Environmental Assessment

In support of an approval of a New Animal Drug Application related to the SBC LAL-C line of genetically engineered chickens (*Gallus gallus domesticus*), which are carrying a single copy of the hLAL rDNA construct integrated at a single genomic site that directs the expression of a recombinant human lysosomal acid lipase protein.

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List of Technical Terms[1]

Diploid	A cell, tissue, or organism with two complete sets of chromosomes, one from each parent.
Feral	An individual or population that has returned to the wild after domestication.
Genetically engineered	A cell, tissue, or organism whose genotype has been modified by recombinant DNA techniques.
Genome	The entire set of genetic instructions found in a cell.
Genotype	Cell's or organism's collection of genes. The genotype also can refer to the two alleles inherited for a particular gene. The genotype is expressed when the information encoded in the genes' DNA is used to make the corresponding RNA and protein.
Hazard	A substance or agent that, upon exposure, might result in a defined harm.
Hemizygous	Having one copy (or allele) of a given gene.
Homozygous	Having two of the same alleles for a particular gene from both parents.
Long terminal repeat	A DNA sequence at the ends of the provirus (integrated DNA) of all retroviruses, derived during reverse transcription by duplication of sequences at the ends of the viral RNA genome. It contains most of the control elements necessary for expression of the proviral genes.
Polymerase chain reaction	A standard technique used to amplify DNA contained within specified regions, often used to confirm genotype
Phenotype	An organism's observable attributes, such as morphology, development, or behavior, which derive predominantly from its genotype.

[1] Based on the list of technical terms provided in NRC, 2002.

Promoter	A regulatory sequence needed to turn gene expression on. The process of transcription (production of RNA from DNA) is initiated at the promoter. Usually found near the beginning of a gene, the promoter has a binding site for the enzyme used to make a messenger RNA (mRNA) molecule.
Risk	An estimate of the probability of an adverse event being realized. Risk is a conditional probability: the likelihood of adverse event provided that exposure has occurred.

1. Overview

1.1 Background

Lysosomal Acid Lipase deficiency is a rare genetic disorder caused by the lack of functional lysosomal acid lipase (LAL), an enzyme that plays an important role in the metabolism of lipids such as cholesteryl esters and triglycerides (Porto, 2014; Reynolds, 2013; Fasano et al., 2012; Grabowski, 2012). The lack of functional LAL leads to accumulation of these lipids in liver, spleen, other organs, and blood vessels of these LAL deficient individuals. LAL Deficiency is a progressive multisystem disease which frequently manifests early in life leading to serious complications (Grabowski, 2012; Bernstein, 2013). In infants, these complications include failure to thrive with progressive liver injury, rapid development of liver fibrosis, and early death. In children and adults, chronic liver injury with liver fibrosis leading to such complications as variceal bleeding due to cirrhosis, and marked disturbances of lipid metabolism leading to dyslipidaemia increasing the risk of premature atherosclerosis, are seen (Bernstein, 2013).

There are currently no safe and effective therapies available to treat patients with LAL Deficiency. In the absence of any effective treatments, management options for affected infants have largely been limited to a variety of supportive therapies, including nutritional support, blood transfusions, and albumin in an attempt to mitigate some of the effects of this rapidly fatal disease. Treatment for children or adults presenting with LAL Deficiency is limited to liver transplant as liver function deteriorates, and attempts to manage dyslipidaemia through diet and the use of lipid-lowering medications (LLMs). The impact of LLMs such as HMG-CoA reductase inhibitors (statins) for the treatment of dyslipidaemia in patients with LAL Deficiency is limited, since they do not address the root cause of the disease, and progression to end-stage liver failure still occurs (Bernstein, 2013).

Using genetic engineering, Alexion Pharmaceuticals, Inc. (“Alexion”) has developed a new approach for the production of lysosomal acid lipase, or sebelipase alfa, in the egg whites (EW) of domestic chickens (*Gallus gallus domesticus*). Purified lysosomal acid lipase (sebelipase alfa) has been used in clinical trials to support licensure as an enzyme replacement therapy for LAL Deficiency (Balwani, 2013; Valayannopoulos, 2014).

Gallus gallus domesticus, commonly known as the domestic chicken, is one of the oldest domesticated species, and the most common avian species worldwide (Richard, 1990). In the last 60 years, livestock producers and genetic improvement organizations have developed highly specialized lines of domestic chickens with phenotypic characteristics desired in commercial production of eggs and meat.

Chicken eggs have a history of use in the commercial production of human medicines (Zhu et al., 2005). Alexion designed a line of genetically engineered (GE) chickens carrying the recombinant DNA (rDNA) construct expressing recombinant human lysosomal acid lipase (rhLAL) in their EW. rhLAL protein is purified from clarified EW for use in treatment of individuals with LAL deficiency.

Genetic engineering technology is now commonly used to manufacture a large number of approved human therapeutic proteins. Most of these therapeutic proteins are expressed in either bacterial or, in the case of glycoproteins, transformed mammalian cell culture systems. Recombinant products of GE animal are approved for use in the United States and include Atryn (recombinant human antithrombin III) (FDA, 2009), and Ruconest (recombinant human C1 esterase inhibitor) (FDA, 2014).

1.2 Proposed Action: An NADA Approval for SBC LAL-C Hens

1.2.1 Regulatory Authorities

Genetically engineered animals are regulated by the Center for Veterinary Medicine (CVM) under the New Animal Drug provisions of the Federal Food, Drug, and Cosmetic Act (FD&CA) using a risk-based hierarchical approach to demonstrate safety and effectiveness described in *Guidance for Industry 187: Regulation of Genetically Engineered Animals Containing Heritable Recombinant DNA Constructs*[2] that is consistent with the FD&CA (221 USC 321 et seq.) and its enabling regulations (21 CFR 511 and 514).

