POINTS TO CONSIDER IN THE MANUFACTURE AND TESTING OF THERAPEUTIC PRODUCTS FOR HUMAN USE DERIVED FROM TRANSGENIC ANIMALS

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Points to Consider in the Manufacture and Testing of Therapeutic Products for Human Use Derived from Transgenic Animals

I. Purpose of the document

This document presents considerations of the Food and Drug Administration (FDA) with regard to the use of transgenic animals to produce FDA-regulated drugs and biological products intended for human use. These considerations include important scientific questions that should be addressed by sponsors during the preparation of IND, PLA, ELA or NDA submissions. This guidance is expected to assist sponsors in determining what scientific data should be submitted in support of applications for new therapeutic products generated in transgenic animals.

In this document, therapeutic products include drugs, biological products and devices, including tissues or cell preparations, derived from a transgenic animal and intended for use in humans.

The purpose of this document is to present points that should be considered in ensuring that biological products from transgenic animals are as safe and effective as biologicals produced by other methods. This document addresses issues such as the structure of the transgene, the fidelity of inheritance, the

consistency of expression, and the avoidance of contamination by drugs, chemicals, and adventitious agents.

It is expected that methods to address specific issues be scientifically rigorous with sufficient validation.

Many issues relevant to the production of therapeutics in transgenic animals overlap previous Points to Consider (PTC) documents (1-5) published by the Center for Biologics Evaluation and Research (CBER). These documents should also be consulted.

II. Regulatory responsibility

The majority of products for human use derived from transgenic animals and intended for diagnostic, preventative or therapeutic purposes will be regulated as biological products. Biological products, including cellular therapies, are regulated by CBER under authority of the Public Health Service Act (42 U.S.C., Sec. 201 et seq.) and the Federal Food, Drug, and Cosmetic Act (21 U.S.C., Sec. 301 et seq.). The regulations are found at Title 21 of the Code of Federal Regulations (21 CFR). Therapeutic products for use in humans not regulated as biologics may be regulated by the Center for Drug Evaluation and Research (CDER) or the Center for Devices and Radiological Health (CDRH) .

In addition, FDAþs Center for Veterinary Medicine (CVM) and Center for Food Safety and Applied Nutrition (CFSAN) and the United States Department of Agriculture (USDA) have regulatory responsibility for veterinary and food safety issues associated with final products and the use of transgenic animals. Regulations at 21 CFR, Parts 58, 210, 211, 600, 680 and 9 CFR, Parts 1, 2, 3 may be applicable to particular aspects of production or disposition of animals.

III. Definition of transgenic animal

A þtransgenic animalþ is defined as an animal which is altered by the introduction of recombinant DNA through human intervention. This includes two classes of animals; those with heritable germline DNA alterations, and those with somatic non-heritable alterations. Examples of the first class include animals with germline DNA altered through methods requiring ex vivo manipulation of gametes, early embryonic stages, or embryonic stem cell lines. Examples of the second class include animals with somatic cell DNA alterations achieved through gene therapy approaches such as direct plasmid DNA injection or virally-mediated gene transfer. þTransgeneþ refers to a segment of recombinant DNA which is either: 1) introduced into somatic cells, or 2) integrated stably into the germline of its animal

host strain, and is transmissible to subsequent generations.

IV. Generation and characterization of the transgene construct

The recombinant DNA construct used to generate transgenic animals should be well characterized in order to help ensure that the final product has the expected characteristics. Details and quality control of the transgene construct assembly, cloning, purification, and final characterization are essential. The Supplement to the Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology: Nucleic Acid Characterization and Genetic Stability (3) discusses issues associated with characterizing expression systems for recombinant proteins which are useful for characterizing the transgene construct.

