Environmental Assessment

for

the Bc6 rDNA Construct in

GTC 155-92 Goats

Expressing Recombinant Human Antithrombin III (rhAT or ATRYN)

Applicant: GTC Biotherapeutics, Inc.

NADA 141-294

For Public Display

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Center for Veterinary Medicine

U.S. Food and Drug Administration

I. Background and Introduction

A. Historical Antecedents of Animals as Sources of Human Medicines

Animals have been traditional sources for the production of biologics, drugs, and devices for use in humans. Insulin has been obtained from swine and cattle pancreas, heart valves have been isolated from pigs, and synovial fluid replacements have been derived from cock's combs. In addition, hen's eggs have served as bioreactors for the preparation of vaccines. Most of these materials have been adequate replacements for human proteins or therapeutic products because of the closely related physiologies of animals and humans. Many of these products, however, are not perfect replacements, as there are molecular differences between animals and humans that result in less than ideal pharmacokinetic, toxicological, and efficacy profiles.

B. The Advent of rDNA-Based Technologies

More recently, investigators have sought to use modern biotechnology, including recombinant DNA (rDNA) techniques, to produce human therapeutic substances, primarily drugs and biologics, in other organisms. For example, much of the insulin now sold is a recombinant human (rh) protein expressed in *Escherichia coli*. Other proteins are produced in bacterial or eukaryotic cell cultures using coding sequences for human versions of these proteins. However, in some cases, those proteins require glycosylation[1] patterns more closely related to human profiles to maximize their therapeutic profiles.

With the advent of the production of genetically engineered (GE) animals, however, there are now possibilities to produce recombinant human proteins with post-translational modifications (e.g., glycosylation patterns) that may more closely resemble human profiles. These "biopharm" animals can be used to produce large quantities of desired recombinant human (rh) proteins in familiar and relatively safe biological matrices (e.g., milk or eggs). These animals can be produced by the introduction of an rDNA construct encoding the production of the human drug or biologic into an early embryo. That construct can be introduced into a one cell embryo and incorporated into the genome of the resulting GE animal. Following the evaluation of selection criteria, a GE lineage may be derived from an animal that is healthy and expressed the introduced trait appropriately.

C. Domestic and Feral Goats

The domestic goat (*Capra aegargus hircus*) is a subspecies of goat domesticated from the wild goat of southwest Asia and Eastern Europe. The goat is a member of the Bovidae family and closely related to the sheep. Goats are one of the oldest domesticated species, having been reared by humans in several locations for as many as 10,000 years. Today there are over 300 distinct breeds of goats. Goats provide many products of value for human use including

[1] Glycosylation is the enzymatic process which links saccharides (sugars) to proteins and lipids.

milk, meat, fiber (e.g., Angora and cashmere wool), and hides. Goat's milk is commonly processed into cheese, butter, ice cream, and other products.

Goats are herbivore generalists, grazing and browsing in a variety of habitats including range/grassland, scrub/shrublands, and even natural and planted forests/woodlands. Goats feed on grasses, forbs, and browse. They tend to feed on the most palatable and nutritious forage available, but can survive on extremely poor-quality forage if required to do so (Hopkins, 1990).

Goats in non-farm environments are usually found in herds of 5-20, although the herd size may range up to 100. In the wild, predation and parasites are the major factors which affect their longevity. Typical predators of goats include coyotes, mountain lions, bobcats, domestic dogs and other large carnivores.

Although closely related to sheep, goats belong to a different genus from sheep (*Capra* vs *Ovis*), and are not able to successfully interbreed with them or any other species. Goats have 60 chromosomes, while sheep have 54 chromosomes. This mismatch of chromosomes generally causes any embryos of a sheep-goat pairing to die at 30-50 days of gestation or for the offspring to be stillborn (Jensen, 2005).

Goats are difficult to confine and populations of feral goats (wild goats derived from domesticated goats), are currently found in several parts of the world including Australia, New Zealand, Ireland, and Great Britain. Feral goats are also often found on islands in many locations where they were originally introduced by man and there are often no natural predators to control them. In the United States, feral goats are found in California and Hawaii. In California, the major populations are found on Santa Catalina Island with small populations in a number of areas of the coastal mountain ranges where they are not a serious problem, likely due to natural predation which limits their numbers (Howard and Marsh, 1986).

D. Recombinant Human Antithrombin (rhAT or ATryn®)

Recombinant human antithrombin III (rhAT), also known as antithrombin III (Recombinant) or antithrombin alfa[2], and by the tradename ATryn® (ATRYN[3]), is a recombinant therapeutic protein produced in the milk of GE goats. It is subsequently isolated and purified to a sterile, lyophilized powder for use in making a solution for intravenous infusion. A formulation of this recombinant protein, known by the name ATryn® for Injection, is the subject of a biologics license application (BLA) that is currently under review by Food and Drug Administration's (FDA's) Center for Biologics Evaluation and Research (CBER). This product is intended to treat patients with congenital Antithrombin III (ATIII) deficiency to

[2] The approved international nonproprietary name (INN) and United States Adopted Name (USAN) for this recombinant protein is antithrombin alfa. The FDA assigned product name is antithrombin III (Recombinant).

[3] Per CVM's convention, all proprietary names of drugs are written in upper case letters. In this case ATRYN is equivalent to ATryn®.

prevent life- threatening clot formation during high risk situations such as surgery and obstetrical procedures. ATIII is a normal plasma protein that inhibits clotting by binding thrombin and clearing it from the circulation. Persons with congenital deficiency of ATIII are at high risk to develop life-threatening clots during high risk situations such as surgery and obstetrical procedures. Abnormal clotting in such persons can be prevented by infusion of an ATIII product. The only antithrombin (AT) currently available in the U.S. is isolated from pooled human plasma (Thrombate III®, hpAT).

E. Environmental Assessments of GE Animals

With this technology come some new concerns for potential environmental impacts. Some of these include the risks of animal escape and establishment in feral populations, the disposal of GE animals once they have completed their useful lives, and the disposal of GE animal wastes and waste products that may contain recombinant genes or gene products.