Part of the approval process (21 CFR 25) requires that the agency meets its responsibilities under the National Environmental Policy Act (NEPA) and determines whether the agency action, in this case, the approval of the regulated article, (the hLAL rDNA construct at the SYN LAL-C site in the SBC LAL-C line of GE chickens), is likely to have a significant impact on the environment of the United States. If the agency determines that no significant impacts are likely to occur, it issues a Finding of No Significant Impact (FONSI). If the agency determines that a significant impact is likely to occur, the agency begins the preparation of an Environmental Impact Statement.

[2] http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/ucm113903.pdf

1.2.2 Purpose and Need for the Proposed Action

Per 21 CFR 514.1 (a)(14), this document is the environmental assessment (EA) that Alexion prepared (with input from CVM) related to Alexion's request for NADA for approval for the SBC LAL-C line of GE chickens producing rhLAL in their EW. As defined in 21 CFR 25.20 (m), the EA was triggered by an anticipated major agency action, the approval of the regulated article (the hLAL rDNA construct at the SYN LAL-C site in the SBC LAL-C line of GE chickens), and addresses potential impacts of this action under NEPA. The EA discusses potential impacts on the quality of the human environment resulting from commercial production of the SBC LAL-C line of GE chickens under the specified conditions of use described in Alexion's NADA and possible hazards or risks that may arise from exposures via the possible escape of these animals from containment.

1.2.3 Alternative Actions

According to NEPA and its implementing regulations, all environmental documents should include a brief discussion of alternatives to the proposed action as well as environmental impacts of these alternatives. This section focuses on the "No Action" alternative and discusses its potential impact on the quality of the human environment of the United States.

A "No Action" alternative implies denial of the NADA for the SBC LAL-C line of chickens. As a result of this action, two possible scenarios are envisioned:

- Cessation of the rhLAL purification and production of the SBC LAL-C line of chickens;
- Production of the rhLAL at locations outside of the United States.

Under the first scenario, the sponsor discontinues production of rhLAL and terminates the SBC LAL-C line of GE chickens. Thus, there will be no production of SBC LAL-C chickens and no potential for their escape or introduction of these GE chickens into the commercial food or feed supply. Therefore, no impacts on the quality of the human environment of the United States are expected.

According to the second scenario, production of rhLAL and maintenance of SBC LAL-C chickens may be initiated at locations outside the United States under the regulatory authority of foreign governments. Therefore, no impacts from the production of rhLAL from SBC LAL-C chickens are expected on the quality of the human environment of the United States.

Thus, should FDA decide to follow the "No Action" alternative (i.e., deny the NADA), there will be no impacts on the quality of the human environment of the United States and therefore, there will be no action for the agency to consider.

2. The Product

2.1 Current Product Definition

The product as defined at this time is the hLAL rDNA construct integrated at a single site (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.

2.2 Molecular Characterization of the rDNA Construct

The *hLAL* rDNA construct is designed on a retroviral vector backbone that has been rendered replication-deficient by virtue of encoding partial nucleotide sequences of the viral *gag* and *env* genes[3] that are incapable of producing functional proteins and rendered self-inactivating due to the lack of intact long terminal repeats (LTRs)[4]. The hLAL rDNA construct contains the nucleotide sequence encoding the full-length recombinant human LAL protein under the control of promoter, enhancer, and other regulatory sequences.

In its review of data and information relative to the construct, FDA has determined that the hLAL rDNA construct does not contain any intrinsic nucleic acid sequences (i.e., hazards) that could pose potential risks to humans, animals, or the environment.

2.3 Characterization of the Insert in the SBC LAL-C Lineage

The SBC LAL-C line of GE chickens descended from a single male founder and lineage progenitor, XLL 109. Multiple assays were used to characterize descendants of the lineage progenitor. These assays were used to confirm the presence, integrity, and the site of insertion, of the rDNA construct in the genome of SBC LAL-C chickens.

In its review of the data and information relative to the construct in the SBC LAL-C line of GE chickens, FDA confirmed: (1) that a single copy of the construct was inserted at a single locus (Southern blot assay), (2) the precise location of the construct in the genome and sequence integrity of the construct (PCR assays and DNA sequencing), and (3) confirmed that the construct was stably-transmitted from one generation to another (genomic DNA analysis).

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[3] *gag*, *pol*, and *env* viral genes are essential for retroviral replication in a host. *Pol* encodes a polyprotein specifying the reverse transcriptase (RT), integrase (int), RNase H, and protease. *Gag* encodes group specific antigen structural proteins, while *env* encodes a precursor envelope polyprotein and a transmembrane glycoprotein.

[4] Long terminal repeats contain sequences essential for retroviral transcription and replication. Disruption of LTRs renders the virus incapable of expressing its genes in mammalian cells.

FDA determined that the integration of the hLAL rDNA construct in the genome of SBC LAL-C chickens does not contain any intrinsic nucleic acid sequences (i.e., hazards) that could pose potential risks to humans, animals, or the environment.

2.4 Phenotypic Characterization

In its review of diverse data and information relative to items such as the management, nutrition, health, growth, egg production, and reproduction in SBC LAL-C chickens, FDA determined that with the exception of the intended expression of rhLAL in EW, the phenotype of SBC LAL-C chickens is equivalent to that of their non-GE counterparts. FDA confirmed that growth, egg production, reproduction, mortality, and general physiology of SBC LAL-C chickens were indistinguishable from that of non-GE comparator chickens and laying hens of the same background genetics commonly used in commercial egg production.

2.5 Food/Feed Safety

In its review of information relative to the food and feed safety, FDA concluded that the likelihood of edible products from Alexion's facilities inadvertently entering the food supply to be extremely unlikely due to the containment conditions and disposal protocols in place at the production facilities; these were confirmed by FDA inspection. FDA also determined that in the extremely unlikely event that edible products were to enter the food supply, the agency's concern level would be low, due to a lack of hazardous components in the rDNA construct, the healthy conditions of the hens, the likelihood for oral breakdown of the rhLAL protein, and similarity of human and chicken LAL, and the non-harmful presence and consumption of chicken LAL in normal U. S. diets.

