chimeric male. The data provided support the conclusion that the genotype is not changing over the life span of an individual animal or across generations.

The Agency concludes that the information provided by the sponsor regarding the Molecular Characterization of the GE Animal Lineage is consistent and in agreement with the Molecular Characterization of the Construct. All the elements described in the rDNA construct have been confirmed to be integrated in SBC LAL-C GE chickens containing the rDNA construct. Data provided on the molecular stability of the hLAL coding region, relative arrangement of functional elements within the rDNA construct, and stability of the insertion site from at least three generations; as well as the DNA sequence of the full length rDNA construct from animals belonging to two non-contiguous generations; indicate genotypic stability and the lack of hazards to the animal from the inserted DNA sequence. Review of the methods, assays, and Standard Operating Procedures (SOPs) for rDNA construct and GE animal generation did not reveal the presence of any hazards due to contaminants, cells, toxins, allergens, other bioactive agents, or chemicals. From the review of the submitted data and information, the Agency did not identify any specific hazards to the animals, humans, or the environment that are intrinsic to the rDNA construct or its insertion into the GE animal. The G_0 male chicken XLL 109 has been established as the founder and lineage progenitor for the SBC LAL-C line of GE chickens. Data and information provided by the sponsor are adequate to support the characterization of the SBC LAL-C GE chicken line.

V. PHENOTYPIC CHARACTERIZATION OF THE GE ANIMAL LINEAGE

A. Production Facilities

The sponsor maintains SBC LAL-C chickens at three production facilities in the US. The SBC LAL-C line is housed, managed, and maintained in the same manner as non-GE laying hens for commercial poultry production. Chickens are co-housed with 3-5 chickens per enclosure, and meet or exceed space requirements for caged laying hens (Federation of Animal Science Societies, 2010; United Egg Layers, 2010). Each animal production facility is designed and operated with biosecurity, containment, and animal health as major considerations. Security systems are in place at all sites to prevent entry by unauthorized personnel. Prevention of external and internal contamination is attained through physical barriers and procedures for material and personnel entry and exit. Environmental controls are established with necessary quality system elements providing appropriate assurances for animal and egg segregation. Environmental conditions are maintained for temperature and humidity throughout the various stages of animal maturation. Controls are in place for feed and water supply, and disposal of waste materials is strictly managed. Individual health monitoring is performed at all facilities in addition to daily observation of all animals for signs of illness or injury. Non-fertile eggs are collected daily for harvest of egg white.

B. Source Genetics and Reproduction

Source genetics upon which the SBC LAL-C chickens were originally produced came from a commercial layer hen line of the White Leghorn breed/type. Once the SBC LAL-C line was established within a production facility (via introduction of fertile eggs from the company's research/production facilities), the line is self-sustained within each production facility by artificial insemination of SBC LAL-C hens with fresh-extended semen collected from SBC LAL-C males at a given facility. Hens used to produce non-fertile eggs for egg white harvest are also used in the breeding campaigns for production of fertile eggs for subsequent hatching/brooding. During the breeding campaigns, eggs collected from production rooms of inseminated hens are not used for egg white harvest. Each production facility is set up to incubate/hatch fertilized eggs and brood newborn chicks. SBC LAL-C hens are maintained for a single cycle of egg production (≤ 90 weeks of age; ~50-70 weeks of egg production), after which hens are removed from production and replaced with hens from subsequent hatches.

C. Animal Identification

Each chicken hatched at the sponsor's facilities receives a unique identification number within one day after hatching (neck tag or wing bands). If original identification is by neck tag, the neck tag is replaced within seven days of hatching with two wing bands (one in each wing). Traceability of animal inventory via physical identification (neck tags, wing bands) and PCR-based genotyping provides for appropriate tracking, control, segregation, and accountability of chickens, eggs, and harvested egg white.

D. Feeding and Nutrition

The sponsor uses a single feed manufacturing source to supply diets for all production facilities. All feeds used at the production facilities are gamma irradiated for bacterial control. Two diets, a chick diet for feeding to growing chicks, and Layer Hen diet for feeding to laying hens and males are maintained at the production facilities.