As specified by 21 CFR 25.20(m), this document is the environmental assessment (EA) prepared by FDA's Center for Veterinary Medicine (CVM) to evaluate the potential environmental impacts that may be associated with the use of GE domestic goats (*Capra aegagrus hircus*) containing the Bc6 rDNA construct that directs the expression of recombinant human antithrombin (ATRYN) in the milk of lactating does. The EA has been triggered by an anticipated major agency action, the potential approval of the regulated article (the rDNA construct) in these GE goats, as described in FDA's Guidance for Industry 187: Regulation of Genetically Engineered Animals Containing Heritable Recombinant DNA Constructs (available at this website: http://www.fda.gov/cvm/Guidance/fguide187.pdf). The EA discusses the anticipated agency action, the possible effects on the environment resulting from the growth and maintenance of these GE goats under the specified conditions of use described in the sponsor's new animal drug application, and possible hazards or risks that may arise from exposures via the possible escape of these GE goats from containment.

The EA focuses on the potential environmental effects of these GE goats and their waste products at, and around, a farm in central Massachusetts where they are raised and used to produce rhAT for use in humans. The EA also addresses the potential impacts from another site in central Pennsylvania where a small number of GE goats are held.

CBER will separately comply with its NEPA obligations arising from its review of the BLA submitted by GTC Biotherapeutics, Inc (GTC).

General risk questions to be addressed in this EA include the following:

- What are the risks associated with the GE goats while under confinement?
- What is the likelihood that the GE goats will escape from confinement?
- What are the likely consequences should the GE goats escape from confinement?

GTC Biotherapeutics has submitted extensive information to the agency as part of its investigational new animal drug (INAD) file, new animal drug application (NADA),

investigational drug application (IND), and BLA. In addition, members of FDA's staff inspected and/or site visited the GTC farm in Massachusetts on at least two occasions. Relevant information from all of these sources, as well as FDA's reviews of the data and information provided by GTC were used in this EA.

II. Description of the Proposed Action and Its Need

GTC Biotherapeutics is requesting approval of an NADA for the Bc6 rDNA construct in the GTC 155-92 lineage of goats that have been genetically engineered to express rhAT in the milk of lactating does. As described in Guidance 187, GE animals are regulated under the new animal drug provisions of the Federal Food, Drug, and Cosmetic Act; major agency actions such as the approval of a new animal drug application (NADA) trigger a requirement to perform an environmental assessment of the outcome of that decision under the National Environmental Policy Act (NEPA).

As described in Guidance 187, CVM has proposed a risk-based hierarchical review strategy that can fulfill sponsor's requirements for demonstrating safety and effectiveness that is consistent with the Act (21 USC 321 et seq.) and its enabling regulations (21 CFR 511 and 514). This approach begins with a product definition, and continues through a series of steps that characterize potential hazards in the rDNA construct and resulting lineage of GE animals. Once those hazards have been identified, the agency can determine whether any significant risks to animal health, humans or other animals via food/feed consumption (if appropriate), and the environmental can occur.

In the following sections, we present our environmental assessment, consistent with the process required by statute and regulation, recommended in Guidance 187, and consistent with the statutory and FDA regulatory requirements of NEPA (21 CFR 25). Rather than reproducing the entire hazard/risk reviews for each step of the process in this document, we will concentrate here on those components relevant to the environmental assessment. A summary of the hazard/risk assessment will be found in the Freedom of Information Summary for the NADA on the CVM website if and when an approval is granted.

III. Risk-Based Review Leading to Environmental Assessment

A. Product Definition

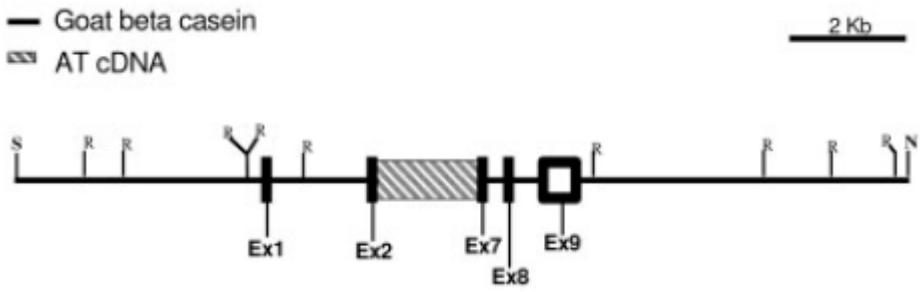
The first step in performing the risk-based review is to establish the identity of the product. As described in Guidance 187, the product that is the subject of the NADA is

> A specific hemizygous diploid line of domestic goats (*Capra aegagrus hircus*), containing 5 copies of the Bc6 rDNA construct located at the GTC 155-92 site, directing the expression of the human gene for antithrombin (which is intended for the treatment of humans) in the mammary gland of goats derived from lineage progenitor 155-92.

B. Characterization of the Bc6 rDNA Construct

The Bc6 rDNA construct is comprised of sequences from three main sources: the plasmid backbone of a common cloning vector (pUC 13) which was removed prior to the microinjection of the intended sequences, the goat beta casein promoter and associated sequences, and the human antithrombin coding region selected from a cDNA library. The β-casein promoter was selected because of its specificity for and demonstrated strong expression in the mammary gland.

Figure 1. Structure of Human Bc6 rDNA Construct.



As part of its overall risk-based review process for GE animals, CVM conducted an evaluation of the submitted information on the Bc6 rDNA construct to address the following questions addressing hazard characterization:

1. Does the rDNA construct intended to generate the GE goats contain DNA sequences that may pose hazards to the animals themselves, to humans, or to other animals consuming food from this goat, or the environment?
2. Does the solution containing the rDNA construct intended to generate the GE goats contain other hazardous material such as hazardous chemicals or live bacteria or viruses?

After reviewing all of the information and data submitted by GTC, including primary DNA sequence information, plasmid construction maps, protocols, and results of analytical tests to characterize the Bc6 rDNA construct, CVM concluded that the Bc6 rDNA construct contains no intrinsic hazards as described in Questions 1 and 2, above.