3. Biopharmaceutical Animals Management

3.1 Production Plan

Commercial production of the SBC LAL-C line of GE chickens is currently carried out at three production facilities in the United States: one in Massachusetts and two in Georgia. Another facility located in Georgia is used only for harvesting EW from eggs laid by GE hens of the SBC LAL-C line. Each Alexion production facility may house one or more consecutive generations of SBC LAL-C line GE chickens. Generations within a facility are propagated from earlier generations via artificial insemination, after the lineage of GE chickens is established at a given facility.

To replace retired GE hens and sustain the size of the GE flock, some GE chickens will be used for reproduction during scheduled breeding campaigns of production hens.

SBC LAL-C hens produce eggs for harvesting EW. These eggs are collected and cracked, EW is separated from the yolk, and bulk raw EW is stored for shipment to contract manufacturing organizations (CMOs) where further processing occurs to purify rhLAL and produce the human therapeutic product sebelipase alfa.

3.1.1 Animal Care

Alexion maintains updated Standard Operating Procedures (SOPs) for animal husbandry to assure that appropriate standards of animal care and well-being are applied at all production facilities. Monthly veterinary inspections, written records of daily animal observations and care provide detailed information on the health status, daily egg production, scheduled breeding to produce fertile eggs for hatching of new hen/male groups, and genetic integrity of all groups of animals. Caretakers document all observations on room charts daily and in the event of unexpected/adverse health observations notify the Director of Upstream Manufacturing, who will contact the facility's consulting veterinarian. The Facility Supervisor is charged with the responsibility of removing ill animal(s) for euthanasia, carcasses of dead animals for disposal, or other actions (including necropsy). Procedures are in place with defined requirements for daily room checks for available water, feed, light, and temperature. Animals receive care 7 days per week.

Environmental and blood samples are collected monthly at each facility and tested for adventitious agents. Alexion has an action plan in place if positive test result(s) are obtained for adventitious agents or other health concerns. This action plan includes follow-up testing and observations, and quarantine of the facility or rooms within a facility.

The Alexion animal facilities are overseen and evaluated by Institutional Animal Care and Use Committees (IACUC). Each IACUC is comprised of members unaffiliated with the sponsor, and includes a Chair, local staff members, a layperson not affiliated with the sponsor, and the consulting veterinarian for that facility as per Office of Laboratory Animal Welfare (OLAW) Public Health Service (PHS) policy on humane care and use of animals. Activities and reports from these committees are coordinated and are submitted annually to the OLAW to provide assurance of Alexion's animal care status and compliance with applicable regulations. Alexion follows IACUC Committee requirements and recommendations concerning the well-being of the animals and retains the services of consulting veterinarians. Alexion utilizes internal Biosafety officers (BSO) for each of its animal facilities. The BSO is the designated position that assures compliance, biosafety/security of genetically engineered animals and the facilities that house them. The BSO provides technical advice to the Institutional Officer (IO), safety committee, production operations and researchers on containment, security, and safety procedures.

3.1.2 Locations of Production Facilities

Alexion facilities (Facilities 1, 2, 3, and 4) involved in egg production and/or EW harvest for the SBC LAL-C GE chicken line are summarized in Table 1.

Facilities 1, 2, and 3 are located in Georgia. The surrounding area around Facility 1 is semi-rural, covered with limited woodland with occasional areas used for agricultural purposes. The closest town is located about a mile away from Facility 1. The rest of the surrounding area has scattered residences. Facility 2 is located approximately 23 miles from Facility 1, in a semi-rural area surrounded by woodland and agricultural land. The area contains scattered residences and a few subdivisions. The closest town is located about a mile away from the site. Facility 3 is located approximately 20 miles from Facility 1. This facility is not involved in the production of SBC LAL-C GE chickens. It is used for EW harvest from SBC LAL-C eggs collected at Facility 1.

Facility 4 is located in Massachusetts. The facility is located in a small industrial park, on the outskirts of a small town. The industrial park is surrounded with woodland and a few private residences and subdivisions.

Table 1. Production Facilities for hLAL rDNA Construct in SBC LAL-C Hens

Facility and Location	Key Activities	Scope of Activities
Facility 1 (GA)	Egg Production	Housing of GE Production Line Egg Production Genetic Backup
Facility 2 (GA)	Egg Production and EW Harvest	Housing of GE Production Line Egg Production EW Harvest and Storage QC Testing and QA release of EW
Facility 3 (GA)	EW Harvest	EW Harvest and Storage QA release of EW
Facility 4 (MA)	Egg Production and EW Harvest	Housing of GE Production Line Egg Production EW Harvest and Storage QC Testing and QA release of EW

3.1.3 Description of GE Animal Production Facilities

All Alexion production facilities have multiple levels of redundant physical containment including battery cages/enclosures with gates, lockable animal rooms, enclosed buildings, perimeter fences, and gated entrances. Gated entrances require a key code for access. Access to the buildings is controlled by an electronic card access system that restricts access to card holders only. All facilities are equipped with break-in detection systems and have security camera systems capable of continuous monitoring inside and outside of the facilities. Entry into the animal housing and EW harvest portions of the facilities has the additional security requirement of a fingerprint scanner.

Chickens of the SBC LAL-C line are housed in all Alexion production facilities except Facility 3. Other lines of chickens (and their products) at Alexion production facilities are uniquely identified, and segregated from SBC LAL-C chickens by management and housing in rooms dedicated to those specific genetic lines. Facility 3 is used only for harvesting EW from eggs produced at Facility 1. Eggs produced at Facility 1 are collected and securely transported to Facility 3 for EWH. All egg shipments are carried out in a van owned and operated by Alexion. All shipments are appropriately labeled and recorded. Facilities 2 and 4 conduct on-site EW harvest and store the harvested EW until it is shipped to the CMO for further purification.

Alexion procedures require that any personnel or visitors entering their facilities are restricted from contact with avian species for 72 hours prior to entry and from traveling outside the United States within the previous seven days. This includes exposure to live chickens at different Alexion facilities, as well as exposure to other domestic or wild avian species.