The diets are comprised of ingredients typical for poultry feeds, contain no animal byproducts, and are formulated to meet the nutrient requirements for chickens of the particular production class (chick or layer hen) as defined by the National Research Council of the National Academy of Sciences (NRC, Nutrient Requirements of Poultry, Ninth Revised Edition, 1994). Diets at the production facilities, nutrient and contaminant analyses (as reflected in certificates of analyses for each lot of feed) and irradiation (as reflected in certificates of processing) are reviewed prior to qualifying the feed for use at the facilities. Nutrient evaluation takes into consideration percentages of protein, fat, calcium, phosphorus, moisture, and ash. Contaminant screening includes assay for heavy metals, mycotoxins, chlorinated hydrocarbons, and organophosphates. Diet formulations and associated nutrient contents are consistent with typical chick and layer hen diets produced for commercial poultry production. Feed is offered to birds to allow for *ad libitum* consumption.

All production facilities are supplied potable water from the local municipality. Drinking water is supplied to chickens via peck-activated valve drinkers to allow *ad libitum* water consumption. Valve drinkers are checked daily for proper operation. Collected water samples are analyzed quarterly for total bacteria, coliforms, turbidity, and nitrates, and on an annual

basis for organophosphates (or certificates of water analysis received from the municipality's water supply departments).

E. Health Management Procedures/Observations

The sponsor has SOPs in place for application of current animal husbandry and management procedures to assure continued good health and absence of disease agents. Each facility is subject to regular oversight by facility-specific veterinarians and Institutional Animal Care and Use Committees.

1. Daily Observations

   At least daily, technicians perform the following tasks, and record their observations related to:

   - Visual observations on animal health and behavior
   - Detect signs of illness or injury, and changes in behavior
   - Provide animal care
   - Check feed and water
   - Check light and temperature conditions
   - Collect eggs

2. Environmental and Serological Sampling/Testing

   To assure continued health of SBC LAL-C chickens, the sponsor follows a rigorous surveillance sampling plan (environmental swab samples from production rooms, serological samples from chickens) at each of their production facilities.

   Monthly environmental swab samples are tested for *Salmonella gallinarum* and *Salmonella pullorum.* Since 2012 when SBC LAL-C chickens were first introduced into the sponsor's first production facility, there have been no positive results returned on environmental samples that have been tested.

   Serological testing for various disease agents is performed for two separate purposes. First, blood samples are collected on a monthly basis from a sampling of birds from each production room, with a different sample of birds tested each month. Second, all hens and males are tested at least once at 16-20 weeks of age as part of their qualification process for inclusion into the production program (production of non-fertile eggs for egg white harvest, breeding campaigns for production of fertile eggs to propagate the SBC LAL-C line). Serological samples are screened for *Salmonella gallinarum, Salmonella pullorum, Mycoplasma gallisepticum, Mycoplasma synoviae,* Avian Influenza (Type A), and Lymphoid Leukosis Virus (subgroups A, B, C, D, and J). Since the first entry of SBC LAL-C chickens into the sponsor's production facilities in 2012, no positive results on serological samples have been obtained.

3. Mortality[1]

At each of the production facilities, chicken mortality is recorded as part of the daily health observations. The summary of weekly mortality for each of the three production facilities is provided in Table 2 below. This summary included mortality rates collected at the three production facilities over a two-year period.

Table 2: Average weekly mortality for SBC LAL-C hens (all three production facilities) and non-GE hens (Facility 1 only).

	Avg. Weekly Mortality (%)	**Standard Deviation**	**Range (%)**
Facility 1	0.66	0.40	0-1.74
Facility 2	0.14	0.14	0-0.52
Facility 3	0.25	0.16	0-0.58
Overall - SBC LAL-C	**0.45**	**0.40**	**0-1.74**
Non-GE hens	**0.40**	**0.58**	**0-2.22**

Note that while mortality at Facility 1 tended to be higher than at the other facilities, comparable mortality rates between SBC LAL-C and non-GE hens at Facility 1 indicate that presence of the hLAL rDNA construct in SBC LAL-C hens does not represent added risk of mortality when compared to non-GE hens.

4. Serology and histopathology

In a study to compare physiological endpoints of SBC LAL-C vs. non-GE chickens, blood samples (n = 10 SBC LAL-C chickens, n = 4 non-GE chickens) and tissue samples (n = 20 SBC LAL-C chickens, n = 11 non-GE chickens) were collected for serological and gross/histological evaluations. No differences between SBC LAL-C and non-GE animals were evident based on the following evaluations: serology and histopathology, including CBC (complete blood count), comprehensive blood chemistry, triglycerides and LDH (lactate dehydrogenase), and histopathological examination of multiple tissues.