C. Characterization of the Molecular Construct in the GE Animal Lineage - Durability of Genotype and Phenotype

The material described in Figure 1 was excised from the pUC13 plasmid, and microinjected into a fertilized egg/early embryo. Following a short period of culture, the early embryos were implanted into surrogate dams. Following genotypic and phenotypic characterization of the resulting live births, one genetically engineered male goat, animal 155-92, was selected to

be the lineage progenitor for establishment of the production herd. The Bc6 rDNA construct was transmitted in a Mendelian fashion, yielding female GE offspring that produced rhAT in their milk and GE males that were subsequently used as breeders to increase the size of the herd. Herd size is currently controlled to meet pre-approval production demands, and would be expanded to meet market requirements should an approval of ATryn® for Injection be granted. The production herd is closely monitored to ensure that the Bc6 rDNA construct is stably inherited and that the production phenotype is maintained.

GTC employs a rigorous genetic testing of all its GTC 155-92 production goats to genotypically qualify them for entry into the milking sub-group. All production goats must have an intact transgene with the correct copy number and with no sign of genetic rearrangement of the transgene. In addition, phenotypic characterization is performed on the milk to confirm expression of the transgene (mRNA from the somatic cells in milk and rhAT levels). Only after passing both the genotypic and phenotypic qualification, as well as having the appropriate health certification, is a rhAT goat allowed to contribute to source material (milk) collection.

The risk-based questions that CVM asked in this portion of the review addressed the incorporation of the Bc6 rDNA construct into the goat genome, its characterization in the lineage progenitor, and subsequent generations. They included the following:

With respect to the inserted sequences and their immediate flanking regions,

- Does the GE animal contain sequences that are likely to pose potential hazards to the animal, humans, or animals consuming food from that animal, or the environment?
- Is the genotype changing over the life span of the animal or product?
- Is the inserted DNA what was expected from the data presented in support of the Molecular Characterization of the Construct?

Also,

- Does the GE animal contain other contaminating or hazardous materials such as viruses, cells, or chemicals?

To evaluate the consequences of the insertion of the rDNA sequence, CVM reviewed (a) the quality of the sequencing, (b) the number of insertion sites, and (c) the insertion site itself, including possible disruption of other genes and analysis of open reading frames (ORFs) within and around the insertion site.

CVM's review of extensive data and information indicated that the insertion site in the goat genome was well characterized in the lineage progenitor and in all subsequent generations. The review also indicated that the information regarding the molecular characterization of the Bc6 rDNA construct in the GE goats is consistent with that provided for the Bc6 rDNA construct. The review of the submitted data did not identify any specific hazards that are intrinsic to the insertion of the Bc6 rDNA construct into the GE goats. There are no sequences in the animal arising from the insertion of the Bc6 rDNA construct that are likely to pose potential hazards to animals, humans, or the environment.

D. Phenotypic Characterization of the GE Animal

CVM reviewed all of the information submitted by GTC to the agency in order to characterize the phenotype of the GE animals to determine whether the insertion of the Bc6 rDNA construct or its expression may have caused changes resulting in the increased risk of adverse outcomes. This review included the results of both of the FDA inspection and the site visit of the GTC farm in Massachusetts. Particular emphasis has been placed on the visit conducted by a CVM veterinarian and a ruminant animal physiologist in November 2008, in order to observe goat management procedures, review original animal health and husbandry records, and obtain copies of standard operating procedures and internal GTC reports related to the health and husbandry of the GTC 155-92 GE goats.

CVM's review indicated that there were no apparent differences in the health, mastitis, nutrition, and reproductive status of GTC 155-92 goats vs. non-GE goats on GTC's goat farm. Other than the presence of rhAT in the milk of the GE goats, which is the intended outcome, the only difference noted was that rhAT female goats had lower daily milk production and shorter lactations than their non-GE herd mates. This is attributable to genetics that originated from a single male founder vs. their non-GE herd mates that had a more diverse genetic background with increased opportunity to introduce superior dairy genetics vs. the rhAT population.

CVM concluded that the insertion of the Bc6 rDNA construct did not pose an increased risk of adverse outcomes to the health of the GE animals; no effects were noted that are anticipated to have an adverse outcome on the environment.

E. Environmental Assessment

In order to conduct the environmental assessment, the results of the previous four evaluations are combined with an analysis of the husbandry and containment of the GE goats, including a review of the animal production facilities and practices; the conditions under which the animals are housed; containment and biosecurity, including animal identification; disposition of animal carcasses, and disposal of animal wastes.

a. GE Animal Production Facilities and Practices

GTC's 155-92 GE goat production herd is housed at a farm owned by GTC in central Massachusetts that is a United States Department of Agriculture (USDA)-registered research facility (9 CFR 2.38). As a result, the goat herd is inspected, monitored, and has been certified scrapie-free by Animal and Plant Health Inspection Service (APHIS) veterinary inspectors in the USDA Voluntary Scrapie Flock Certification Program (VSFCP). The herd was closed to the introduction of animals from outside the facility in 2000.

The GTC farm also has an Animal Welfare Assurance on file with the National Institutes of Health, Office of Laboratory Animal Welfare and has been inspected and accredited since 1997 by the Association for Assessment and Accreditation of Laboratory Animal Care

International (AAALAC Int.). As mandated under the Animal Welfare Act, all animal activities and related husbandry, facilities and veterinary care are overseen by GTC's Institutional Animal Care and Use Committee (IACUC). All of these certificates and inspection reports have been confirmed by copies submitted to FDA or during site visits performed by FDA staff.

The current 155-92 GE goat production herd includes several hundred male and female goats, whose genetics stem from Alpine, Saanen, Toggenburg, and Nubian breeds. CVM scientists conducted a site visit of the GTC farm in Massachusetts in early November 2008. The conditions and general practices at GTC's farm are described below based on information submitted by GTC in the IND, BLA, and NADA; standard operating procedures (SOPs) and other documents collected during the CVM site visit; and personal observations made by CVM scientists during the site-visit.