In addition, personnel with signs of illness are denied access to the animal production areas. Entrance of Alexion personnel or visitors who meet the exclusion criteria described above into the animal production area of each facility is allowed only after a shower and change into facility dedicated clothing and footwear.

All Alexion facilities implement rodent, insect, and wild animal control programs. Wire mesh, ultrasonic bird repellant, and predatory call making devices are used to keep wild birds away from the facilities. Commercial pest control companies at the respective production facilities service rodent/pest control traps on the inside and outside of the buildings, and the sponsor maintains insect traps within each of their facilities. The areas surrounding the facilities are not rich in wild bird nesting habitats and migratory birds are not expected to preferentially roost in these areas.

3.2 Individual Hen Identification

Facility design, labelling, and procedural controls are established at all Alexion production facilities to ensure segregation and monitoring of GE chicken lines. A PCR-based method is used to verify the GE hens' genotype and individual identification allows traceability throughout their lifespan. Hen inventories are maintained in a continuously updated database. All hatched GE and non-GE chicks at Alexion production facilities are provided with unique identification numbers using neck tags or wing bands (2 bands, one in each wing) within one day of hatching. If initial identification is via neck tag, then chicks receive wing bands within 7 days post-hatch. Missing or illegible individual ID tags are replaced if an animal has at least one form of identification (i.e., a single wing band). If an animal loses all forms of identification, it is removed from production. After identification of each GE hen by the rhLAL real-time PCR assay, the SBC LAL-C production line is subjected to the following genetic assays: the rhLAL gene is PCR-amplified from blood DNA and sequenced to confirm 100% homology with the human sequence and the location of the rDNA construct integration site is confirmed by PCR. Identical standards for qualification of each new generation of GE hens are maintained at all facilities.

3.3 Disposal of Waste and Carcasses

Each building has a fog room used for removal of municipal waste and biosecure transfer of large equipment and supplies in and out of the building. Animal and biological waste (e.g., animal carcasses, unused eggs, hatch residue, and waste from EWH) is bagged and removed from facilities through separate dedicated waste exits, then placed outside into refrigerated trailers which are removed off site by a licensed medical waste service.

Facility 1 does not have a separate waste exit and bagged municipal and animal waste is removed through the fog room; municipal waste is placed into the municipal waste container emptied by a contracted municipal waste service and animal biological waste is incinerated in the on-site incinerator.

Procedures are in place for waste disposal and records of disposal are maintained by Alexion for a period of two years.

3.4 Manure and Other Waste Disposal

Fecal waste is collected into bags in each production room and removed from the facility via the waste exit dock. Waste from Facility 1 is disposed of using the onsite incinerator owned and operated by Alexion. Other facilities contract with a licensed medical waste disposal service that transports the waste in a refrigerated trailer to a licensed medical waste disposal facility for incineration.

3.5 Labeling, Packaging, and Shipping

3.5.1 Transportation of Eggs Between Facilities

Eggs containing rhLAL are collected on a twice per day basis – in the morning and again in the afternoon. Only animals that have been released for product production (based on genetic and health testing described in Section 3.2) enter into egg production for EW harvest. Because the production facilities are multi-product facilities, several precautions are taken to ensure proper storage and segregation of eggs for each product. These measures include dedicated animal rooms (by GE animal line, zygosity, hatch, etc.) and identification/segregation of eggs from each hen line at all production facilities from the time of collection through EW harvest and bulk EW storage.

In Facility 1, eggs are labeled prior to their transfer for EW harvest. Eggs containing rhLAL are transported from Facility 1 to Facility 3 for EW harvest. Transportation is carried out using a van, owned and operated by Alexion. Eggs placed on color coded egg flats are packaged in reusable coolers with ice packs and a temperature recorder. Each egg transport cooler is numbered and carries a label that includes the date range during which the eggs were produced and the identification number of the production room where the eggs were produced. A shipping invoice containing the following information is included with the shipment:

1. Date of shipment
2. Product being shipped
3. Production room number
4. Lay date for eggs packaged in each cooler
5. Number of eggs in each cooler

A copy of the shipping invoice will be maintained by Alexion for at least two years after approval, should an approval be granted (21 CFR §511.1 (b) (3). The cooler is sealed with shrink wrap and transported by an Alexion owned vehicle to Facility 3 for EW harvest.

Eggs produced in Facility 2 and 4 are placed on product-specific, color coded egg flats, packaged into reusable plastic boxes in production rooms, sealed, labeled, and moved to refrigerated storage in the EW harvest area within the same facility.

4. Affected Environment

4.1 Climate

The production facilities are located in Massachusetts and Georgia. The state of Massachusetts is located in in the northeastern part of the United States. The climate of Massachusetts is defined by a number of mountain ranges spanning the central and western part of the state as well as by its proximity to the Atlantic Ocean. The overall pattern can be described as a humid continental climate. Summers are usually warm and humid with average temperatures (in Fahrenheit) ranging between upper 70s (high) and lower 60s (low). In winter, the region is open to northern winds that bring cold, arctic air from Canada leading to average temperatures between mid 30s (high) and upper 10s (low). These winds also bring a considerable amount of snow. Average monthly temperatures and precipitation at the location closest to Facility 4 for which data are available are summarized in Table 2.

The state of Georgia is located in the southeastern part of the United States. The Gulf of Mexico and the Atlantic Ocean have a considerable influence on the climate of Georgia. With the exception of the northern part of the state, the climate of Georgia is fairly uniform. Summers are typically hot and humid with average temperatures ranging from lower 90s for highs to upper 60s for lows. Winters are relatively mild with average temperatures between mid 50s (high) and mid 30s (low). Average monthly temperatures (in Fahrenheit) and precipitations at the nearest location to Facilities 1-3 for which data are available are summarized in Table 3.

Table 2. Average monthly high and low temperatures (in Fahrenheit) and precipitation values (in inches) in Massachusetts recorded at the station closest to the production facility (adapted from: U.S. Climate Data).