F. Growth and Egg Production

Body weights on a sampling of SBC LAL-C and non-GE chickens (both hens and males) were collected at 18, 23, and 28 weeks of age. Results from this study are provided in Table 3 below:

[1] Mortality as attributed to animal death and euthanasia for items such as broken legs/wings, cloacal prolapse, or other injury.

Table 3: Body Weights (in grams) of SBC LAL-C and Non-GE Hens and Males Collected at 18, 23, and 28 Weeks of Age (Mean ± standard deviation (SD)).

LINE	Number of Animals	18 Weeks of Age Body Weight (in grams) Mean ±SD	23 Weeks of Age Body Weight (in grams) Mean ± SD	28 Weeks of Age Body Weight (in grams) Mean ± SD
SBC LAL-C Hens	77	1420 ± 219	1635 ± 202	1732 ± 231
SBC LAL-C Males	23	1815 ± 207	1975 ± 112	2031 ± 131
Non-GE Hens	6	1265 ± 163	1380 ± 171	1375 ± 192
Non-GE Males	7	1569 ± 158	1666 ± 137	1778 ± 68

SBC LAL-C chickens grew at similar rates as non-GE chickens, providing further evidence to support the Agency's overall conclusion that these chickens were indistinguishable from non-GE comparator chickens.

As part of the normal practices at each of the production facilities, non-fertile eggs are collected, with the number of eggs collected recorded for each room in production. From the daily egg production data for each room, the sponsor calculated egg production on a per hen basis. Table 4 below provides cumulative egg production, from 20 to 70 weeks of age, calculated on a per hen average.

Table 4: Cumulative, per hen egg production from 20 to 70 weeks of age in SBC LAL-C hens (means and standard deviations from eight production rooms) and non-GE hens (one production room).

Hen Age (weeks)	SBC LAL-C Hens (Average ± SD) [1]	Non-GE Hens (Facility 1)
25	28.9 ± 4.3	31
30	58.4 ± 4.3	63
35	87.4 ± 5.3	93
40	116.2 ± 6.3	118
45	145.9 ± 7.7	147
50	174.4 ± 8.7	173
55	201.6 ± 9.8	198
60	224.2 ± 11.3	222
65	247.5 ± 13.7	248
70	272.1 ± 14.7	274

[1] Based on data from eight production rooms at Facilities 1 and 2.

Cumulative, per hen egg production through 70 weeks of age for the eight production rooms of SBC LAL-C hens averaged 272.1 eggs per hen (range 260-300 eggs/hen). This compared to a cumulative, per hen egg production of 274 eggs per hen for non-GE hens. Therefore, SBC LAL-C hens had egg production that was comparable to that of the non-GE hens maintained at the sponsor's facility, and to that observed in commercial layer operations. This supports the Agency's overall conclusion that these chickens were indistinguishable from non-GE comparator chickens.

G. Conclusions on Phenotypic Characterization

Based on an evaluation of data and information relative to observations on the management, nutrition, health, growth, egg production, and reproduction, the Agency determined that the SBC LAL-C chickens were indistinguishable from non-GE comparator chickens. Therefore, the Agency concludes that the SBC LAL-C GE laying hens are as healthy as conventional laying hens. They do not differ from conventional hens in any observable manner, except for the presence of the hLAL rDNA construct added by design, and are, for all intents and purposes, equivalent to laying hens of the same background genetics commonly used in commercial egg production.

VI. FOOD SAFETY

The sponsor indicated that they do not intend for the hLAL rDNA construct in SBC LAL-C chickens, nor any materials derived from this GE line, 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, eggs, and waste including any eggs that do not meet quality standards for use in producing the human drug. Thus, the likelihood of edible products from SBC LAL-C chickens 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 animal, egg, and waste flow;
- SOPs for animal identification and disposal that include procedures to ensure that
  - all SBC LAL-C animals are uniquely identified by neck tag or wing bands within a day of birth;
  - all animals are traceable via an inventory database maintained based on their unique animal ID;
  - all animals, unused eggs, and waste are incinerated at termination of use.