GTC also maintains a small 155-92 GE goat herd at a facility in central Pennsylvania. This secondary herd is managed as a source of animals to renew or expand the existing production herd should the need arise. The secondary herd is maintained entirely indoors under secure conditions. It was not inspected by FDA; however, GTC represents that it is maintained under conditions very similar to those for the Massachusetts production herd; the sponsor has provided additional information to support this. Details on the biosecurity and containment conditions at the Pennsylvania facility are presented in Section *g* below.

b. GTC's Massachusetts Farm, Goat Housing and the Surrounding Environment

Acquisition of the GTC farm site in Massachusetts was governed by a series of strict selection criteria to minimize risks of disease spread and assure containment:

- No occupation by bovine species for at least five years prior to purchase to reduce the risk from environmental pathogens;
- No evidence of occupation by sheep or goats to minimize the risk from scrapie or other species-specific pathogens;
- Suitability of the terrain for agricultural operations;
- No activities on abutting properties that would pose herd safety or health concerns;
- No significant environmental risks on or close to the property; and
- Water that meets National Primary Drinking Water Standards

Once the site was selected and purchased by GTC, animal housing was designed and built to address animal comfort, efficiency of logistical operations, and to comply with animal care and welfare regulations for animal spaces, including USDA's regulations for research facilities (9 CFR 2.38). Additionally, the potential impact of seasonal extremes and weather conditions on goat health and welfare were taken into consideration in the design of the buildings.

Most of the animal housing consists of state of the art large barns, employing dry lot design, and a center alley with internal penning. Surface materials are designed to withstand cleaning with detergents, disinfectants and high-pressure water. Passive ventilation is provided through screened ventilation curtains, and active ventilation is provided in some buildings for

which passive ventilation is insufficient to maintain appropriate conditions. Goats can enter fenced outdoor paddocks via doors in each pen; outdoor paddocks are surfaced with gravel and stone dust. Goats are allowed free access to these outside paddock areas unless inclement weather dictates internal housing. No free-range pasturing is allowed for any goats. Pens are equipped with structures and materials to engage the goats, including climbing steps to simulate hills, and various entertainment devices.

All on-site GE goats are contained by duplicate barrier systems. External fencing encompasses the entire campus of buildings, while internal fencing and barriers maintain each group of animals within their barn and the adjoining paddocks. Access to this site is highly restricted with both physical and electronic access restrictions in place. Additional details are provided below in Section *e* on physical security and animal containment.

The GTC farm is surrounded on its four boundaries by a highway and GTC-owned land to the east, by railroad tracks to the west, by a wooded grove to the north, and by a large hill to the south. Outside of the immediate farm perimeter, the surrounding area is rolling, densely wooded, and semi-rural, with a few isolated agricultural fields and open meadows. The area contains many scattered residences along the nearby roads and in a few isolated subdivisions. Many of these residences consist of large homes and relatively large lots. Several large ponds are found in the area, many surrounded by residences.

c. Animal Identification, Segregation, and Husbandry

The following description of animal identification and husbandry is provided to indicate the risk mitigation measures in place to address potential misclassification of GE animals as non-GE, and contributes to an assessment of the overall farm security and the potential for long-term escape and misidentification of animals.

Each goat in the herd is assigned a unique identification number at birth, and a master list of all assigned numbers is maintained at the facility to ensure traceability. That unique number is associated with each animal in three redundant ways:

1. Permanent ear tattoo applied less than 24 hours after birth;
2. Subcutaneous transponder (electronic implant); and
3. Physical tag attached to a neck chain or Velcro collar.

Members of GTC's farm staff conduct a monthly herd-wide inventory to identify any animals that have missing or illegible tags, which are then replaced. Farm staff also conduct an annual herd-wide transponder check, which replaces non-functional transponders.

All goats, regardless of their GE status, are segregated before sexual maturity into age and size cohorts to encourage socialization and maintain a healthy juvenile group. Young goats are housed primarily in dedicated nurseries and kid spaces. Male goats are segregated from females before they reach sexual maturity to prevent unplanned pregnancies. When an rhAT GE doe begins lactation, she is moved to a dedicated dairy building on the farm where she is qualified, milked, and housed.

GTC farm staff are trained (with appropriate documentation) to perform all routine animal husbandry activities including, but not limited to, daily feeding, milking, watering, grooming, and breeding of goats. Execution of farm tasks are conducted in accordance with documented procedures that provide guidance of all animal interactions as well as non-animal related activities.

d. Animal Health and Biosecurity

The following description of animal health and biosecurity is part of the assessment that evaluates the potential for environmental risks resulting from the spread of disease due to housing and biosecurity practices at the GTC facilities, or to help determine the potential for disease status in the event that an animal escapes from the facility.

GTC strictly adheres to currently accepted guidelines regarding animal health and welfare. This includes those outlined in the Animal Welfare Act, *The Guide for the Care and Use of Laboratory Animal* (including all amendments established by the National Institute of Health as combined in the *Public Health Service Policy on the Humane Care and Use of Laboratory Animals*), and also the *Guide For the Care and Use of Agricultural Animals in Agricultural Research and Teaching* published by the Federation of Animal Science Societies and accepted by the USDA-APHIS-AC and AAALAC Int. GTC also complies with all other federal, state and local requirements for the responsible use and care of animals.

A quarterly review of morbidity and mortality (M&M) data is performed to evaluate the health status of the herd, with the aim improving the overall animal health and welfare of the animals, and decreasing M&M within the general herd. Several full-time veterinarians are employed by GTC to provide 24/7 coverage year round. The veterinarians monitor and track both individual animal and herd health on a daily basis and can detect subtle changes that may be indicative of potential clinical or sub-clinical issues or possible adventitious agent concerns. For each goat, a permanent health record is initiated immediately after birth and is maintained throughout its life. This record contains a complete history including date of birth, sex, breed, results of routine and diagnostic testing, records of vaccinations, preventative care and treatments, surgical procedures and breeding and reproductive data.