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Average high, ^{0}F	31	35	43	55	66	74	79	77	70	58	48	36
Average low, ^{0}F	17	19	26	37	47	56	62	60	53	42	33	23
Average precipitation, inches	3.5	3.2	4.2	4.1	4.2	4.2	4.2	3.7	4.0	4.7	4.3	3.8
Average snowfall, inches	17	16	11	3	0	0	0	0	0	0	3	14

Table 3. Average monthly high and low temperatures (in Fahrenheit) and precipitation values (in inches) in Georgia recorded at the station closest to the production facility (adapted from: U.S. Climate Data).

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Average high, ^{0}F	54	58	66	74	82	89	91	90	84	74	65	56
Average low, ^{0}F	33	36	42	49	58	66	70	69	63	51	42	35
Average precipitation, inches	4.1	4.5	4.5	3.2	3.0	4.2	4.5	3.5	3.9	3.5	3.8	3.7
Average snowfall, inches	1	1	1	0	0	0	0	0	0	0	0	0

4.2 Landscape and Predators

The landscape surrounding the production facilities is semi-rural or industrial and located near suburban towns or cities. Open and limited forested areas interspersed with buildings are the norm around the facilities.

Although there are no known feral[5] populations of *Gallus gallus* in the areas surrounding the facilities, Massachusetts and Georgia have populations of wild turkeys, pheasants, and partridges (DeGraaf, 1986; Georgia DNR, 2015; Massachusetts EEA, 2015). Feral populations of chickens around the facilities would have limited areas of cover making them easy prey for carnivorous species. Local predators in both states include canine species (coyotes, foxes, and dogs), raptors (hawks, eagles, and falcons), feline species (domestic and feral cats, bobcats), mustelids (weasels, ferrets, martins, mink, and Fisher cats), and raccoons (DeGraaf, 1986; Georgia DNR, 2015; Massachusetts EEA, 2015).

4.3 Occurrence of Natural Disasters

Alexion's production facilities are located in seismically inactive regions. The survey of earthquakes during 2000-2015 in Massachusetts and Georgia did not identify any events that were capable of significant damage that would compromise the integrity of production facilities (USGS, 2015).

Although Massachusetts is frequently affected by rain and snow storms, it has rarely been a subject to significant climatic events. National Climatic Data Center at the National Oceanic and Atmospheric Administration reports only a single major (EF3) tornado in the vicinity of the production facility in Massachusetts during 2000-2015 (NOAA, 2015b). During the same period, no major tornadoes were reported in the vicinity of production facilities in Georgia (NOAA, 2015a).

[5] *Gallus gallus domesticus* is a domesticated version of the Red Jungle Fowl (*Gallus gallus*), which can still be found in the wild in Southeast Asia. There are no known wild populations of *Gallus gallus* in the United States.

5. Mitigation of Exposure: Prevention of Unauthorized Access and Escape

5.1 Physical Containment

All Alexion's facilities are equipped with multiple redundant physical barriers to prevent escape of GE animals into the environment. These barriers include perimeter fences and gates, buildings and rooms, and housing enclosures.

5.1.1 Perimeter Fence and Gates (Tertiary Physical Barrier)

The entire perimeter of each facility has a six foot high chain link fence topped by barbed wire and monitored by security cameras. The gated entry, which requires key-code activation, is kept closed at all times and is locked with a chain when employees are not in the facility. Ultrasonic wild bird repellant and predatory call making devices are installed at some facilities to further restrict wild birds from approaching the building. These physical barriers prevent wild birds from nesting or roosting around the eaves of the building and prevent wild bird-derived adventitious agents from contaminating the facility. Each facility has contracted a licensed pest service providing continuous pest prevention at the perimeter of each facility.

5.1.2 Facility Buildings and Rooms (Secondary Physical Barrier)

Each production facility consists of a single building. The entrance to the building is through a door equipped with an electronic card access system and a security camera. Each building has several emergency exits with alarms. For each facility, doors to each animal room carry a sign that identifies the GE chicken line housed in that room, zygosity, hatch date, generation, type of feed to be used, and the color code used for labeling the eggs produced by the line of chickens housed in the room. Rooms are accessed through an anteroom that has two doors. The first door allows access from a central corridor into the anteroom where personnel put on additional personal protective clothing. The second door allows personnel entry into, but not exit from, the animal production room; exit of personnel is via an exit door at the opposite end of the room. Only one door of the anteroom should be opened at any given time. Rooms housing animals in Facilities 2 and 4 are separated from the rest of the facility by air-locked doors. To maintain the air pressure and prevent the intrusion of airborne contaminants only one door can be opened at a time.

5.1.3 Hen Housing Enclosures (Primary Physical Barrier)

GE hens and males are housed individually or in groups on 3 or 4 levels (tiers) in commercial grade enclosures at all production facilities. Each enclosure is equipped with a latch to lock doors. Facility 1 includes enclosure batteries with mechanized waste removal and conventional poultry enclosures with manual waste removal and manual feed distribution.

Production animal rooms in Facilities 2 and 4 have the same design and equipment (custom built hen housing enclosures in 4-tiered batteries). All enclosure systems in Facilities 2 and 4 include automated feed and water distribution and mechanized waste removal from each enclosure tier. Removal of waste from each production room and facility is manual.

Facility 3 is used for harvesting EW and does not house any SBC LAL-C GE chickens involved in the commercial production cycle.

5.2 Procedural Containment

5.2.1 Personnel and Training

Procedural containment implemented at Alexion production facilities includes restricted entry and specific precautions taken by each person entering the facility or entering each animal room. This controlled personnel flow prevents introduction and/or spread of potential contamination within the facility, and ensures segregation of hens and eggs for each product. The facility entry standard operating procedure requires that a person should not have entered any other facilities housing avian species nor should a person have had any contact with avian species for at least 72 hours prior to entering the Alexion facility or traveled outside the USA in the 7 days prior to facility entry. Examples of prohibited facilities include pet shops, poultry farms, families with pet birds and back-yard chickens etc. Furthermore, persons with flu-like symptoms, fever, chills and any symptomatic respiratory or digestive illness are not allowed to enter the facility. Only limited numbers of visitors, such as a veterinarian, or other consultants are authorized to enter the facility with prior authorization from Alexion management.