Based on the high degree of containment at the sponsor's facilities, it is unlikely that any SBC LAL-C chickens would escape the facilities in which they are raised and housed. In the extremely unlikely event of escape, each chicken has a unique form of identification (neck tag or wing band), and even if any of those form of identification were lost, and a chicken of unknown provenance were discovered near the facility, there is a regulatory analytical method for identity in place at the Agency (see next section) to determine whether it is an SBC LAL-C GE chicken.

The hazard likely to be posed by inadvertently consumed and digested hLAL protein is also negligible: hLAL is a necessary enzyme for normal human growth, and the reason for the production of the hLAL protein in SBC LAL-C GE chickens is to supplement it intravenously in individuals who have a hereditary LAL deficiency. In addition, LAL is a highly conserved protein, with an approximately 76% amino acid identity between chickens and humans. The Agency, therefore, expects that hLAL would be digested as would the chicken LAL protein, which is consumed regularly as part of the edible chicken products eaten.

It is concluded that there is a reasonable certainty that SBC LAL-C GE chickens will not be introduced into the human or animal food supply. Based on Agency evaluations there is a low[2] level of concern should edible products of SBC LAL-C GE chickens inadvertently enter the food supply.

VII. REGULATORY ANALYTICAL METHOD OF IDENTITY

A. Description of Regulatory Analytical Method for Identity

The regulatory analytical method for the presence of the hLAL rDNA construct in muscle tissue is a polymerase chain reaction (PCR) method which provides acceptable sensitivity for routine monitoring to identify and confirm whether the tissue is from SBC LAL-C GE chickens. The method detects the presence of the hLAL rDNA construct at its SYN LAL-C integration site in the genome of SBC LAL-C GE chickens.

B. Availability of the Method

The validated regulatory method for detection of the hLAL rDNA construct is kept on record at CVM- Office of Research, FDA, 8401 Muirkirk Road, Laurel, MD.

VIII. ENVIRONMENTAL SAFETY

Based on the Agency's review of information and analyses presented in the sponsor's Environmental Assessment (EA), data submitted by the sponsor in support of other steps of the risk-based review process, and FDA inspection of the sponsor's production facilities, it is concluded that the approval of the NADA related to the SBC LAL-C line of GE chickens will not

[2] 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".

have a significant impact on the quality of the human environment in the United States. A Finding of No Significant Impact (FONSI) has been prepared summarizing the basis for this decision. The EA and FONSI may be seen in the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852.

IX. GENOTYPIC AND PHENOTYPIC DURABILITY

Data were provided that demonstrate that both the genotype and phenotype of the SBC LAL-C lineage are conserved over seven consecutive generations. These data adequately demonstrate that the genotype and phenotype of these chickens is durable, the rDNA construct for hLAL 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 SBC LAL-C 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 SBC LAL-C lineage of GE chickens. Together, the data and information the sponsor provided assure that the SBC LAL-C lineage will continue to be equivalent to those chickens evaluated prior to NADA approval.

X. CLAIM VALIDATION

The sponsor's Western immunoblot assay data on egg white from individual GE chickens indicate the presence of hLAL protein of expected protein size and immunoreactivity to a human LAL antibody on a per egg basis. The immunoblot data are corroborated by data from a routine, high-throughput lipase activity assay conducted on pooled, clarified, SBC LAL-C egg whites used to produce the human therapeutic Sebelipase alfa, KANUMA (reference BLA 125561). Results from this lipase activity assay indicate the reliable presence of bioactive hLAL based on its ability to hydrolyze the *in vitro* fluorogenic substrate 4-methylumbelliferyl oleate (4-MUO), a fatty acyl ester of 4-methylumbelliferone (4-MU).

Based on the review of data and information submitted in support of the Claim Validation section of the hierarchical review process and confirmed during FDA inspections of the sponsor's research and egg production facilities, the Agency concluded that the data demonstrate the biological activity of hLAL in egg white derived from SBC LAL-C GE chickens that is not present in egg white derived from non-GE comparator chickens. These data related to the 4-MUO assay support the Claim Validation step and have also been reviewed and found to support the phenotypic durability aspect of "Genotypic and Phenotypic Durability" for SBC LAL-C GE chickens.

XI. 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 hLAL rDNA construct in SBC LAL-

C chickens, when used according to the label, is safe and effective for the expression of a recombinant human lysosomal acid lipase (hLAL) encoding gene such that hLAL protein (intended for the treatment of humans) is present in their egg whites. Food or feed from SBC LAL-C chickens is not permitted in the food or feed supply.

XII. REFERENCES

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