Control starts at the level of the farm with extensive, well-documented, written standard operating procedures employed for the majority of operations involved with maintaining the site and for caring of the animals. Similar practices apply to incoming materials, which at a minimum, include all of GTC's hay, grain, and bedding materials. Lastly, this control encompasses monitoring, and restricting where necessary, flow of personnel/visitors and vehicular traffic.

GTC's quality assurance (QA) documentation system utilizes a number of different categories of documents to encompass the activities that occur at the level of the farm and animal; standard farm practices, standard veterinary practices, and other standard operating procedures and good manufacturing practices that are aimed at defining best practice in an agricultural/pharmaceutical setting. These documents provide the exact procedure to be followed by trained personnel. Similar to recombinant production systems established for other rDNA organisms, it is these practices and the documentation system that allows for a

highly controlled, well characterized, and consistent product to be produced from the rhAT goat herd.

A variety of general adventitious agent risk minimization (i.e., biosecurity) measures have been implemented at the GTC farm. Many of these measures are derived from generally accepted principles used to minimize risk of adventitious agent introduction into recombinant expression systems, downstream processing operations, and specific recommendations for animal-derived products in regulatory guidance documents. The primary basis for such measures is the definition of an appropriate level of segregation and control at the level of the environment, the equipment, the raw materials and the manufacturing process. A similar approach has been taken for production of rhAT in GE goats.

The risk minimization started at the level of the farm with strict selection criteria for the farm site and the goats. Following establishment of the base herd, closure of the herd reduced any potential for entry of adventitious agents into the herd via outside animal introduction resulting in the current certified scrapie-free and specific-pathogen free goat herd. A comprehensive biosecurity program was implemented which covers both internal and external aspects of farm operations and the overall animal care program.

In the event of the suspected presence of an infectious disease, a goat or group of goats may require isolated to avoid the possible disease spread. Based on the presumptive clinical diagnosis, the goat(s) will be hospitalized, quarantined, or isolated. Several infirmaries are located throughout the GTC farm, and two isolation suites are located in the main clinic.

From the perspective of the introduction of infectious agents from the outside, the program encompasses all personnel and visitors or service personnel/contractors. The program addresses known wildlife that exists in the surrounding environment and appropriate monitoring and controls to limit that population, where possible. The biosecurity program includes an Integrated Pest Management (IPM) Program that monitors and controls incursions by birds, rodents and insects. Extensive use of bird netting, rodent traps, and electronic insect light traps is part of the IPM program.

Internal aspects of the biosecurity program focuses on the goat herd itself and addresses herd closure, evaluation of raw materials provided to the goats (hay, grain, water, bedding, etc.), and the monitoring of overall clinical health as a tool for detecting potential disease entry.

Although focused on maintaining animal health, many of the extensive biosecurity measures in place at the GTC farm also directly or indirectly contribute to animal containment and tracking (i.e., physical security) and reduce the risk of long-term escape.

e. Physical Security and Animal Containment

The GTC farm is surrounded by a double barrier between all 155-92 GE goats in the production herd and the outside environs. The entire farm site is surrounded by a heavy, 6-foot high, chain-link perimeter fence with gated access. The fence also extends 18 inches below ground level to prevent the ingress of external wildlife or the egress of farm animals. At locations where there are gates for access to the farm and the fencing cannot be buried,

there are additional barriers present. Each barn that houses 155-92 GE goats has an external paddock/exercise yard with wood and wire fencing that limits the goats' access to the farm grounds. Inside each barn there are other physical containment structures preventing escape. The perimeter and paddock fencing was examined during the site visit by CVM staff and found to be appropriately strong and secure with no obvious gaps or openings that would allow entry of large wildlife or the escape of goats.

Access to the farm from the perimeter is highly regulated. The vehicle gates and building entrances are all electronically controlled with video surveillance. All personnel and visitors are required to fill out a detailed biosecurity questionnaire prior to or upon initial entry. Site access may be restricted or denied depending on the person's biosecurity risk. The only vehicles allowed within the perimeter fencing are those essential for servicing the farm as there are already dedicated vehicles on the farm for movement between the buildings on the site. All persons' footwear and vehicle tires must receive an appropriate treatment (e.g., disinfectant foot bath) with a biosecurity solution prior to entry onto the farm site.

A video surveillance system is in place which focuses on key entry points and remote sections of the farm perimeter. Recording is performed around the clock and is available for review as needed. The farm is staffed with non-security personnel around the clock, 7 days a week. In addition, there is a dedicated security force on site all nights, weekends, and holidays. Daily checks by farm staff and veterinarians during feeding and milking operations help insure that the absence of any goats would be quickly identified.

All told, at least five levels of physical containment are considered to be present at the GTC farm including two independent fencing systems, 24-hr staffing/security, multiple daily staff checks, and video surveillance.

f. Disposal of Waste Products and Carcasses

Farm wastes including liquids, solids, or by-products from processing, are collected and disposed of in a safe and sanitary manner, in accordance with local, state, and federal regulations. Liquid wastes from barns include goat-housing wash water, milk equipment cleaning solutions, and milk. Each building that generates liquid waste has a system that consists of a drain and collection tank. Goat-housing wash water is captured in a tank for removal, diverted to a grass filter. Milk-equipment cleaning solutions are captured in a holding tank for settling and bacterial degradation. Remaining wastes are subsequently pumped to an organic bed disposal system (percolation bed) for further degradation. Small amounts of waste milk combined with spent equipment cleaning solutions are directed to a dedicated collection tank until the contents are removed by a certified waste removal service for off-site disposal at a local publicly owned treatment works (i.e., sewage treatment plant) or at another acceptable disposal site consistent with all federal, state, and municipal regulations.

The bedding and manure from the barns is collected every few days or at appropriate intervals and trucked outside the perimeter fence line to a dedicated manure transfer station.

It is composted at this location until a certified contractor collects and removes the material from the farm for further composting.