All company employees with daily duties at production facilities are required to participate in a training program on procedures applicable to the employees' scope of work duties. A selected set of procedures require in-person training conducted by a trainer and include scored evaluation and other procedures require the employee to read and understand documents and confirm this by signing the training forms. Newly hired employees are provided hands-on training and support for some duties and functions by experienced employees including temporary assignment of a trainer/technician. All workers are required to undergo annual refresher training. Procedures are reviewed and revised annually using the Document Change Order process and all affected employees are retrained.

Prior to entering the facility, street clothes and shoes are removed and stored in a locker outside the shower barrier. Each person is required to take a timed, full body shower and wear facility dedicated clothing and shoes. All facility dedicated clothing and shoes are laundered on site. Each exit and re-entry into the facility requires a shower and attire change.

Prior to entering a production room, a person is required to spray their hands with 70% ethanol, wear disposable gloves, face mask, full body coveralls, and hair nets. Finally, the person is required to wear disposable foot covers as he/she crosses through the entrance door from each ante room in to each production room. Any inanimate object carried into a production room is sprayed with a sanitizing agent and placed just inside the room beside the door. When the employee leaves a production room, all disposable gowning is removed and discarded in a trash can.

5.2.2 Staff and Visitor Access

Alexion's facilities are equipped with restricted card access for the building and monitoring by exterior and interior security cameras. The front door is the primary entrance designated for entry into the building. This door is fitted with an electronic card access system. Each authorized employee is provided an electronic access card with a unique number. No one can enter the facility without an authorized card. Use of this card to enter the facility automatically creates a record of the time and the name of the employee entering the facility. The daily record of personnel who have entered the facility is routinely reviewed by the facility supervisor. The access card for any given employee can be electronically deactivated within minutes (without the physical card) if the employee is terminated or resigns. Entry into the animal housing and EW harvest sections of the facilities is equipped with a fingerprint scanner system.

5.2.3 Facility Security and Alarms

Alexion's facilities are equipped with a camera system which is capable of continuous video monitoring. There are cameras inside the facility allowing viewing of hen housing rooms 24 hours a day, 7 days a week. Each Alexion facility is fitted with a variety of security devices to prevent entry of unauthorized personnel, monitor the entry and exit of authorized personnel, monitor temperature at various locations within the facility, prevent entry or exit of unauthorized materials, or wild birds. These systems are designed to immediately alert designated facility personnel if any security system breach occurs. Key security barriers include a fenced perimeter; restricted card access for the building; monitoring by exterior and interior cameras; pest control for rodents, insects, and wild birds; through appropriate use of pesticides, nets, traps, and an ultrasonic emitter.

Each production facility is equipped with a break-in alarm system that can be activated or deactivated using a unique code. The system is activated when the last authorized employee leaves the facility, and is deactivated when the first authorized employee enters the facility. In the event of a security breach or fire, the security monitoring company is called via an automatic dialer. Should an intrusion occur, security cameras can be accessed 24/7 to determine the nature of the occurrence and the appropriate action required.

5.2.4 Disaster Guidelines

Each facility is equipped with a backup generator which automatically turns on when electrical power is lost.

Alexion facilities located in different geographic areas provide redundancy for the SBC LAL-C line of GE chickens in cases of a natural disaster or disease/infection of hen populations. Each facility can produce sets of fertile eggs and/or chicks and/or near lay hens for re-stocking and re-starting hen populations at other facilities. Facilities are located away from large animal agriculture units, food processing/manufacturing, and high traffic pathways to provide isolation.

5.2.5 Animal Monitoring

Caretakers document all observations on charts daily and notify Facility Supervisor and the Director of Upstream Manufacturing in the event of unexpected/adverse observations. The Facility Supervisor is charged with the responsibility of removing and euthanizing ill hens including necropsy as warranted based on the decision of the Director of Upstream Manufacturing. Daily procedures are in place to define requirements for production room checks for water, feed, and light and temperature requirements. Hens receive care 7 days per week. Feed and water is checked daily. Record of room checks is documented by the caretakers on a daily basis.

Initial inventories of each production room are established with individual hen IDs and are updated continuously and numbers are verified weekly. Every mortality is recorded and the facility supervisor and the Director of Upstream Manufacturing are notified.

6. Risk Assessment

6.1 Risk-Related Questions-Environmental Assessment

The current EA relies on a risk-based approach that identifies potential hazards (i.e., elements or actions that may cause an adverse effect) and risks to the environment. In the environmental assessment, risk may be defined as the likelihood of an adverse effect provided that exposure to a hazard has occurred. Therefore, risk is a function of adverse effects and exposure.

Because SBC LAL-C chickens are intended to be kept under strict conditions of containment, most of the estimation of risk to the environment can be inferred from the probability of the exposure. In the context of evaluation of environmental impacts associated with GE animals, exposure is more than a release or escape of these animals into the environment. Escaped or released GE animals may not be able to establish in the ecosystem or may be quickly eliminated through natural selection. For a GE animal to cause an adverse effect, it must spread and establish in the ecosystem in which it has escaped. Thus, it would be more appropriate to define exposure as an establishment of GE animals in the ecosystem (NRC, 2002).

As defined earlier, risk is a function of adverse effects and exposure. Therefore, there must be both exposure and an adverse effect to pose a risk to the environment. If one of the components is missing, the risk will be negligible. Exposure can be minimized through ensuring appropriate physical containment, standard operating protocols, and training programs for personnel. Therefore, our risk-based assessment primarily focuses on physical and procedural containment of the SBC LAL-C line of GE chickens, potential establishment of these GE chickens in the environment, and potential adverse effects on that environment.

To assist in evaluation of the risks associated with commercial production of the SBC LAL-C line of GE chickens, Alexion developed the following risk-based questions:

- What are the risks to personnel involved with animal husbandry, other animals, and environment associated with production of the SBC LAL-C line of GE chickens while under containment?
- What is the likelihood that SBC LAL-C GE chickens will escape the conditions of containment?
- What is the likelihood that SBC LAL-C GE chickens will survive and disperse if they escape the conditions of containment?