No 155-92 GE goats or goat by-products enter the public food supply or are offered for rendering. Goat carcasses are disposed of in accordance with state and federal laws, and all GE goat carcasses and other goat tissues (e.g., placental and reproductive tissues) are removed by a contractor for incineration. Manifests and carcass pick-up records are maintained.

During the CVM site visit, GTC farm staff confirmed that no compost, manure, or GE animal carcasses were applied to the land or buried at the GTC farm. As stated in GTC's written standard operating procedures and documented via records collected during the site visit, commercial entities that specialize in waste disposal remove almost all of the liquid and solid waste generated at the GTC farm for off-site disposal at a local sewage treatment plant. The only materials retained on the farm are the materials that enter the sanitary septic system (e.g., waste from the bathrooms used by the farm staff) and a small amount of milk from GE animals that is diverted to the organic bed disposal field. Although GTC has constructed this organic bed disposal field, their staff noted that virtually all of the milk from the GE goats is retained for use in manufacturing or additional research activities.

g. Pennsylvania GE Goat Facility

A small herd consisting of few dozen of GTC's 155-92 goats is maintained at a USDA-registered research facility in central Pennsylvania. This facility is also accredited by AAALAC Int. The goats at this facility are maintained indoors within a biosecure barn at all times and are not allowed outside access. Physical security consists of stalls and pens within this barn, plus a room/door system that prevents the goats from exiting the building, thus there are effectively two levels of physical containment. In addition, much of the farm complex, including the barn where the GE goats are housed, is surrounded by fencing; however, because it is not 100% contiguous, it cannot be considered to provide full containment. The GE animals at this site are identified in the same redundant manner as those at the GTC farm in Massachusetts and are subject to the same daily checks at feeding times. There is no video surveillance at this facility; however, as with the Massachusetts farm, there is a physical presence at the site 24 hours a day, seven days a week.

Written standard operating procedures are in place and are followed at the Pennsylvania facility to insure that the animal husbandry and biosecurity conditions are very similar to those for the Massachusetts farm. For example, the animal feeds at the two sites are identical and come from the same source. Disposal procedures for goat wastes are similar to, though not identical, to those followed in Massachusetts. All manure/bed pack is first composed and subsequently spread over on-site fields that are not used for hay or feed production. Does are not milked at this location so disposal of waste milk is not an issue. All animal carcasses are disposed of on-site per USDA regulations using a six foot deep trench which is layered with and then covered in lime.

The surrounding environment at the Pennsylvania goat facility is also similar in many ways to that at the GTC farm in Massachusetts. The area outside of the farm is largely rural and wooded with scattered residences in the vicinity.

Overall, the level of containment at the Pennsylvania facility is considered comparable to that in Massachusetts. Other conditions at the two facilities are also similar and comparable.

IV. Analysis and Risk Characterization of Potential Environmental Impacts

The science-based concerns posed by GE animals, including risks to the environment, have been considered by several panels of experts in recent years, including a committee of the National Research Council (NRC) of the National Academies of Science in 2001. This committee's report, *Animal Biotechnology: Science-Based Concerns* (NRC, 2002) specifically addresses, among others, environmental hazard and risk issues. Others (Devlin et al. (2006), Hallerman et al. (1999), Muir and Howard (1999, 2002) and Kapuscinski (2005) have also presented general methods and models for evaluating the ecological risk of these animals. To date, most of the emphasis has been on GE finfish because of the potential risks associated with their widespread escape or release, but many of the general approaches have broad application and were used in this environmental assessment of the GE goats.

The potential for environmental risk are expected to increase as a function of likelihood of escape, and the inherent likelihood of becoming feral if escape should occur. Based on a ranking of the number of citations in the scientific literature, the animals considered most likely to become feral are insects, fish, domestic cats, and pigs (NRC, 2002). Although ranked somewhat lower than these animals, NRC ranked goats as having a high ability to become feral, and as having a moderate likelihood of escape from captivity based on a subjective evaluation of the animal's ability to evade confinement measures (NRC, 2002).

Although the NRC report gave the goat an overall moderate level of concern for the ability to escape, disperse, and become feral, they noted that the receiving ecosystem also plays an important role in determining the nature and extent of risk even if the GE animals were to escape from confined conditions. For example, if there were no wild or feral relatives in the area, then the potential for interbreeding would not be an issue. Likewise, the presence of predators in the area of escape limits the probability that the GE animal will survive and reproduce.

Most of the potential hazards and harms associated with environmental releases or escapes of GE animals relate to the gene construct or transgene being present in the GE animal, its establishment in the wild, and its ability to spread to other animals of the same or similar species. These need to be considered on a case-by-case basis. For example, there may be adverse outcomes to receiving wild populations associated with GE animals that have increased survival traits, but GE animals that have different color patterns may pose minimal, if any, adverse outcomes to wild populations.

Environmental impacts from the release or escape of GE animals may also occur even if there is no gene flow or spread of the transgene. These may arise from the actual properties of the GE animal, including the effect that disposal of its wastes or carcass may have on the environment.

This analysis focuses on the following risk-related questions specific to the product definition specified earlier in this assessment:

1. What are the risks associated with the GE goats while under confinement?
2. What is the likelihood that the GE goats will escape from confinement?
3. What are the likely consequences should the GE goats escape from confinement?

A. Risks Associated With 155-92 GE Goats While Under Confinement

The hazards and risks associated with GE animals in confinement are highly dependent on the gene expression product and the ability of the inserted gene construct to mobilize and spread to other animals. In the case of the 155-92 GE goat production herd at the GTC farm in Massachusetts, the following environmental risks were identified and considered:

- *Risk of gene flow via mobilization of the Bc6 rDNA construct.* The Bc6 gene construct was introduced into the embryo of the founder goat using microinjection (i.e., without use of a viral vector). The rDNA construct does not contain viral or other sequences that would render it mobilizable or likely to spread.

- *Risk of direct toxicity resulting from increased environmental concentrations of rhAT.*
  - *Hazard.* The product of the Bc6 rDNA construct in the GE goats (i.e., rhAT) is similar to naturally occurring forms of this protein in other species and poses no intrinsic hazard. Based on its structure and composition, rhAT is expected to rapidly and readily degrade in the environment.
  - *Exposure.* The protein rhAT is expressed in significant amounts only in milk of lactating does. Expression in other tissues is either very low or absent. This milk is a valuable commodity and is processed to remove the rhAT for further purification and use in ATryn® for Injection; therefore, no change in the concentration or distribution of this compound in the environment is expected.

- *Risk of disease spread from confined housing of 155-92 goats.* The extensive internal and external biosecurity measures in place at the GTC farm protect the GE goats from introgressing adventitious and infectious agents and from coming into contact with outside wildlife. Thus, the possibility for disease transmission (and also for gene flow), both from and to the GE goats, is low.

- *Risks that may be associated with the disposal of GE animal wastes or carcasses.* Even though no hazards that could be present in animal wastes were identified, exposure to other organisms is minimal due to the highly-controlled measures currently in place for the disposal of the GE goats' waste products. For example, the

carcasses of all 155-92 GE goats at the GTC farm are incinerated. Furthermore, we are not aware of any evidence of horizontal transfer of genes from vertebrates via their manure, byproducts, or carcasses to other organisms in the environment. We also are not aware of any reasons that any such transfer of the Bc6 rDNA construct, even if it were to occur, would cause greater or different environmental effects than transfer of the native goat antithrombin gene from conventional goats.

In summary, the Bc6 gene construct is not likely to mobilize and spread to other organisms, and the gene product does not pose an intrinsic hazard; therefore, 155-92 GE goats in confinement do not appear to present any significant risk to the quality of the human environment.

B. Likelihood that 155-92 Goats Will Escape from Confinement

As discussed previously, there are at least five levels of containment on the GTC farm to prevent the escape of the GE goats including two independent physical barriers, 24-hr security, daily staff checks, and video surveillance. One of the physical barriers is a heavy chain-link fence that is 6 feet high, is buried 18 inches below grade, and surrounds the entire farm perimeter. In addition to the video surveillance system, there is around-the-clock staffing by farm personnel and a security guard presence all nights, weekends, and on holidays. These not only greatly reduce the possibility of escape, but also ensure a high likelihood that any escaping animals will be rapidly identified and recaptured.

All of the GE goats have redundant identification systems (ear tattoos, neck tags, and electronic transponders) that allow them to be identified easily and quickly. Taken as a whole, the containment and security systems indicate that it is highly unlikely that any 155-92 GE goats will be able to escape from the GTC farm. In the unlikely event of an escape, the presence of redundant identification systems reduces the possibility that any of the goats will remain at large for an extended period of time.

C. Likelihood of Harm in the Event that GE Goats Escape from Confinement

Assuming that one or more 155-92 GE goats were able to escape the confines of the GTC farm, there are several risk-related questions that may be asked in order to evaluate the potential environmental harms that could result. These include the following:

- What is the likelihood of survival, reproduction, and establishment of the 155-92 goats in the area outside the GTC farm?
- What is the likelihood of dispersion to new habitats?
- What is the likelihood of survival, reproduction, and establishment in the new habitats?
- What direct and indirect effects may result in receiving habitats?

- Are these effects likely to produce significant impacts on target resources? For example, to alter gene pools, harm species of special concern, or cause a loss of stability or resilience in receiving habitats or communities?

There is very little reason to believe that 155-92 goats would be able to survive, reproduce, and establish a population in the environs outside the GTC farm, or that they would be able to migrate to, and establish in, another nearby habitat. First, in order to establish a population, two or more animals would need to escape at one (or approximately the same) time. Given the high value of these animals, and the intensive surveillance systems in place, the animals would likely be found missing and recaptured within a very short period of time and quickly returned to the farm. The presence of numerous scattered residences in the general vicinity of the GTC farm and the fact that the GE goats have redundant identification systems strengthens this assurance.

In the highly unlikely event that the escaped animal(s) were not recovered, the likelihood of long-term survival, reproduction and establishment is extremely low based on the fact that there are no known populations of feral goats in the northeastern United States despite the presence of numerous herds of commercial domestic goats, as well as pet goats, throughout this area. Although there are several feral goat populations present in the U.S., they are confined to isolated locations in the foothills and coastal habitats of California, Santa Catalina Island, and the Hawaiian Islands (Hopkins, 1990).The harsh winter climate of Massachusetts and the presence of potential predator species (e.g., dogs, coyotes) makes survival and establishment highly improbable. Coyotes originally moved into the central and western regions of Massachusetts in the 1950s. They are currently well established throughout the state and the eastern U.S. in general, and have adapted well to all habitats including urban and suburban areas (MDFW, undated). Depredation of livestock, including goats and sheep, by coyotes has become such as significant problem that several eastern states have set up integrated predation management programs to help reduce these losses (Houben, 2004).

Reproduction by escaped GE goats in the wild is particularly unlikely because adult male and female 155-92 goats are housed separately on the GTC farm, and thus are unlikely to escape at the same time should escape actually occur. In addition to the above, there is no evidence to indicate, and little reason to hypothesize, that the addition of the Bc6 gene construct to their genome has increased their fitness and made these goats any more likely to survive and establish in the wild than normal domesticated goats. Extensive studies on the fitness of genetically engineered mammals are currently lacking, but studies on GE fish (coho salmon) have not generally shown them to have increased overall fitness over their natural counterparts under simulated natural conditions, although certain individual fitness components may be enhanced (Sundström et al., 2007; Devlin et al., 2004a). In fact, the GE coho salmon are less fit and less likely to survive under some simulated natural conditions, for example when food availability is limited (Devlin et al., 2004b).

Even if one or more 155-92 GE goats were able to escape and survive for an extended period of time outside the GTC farm, it is hard to postulate any significant adverse effects that they might have on the local environment. Goats can be highly destructive to natural vegetation

and have been reported to contribute to erosion, the spread of deserts, and the disappearance of wildlife in Middle East and Mediterranean areas, Hawaiian islands and other island locations (Hopkins, 1990). However, a significant level of habitat destruction is extremely unlikely in Massachusetts because of the local climate, topography, and vegetative conditions in the area of the GTC farm and because it would require more than just a few animals.