- What is the likelihood that SBC LAL-C GE chickens will reproduce and establish if they escape the conditions of containment?
- What are potential impacts on the environment of the United States should SBC LAL-C GE chickens escape the conditions of containment?

6.1.1 What are the risks to personnel involved with animal husbandry, other animals, and environment associated with production of the SBC LAL-C line of GE chickens while under containment?

Risks associated with production of SBC LAL-C GE chickens while under containment largely depend on the expression product of the hLAL construct and the ability of the hLAL construct to mobilize and spread to other animals. The following risks associated with production of SBC LAL-C GE chickens were considered:

Risk of gene flow to personnel involved with animal husbandry and other animals

The hLAL construct is based on a replication-deficient, self-inactivated retroviral vector that contains only partial nucleotide sequences for the viral *gag* and *env* genes (Sections 2.2 and 2.3). FDA has determined that the hLAL construct is not likely to mobilize to other animals and does not contain any intrinsic hazards that could pose potential risks to humans, animals, or the environment.

Risk of disease spread to other animals

To ensure that SBC LAL-C GE chickens do not come in contact with wildlife, Alexion has implemented a comprehensive set of biosecurity measures that includes rodent, insect, and wild animal control programs (Section 3.1.3). In addition, GE chickens are maintained in a tightly controlled environment and are regularly tested for adventitious diseases (Section 3.1.1). Therefore, it is highly unlikely that they could harbor any diseases. Thus, the possibility for disease transmission to and from SBC LAL-C chickens is low.

Risk of direct toxicity associated with increased environmental concentration of rhLAL

rhLAL encoded by the hLAL construct is similar to naturally occurring forms of this protein found in humans, chickens, and other species and poses no intrinsic hazard. This recombinant human protein is purified from the EW and further processed for use in treatment of individuals with LAL deficiency, therefore, no change in the concentration or distribution of this protein in the environment is expected.

Risks associated with disposal of GE animal waste and carcasses

Although neither the hLAL construct nor rhLAL are inherently hazardous, Alexion has implemented comprehensive GE animal waste procedures (Sections 3.3 and 3.4). All GE animal waste including animal manure, unused eggs, and animal carcasses is incinerated by a licensed waste management company or onsite. Therefore, risks associated with disposal of GE animal waste are low.

Thus, the hLAL construct does not pose any intrinsic hazard and is not likely to mobilize to other animals. rhLAL does not pose any hazards as well. Therefore, production of SBC LAL-C chickens under conditions of containment is not expected to pose any significant risks to personnel handling the animals, other animals, or the environment.

6.1.2 What is the likelihood that SBC LAL-C GE chickens will escape the conditions of containment?

As noted in the NRC report, chickens have a moderate ability to escape the conditions of containment (NRC, 2002). In general, chicken's ability to escape largely depends on their ability to fly, jump, and run. Therefore, the likelihood that SBC LAL-C GE chickens will escape from production facilities will depend on the adequacy and extent of the physical and procedural containment implemented at these production facilities.

Production of the SBC LAL-C line of GE chickens is carried out in facilities with multiple, redundant levels of physical containment that include perimeter fencing topped with barbed wire, buildings/production rooms, and housing enclosures. These measures are described in greater detail in Section 5.1. The entire production cycle in Facility 2 and 4 is closed, i.e., GE animals are born, live, and disposed of without leaving the production facility. EW from the eggs produced by these GE animals is processed on the premises as well. Thus, the production cycle design also contributes to the effectiveness of containment. The production cycle at Facility 1 is similar to production cycles at other Alexion's production facilities with the exception of EW harvesting. Eggs are shipped for EW harvesting to Facility 3 by a van owned and operated by Alexion in accordance with existing SOPs. These measures ensure that eggs will not accidently enter a commercial food or feed supply. Procedural containment and surveillance systems implemented at the production facilities serve as additional measures to ensure adequate control over production animals and are described in Section5.2.

It is highly unlikely that SBC LAL-C chickens will be introduced into the environment as a result of a catastrophic event (e.g., tornados, earthquakes) due to redundancies in physical containment and rare occurrence of these types of events in the vicinity of production facilities (Section 4.3).

If in a highly unlikely event birds surviving a hypothetical catastrophic event are introduced into the environments around production facilities, they will not be able to survive, disperse, reproduce, and establish as discussed in Sections 6.1.3 and 6.1.4.

Information reported by Alexion regarding their production facilities was verified during Bioresearch Monitoring inspections conducted at the Georgia and Massachusetts facilities in January 2015. These inspections confirmed that the facilities are well maintained with multiple, redundant forms of physical containment as described in the sponsor's submissions to CVM. Descriptions and schematic diagrams of production facilities are accurately represented. All production facilities were found to be in compliance with FDA regulations. No Form FDA 483 was issued by CVM at the conclusion of the inspections[6] for any of the production facilities visited.

Thus, the likelihood of escape of GE chickens from any of the Alexion facilities is extremely low due to multiple redundant levels of physical containment and surveillance procedures.

6.1.3 What is the likelihood that SBC LAL-C GE chickens will survive and disperse if they escape the conditions of containment?

Survival and dispersal of the species in the environment depends on its ability to adapt to the natural conditions. Highly domesticated species like poultry are not well adapted to the natural conditions and more likely will not be able to survive and disperse in the natural setting as they lack characteristics of truly "wild" or feral populations. Domestication processes and artificial selection for a particular trait (e.g., increased meat or egg production) likely lead to a situation where other traits required for survival (e.g., foraging, predator evasion) are no longer needed and are selected out. When animal's resources are allocated maximally to the trait they are selected for, it leaves the animal unable to adequately respond to additional environmental stresses and challenges and decreases its chances for survival. SBC LAL-C GE chickens are housed in specialized production facilities with controlled climates and are completely dependent on human handlers for food and water. These animals will not likely be able to survive winter conditions in Massachusetts. Because winters in Georgia are mild compared to those in the northeastern U.S., there is a theoretical possibility that these GE animals could survive winters in Georgia. But the presence of carnivorous predators in the environment around production facilities in Georgia and Massachusetts dramatically decreases their chances for survival and dispersal.