There is no reason to believe that the Bc6 gene construct would spread to other populations of feral goats as none are known to exist in Massachusetts or in any of the surrounding states. Interactions with domesticated goats in the vicinity of the GTC farm are also not expected because the GTC goats would be quickly recognized by their ear tattoos and neck tags, captured, and returned to the GTC farm. In addition, there are no known livestock farms in the area surrounding the GTC farm; therefore, interactions with other domestic goats are unlikely.

Because the Bc6 rDNA construct is not mobilizable, even if interactions were to occur with domesticated animals or wildlife species in the area, there is no realistic pathway for the gene to spread to these animals. Direct transfer to a related species, such as sheep, is not expected since the offspring of goat-sheep matings are generally stillborn or die as embryos (Jensen, 2005), and goats do not interbreed with any other species. Thus, the probability for the Bc6 rDNA construct to spread to any animals other than goats is negligible.

Finally, because of the containments provided for the GE goats and the low probability of escape, survival and inbreeding with feral or domestic goats in the area, the likelihood of the transfer of the Bc6 rDNA construct to wild or other domestic goats is negligible.

D. Risk Analysis for the Pennsylvania GE Goat Facility

In almost all respects, conditions which affect the risk analyses described above for the GTC farm in Massachusetts are similar or identical to those for the Pennsylvania goat-holding facility. Major differences between the two are in the number of GE goats (several hundred in Massachusetts versus several dozen in Pennsylvania) and in the production of milk containing rhAT (none is produced in Pennsylvania). As previously discussed, the GE goat facility in Pennsylvania has a similar level of physical containment to the GTC farm in Massachusetts. In fact, it can be argued that containment is greater at the Pennsylvania facility because the GE goats there are never allowed outside of their barn. Procedures for animal husbandry and to insure biosecurity are also very similar to those for the Massachusetts farm and the animal identification systems are identical for both. Disposal procedures for goat wastes are similar to, though not identical, to those followed in Massachusetts. One additional major difference between the two facilities is in the disposal of animal carcasses. They are incinerated off-site in Massachusetts as opposed to being buried 6 feet underground and treated with lime in Pennsylvania; however, both are USDA-acceptable means of destruction and neither should present a risk to the environment because the gene construct does not pose an intrinsic hazard and is not likely to mobilize and spread.

An analysis of the available information indicates that the same conclusions should apply for the Pennsylvania facility as for the GTC Massachusetts farm with respect to the risks associated with confinement, the likelihood of escape, and likelihood of harm in the event that the GE goats should in fact escape from confinement. The risks associated with confinement in Pennsylvania are minimal because the GE animals are identical and conditions of confinement are comparable to those at the farm in Massachusetts. Considering the high level of containment, the likelihood of escape at the Pennsylvania facility is also very low. As for the Massachusetts farm, in the unlikely event of an escape, the presence of redundant animal identification systems reduces the possibility that any of the goats will remain at large for an extended period of time. The environments surrounding both facilities are quite similar, largely wooded and semi-rural in nature. As in Massachusetts, coyotes are abundant in Pennsylvania and have caused significant losses in sheep/lamb flocks in the state (Houben, 2004). Therefore, should one or more of these animals escape, the likelihood for survival, reproduction and establishment of the GE goats (or the probability they will cause adverse effects on the local environment) is very low and no greater in Pennsylvania than in Massachusetts.

E. Risk Analysis for Animal Transport

From time to time, a few 155-92 GE goats, generally 8 – 12 at a time, may be transferred between the GTC farm in Massachusetts and the goat facility in Pennsylvania via truck transport under the control of a licensed animal hauler with at least two persons accompanying the animals during transfer. The likelihood for escape during transport is very low. In addition, the likelihood for survival, reproduction and establishment of the GE goats in the environment due to escape during transport (or through an accident occurring during transport), is extremely remote. Because of the redundant animal identification systems in place for the 155-92 GE goats, any escaping animals would be expected to be quickly identified and recaptured.

IV. Conclusions

Based on the information considered by FDA, including site visits by FDA staff, there is substantial evidence to conclude that the 155-92 GE goat herds at the GTC farm in Massachusetts and the holding facility in Pennsylvania are unlikely to result in significant effects to the quality of the human environment. The Bc6 rDNA construct is not likely to mobilize and spread to other organisms, and the gene product does not pose an intrinsic hazard; therefore, 155-92 GE goats in confinement are not believed to present any significant risk to the environment. At least five levels of containment (two physical barriers, multiple daily staff checks, 24-hr staffing/security, and video surveillance) are in place to insure that escape of any 155-92 GE goats from the GTC farm is highly improbable. In the unlikely event of an escape, the presence of redundant animal identification systems reduces the possibility that any of the goats will remain at large for more than a brief period of time. In addition, even if one or more GE goats were to escape and not be recaptured, there is very little or no reason to believe that they would be able to survive, reproduce, and establish a

population in the environs outside the GTC farm, or to cause significant adverse effects on the local environment. Furthermore, because of the containments provided for the GE goats and the low probability of escape, survival and inbreeding with feral or domestic goats in the area, the likelihood of the transfer of the Bc6 rDNA construct to wild or other domestic goats is negligible. Finally, the probability for the Bc6 rDNA construct to spread to any animals other than goats is also negligible.

V. Proposed Alternatives and Their Potential Environmental Impact

Because the proposed action is not expected to significantly affect the quality of the human environment, no proposed alternatives or additional mitigating measures beyond those currently in place were considered or evaluated.

VI. Agencies and Persons Consulted

No outside state or Federal agencies were consulted. Several scientists within the Center for Veterinary Medicine with expertise in areas of veterinary medicine, animal science, molecular biology, environmental science and risk assessment were consulted; those making substantive contributions were Larisa Rudenko, Jeff Jones, and Charles Eirkson. Officials at GTC Biotherapeutics were also consulted, including Richard Scotland and William Gavin.

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