[6] Form FDA 483 is used to communicate investigational observations that may need correction.

Thus, the likelihood that GE chickens of the SBC LAL-C line will survive and disperse if they escape the conditions of containment is extremely low.

6.1.4 What is the likelihood that SBC LAL-C GE chickens will reproduce and establish if they escape the conditions of containment?

In the highly unlikely event that SBC LAL-C line GE chickens escape the conditions of containment and survive in the environment surrounding production facilities, the likelihood that they would be able to reproduce and establish will largely depend on availability of the suitable mates for the escapees. It is highly unlikely that escaped chickens would breed with wild members of the *Phasianidae* family such as partridges, pheasants, grouses, and wild turkeys found in Massachusetts and Georgia. Because production facilities are located relatively close to populated areas, interaction of GE chickens with these species is very unlikely. Although theoretically this interaction may take place, the likelihood of interbreeding with related species and producing viable progeny is extremely low. Hybrids of domestic chickens with members of *Phasianidae* family are very rare and have been predominantly produced by artificial insemination. The offspring of such crosses were usually nonviable (McCarthy, 2006). Because local environments around production facilities do not have any known populations of feral chickens, the primary concern is the reproduction potential between escaped GE chickens.

Although it is theoretically possible that GE hens and males may escape and reproduce, it is highly unlikely that this would lead to their establishment in the ecosystem. Establishment implies a long-term survival and sustained reproduction of the animals in the environment that would require the escape of a significant number of animals of both sexes which is highly unlikely in the absence of a catastrophic event (e.g., tornado, earthquake). In addition, escaped GE chickens and their potential offspring will not be able to withstand to environmental stressors (e.g., temperature, food scarcity, etc.) and predation and will be rapidly eliminated from the environment.

Thus, the likelihood that SBC LAL-C GE chickens will reproduce and establish if they escape the conditions of containment is extremely low.

6.1.5 What are the potential impacts on the environment of the United States should SBC LAL-C GE chickens escape the conditions of containment?

In the highly unlikely event that SBC LAL-C chickens were to escape, their potential impacts on the environment may be divided into three broad categories (Table 4). These categories are similar to general areas of concern identified by Snow et al., 2005 for GE organisms.

Establishment of a feral GE chicken population and interbreeding with related species may affect the genetic diversity of the ecosystem. As discussed in Section 6.1.3, these consequences are highly unlikely due to inability of escaped GE chickens to withstand to environmental stressors, predation, and inability to produce viable offspring with closely related species; and therefore, escaped GE chickens are not expected to cause any significant effects on the environment.

Disease transmission to wild populations of non-target animals is another theoretical impact that could be considered. Although disease transmission to wild birds and other animals in the environment may be a source of concern at avian facilities, it is not the issue at Alexion's production facilities for several reasons. First, all production facilities are maintained under strict standards that include regular monitoring for common disease agents and overall evaluation of GE chicken health, so that in the highly unlikely event that an SBC-LAL chicken escaped, it would be healthy. Second, there are no data suggesting that GE chickens are more susceptible to diseases than non-GE chickens; again, decreasing the potential for any disease spread in the highly unlikely event of escape. And third, partial viral sequences present in the rDNA construct are non-functional and are incapable of forming active virions within GE chicken's cells. Therefore, the emergence of new viral diseases that may be transmitted to other non-target animals is highly unlikely in the highly unlikely event of escape, and will not result in any significant impacts on the environment.

Thus, overall, in the highly unlikely event that SBC LAL-C GE chickens escape the conditions of containment at Alexion's production facilities, the escape is not expected to result in significant impacts on the environment of the United States.

Table 4. Potential environmental impacts associated with production of SBC LAL-C GE chickens (adapted from (Snow et al., 2005)).

Impacts	Potential consequences (effects)	Risk Associated with SBC LAL-C GE chickens
Establishment of a feral population	Disruption ecosystem leading to a loss of biological diversity; competition for resources; loss of habitat; changes in the ecosystem function	Escape of GE chickens is mitigated by multiple, redundant levels of physical containment; low probability of spread and establishment due to inhospitable environment, lack of wild or related species and the presence of carnivorous predators around production facilities will not allow establishment of GE chickens in the environment. **NO SIGNIFICANT RISK**
Interbreeding with related species	Gene flow to related species may result in the loss of genetic biodiversity	There are no wild or related species in the area around production facilities. **NO SIGNIFICANT RISK**
Viral disease transmission	Use of viral plasmids to produce GE animals may lead to emergence of new viral diseases that may transmit to non-target animals	The rDNA construct incorporated into genome of SBC LAL-C chickens contains partial, non-functional viral sequences; recombination and emergence of new viral diseases due to the rDNA construct is not possible. **NO SIGNIFICANT RISK**

6.2 Conclusion

As defined earlier, risk is a function of both exposure and adverse effects. If one of the components is negligible, then the overall risk would be negligible as well. Evaluation of adequacy and the extent of the physical and procedural containment established that the escape of SBC LAL-C GE chickens into the environment is highly unlikely due to multiple, redundant forms of physical containment and surveillance procedures implemented at Alexion's production facilities. The likelihood that GE chickens of the SBC LAL-C line will disperse, reproduce, and establish in the environment is very low

In addition, potential consequences on the environment are negligible as well. As a result, the overall risk to the environment from commercial production of SBC LAL-C chickens is negligible. Therefore, approval of the NADA for the SBC LAL-C line of GE chickens is not expected to have significant effects on the quality of the human environment of the United States.

7. Agencies and Persons Consulted

This Environmental Assessment was prepared by Alexion BioPharma, Inc. with advice and direction from the Center for Veterinary Medicine at the Food and Drug Administration. No other state or Federal agencies were consulted.

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