A. Transgene and expression system (Heterozygous gain of function)

Detailed characterization of the original gene intended for introduction into the animals should be provided. The natural protein and its functions should be described along with a description of its pattern of expression. The methods used to clone and isolate the gene should be

reported. Description of the transgene should include properly scaled maps and all nucleotide sequences either previously published or newly determined. Description of large segments of DNA in vectors such as yeast artificial chromosomes (YAC) should include detailed restriction maps if the entire nucleotide sequence has not yet been determined. In these cases, nucleotide sequences of cDNAs should be determined.

The strategy used to create the final transgene construct should be described in detail. Both the original vector sequence and the transgene construct should be extensively characterized by restriction maps and nucleotide sequences. The origin and characteristics of regulatory elements are of particular interest and should be carefully described. Information on transcriptional control of the transgene including elements such as enhancers, promoters, suppressors, and presence or absence of dominant control regions should be reported if these elements are included in the transgene construct and will contribute to targeted expression. In addition, introduction of novel transcription factors which can be positively or negatively regulated should be fully described.

B. Gene targeting by homologous recombination (Homozygous loss of function)

The process of homologous recombination can be used to target specific regions of an animalþs genome for interruption or removal, thus creating a null allele. In cases where this approach is used, it will be important to show that the alteration has resulted in an adequate loss of function of the targeted gene. Several instances of incomplete loss of function of targeted genes have been documented and may be explained by a variety of mechanisms. Therefore, demonstration that the product of the targeted gene is not present in any potentially functional form will be important.

V. Creation and characterization of the transgenic founder (G0) animal

A. Donor and foster animal characterization

The history of the animals which donate gametes or embryonic stem cells (donor) and foster or recipient animals should be presented in detail and should include the species, breed,

country of origin, general health, and other available genealogical information. Animals should have detailed veterinary evaluations of health, including specific tests for species and breed-related disease problems. The use of animals from countries where transmissible spongiform encephalopathy (TSE) agents have been identified to have affected the species should be avoided. For the control of adventitious agents, the donor and foster animals should meet the same criteria as required for adventitious agent screening required for admission to the production herd (Section VII C.)

B. Method of transgene introduction

The methods used to introduce recombinant DNA into animals should be described in detail. For example, all procedures used during generation of animals with germline alterations should be presented including techniques used in: isolation of the ova, in vitro fertilization, microinjection of blastula or of the embryonic stem cell line, embryo development and transfer and other established or novel techniques. The methods used to generate animals with somatic alterations should also be described in detail.

C. Characterization of the transgenic founder (G0)

1. Analysis of the transgenic founder

The methods to be used for identifying founder transgenic animals and transgenic animals in subsequent generations should be reported. The sensitivity of the test for presence of the transgene in founder animals should be established. Animals that have taken up exogenous DNA but are not producing the intended product should be distinguished from animals that have not incorporated any exogenous DNA. Disposition of animals to be used as food will be regulated by the FDA (CVM or CFSAN) or USDA Food Safety and Inspection Service (FSIS) when they are of an inspected species being offered for human food. (See Section VIII.E.).

A description of the methods used to confirm that the founder animal is producing the desired product within accepted criteria should be presented in detail. The yields of the desired product should be reported as well as any seasonal, age-related, or other variations in production. There should be verification of transgene expression at the expected tissue sites

and/or at appropriate times in the host's life. This tissue should be carefully monitored and verification of normal processing should be undertaken. The source tissue may not be the natural site of production for a given biological and could result in different post-translational modifications of the product. Since different forms of processing may occur, the effect on biological and immunological activity should be assessed. In addition, high levels of transgenic protein expression may cause adverse side effects or may affect expression levels of endogenous proteins, (i.e., by interfering with or modifying their function) leading to adverse consequences that compromise the health and usefulness of the animals.

2. Genetic stability and expression

Continued availability of the product depends on two aspects of stability of the transgene, the structural stability or integrity of the transgene and the stability of expression of the transgene. Once the founder animal has been identified, stability of the transgene must be demonstrated in animals used in production or until stability has been demonstrated

through multiple generations.

a. Genetic Stability

The process of exogenous DNA insertion into the germline of a host animal often involves integration of multiple copies of the DNA, usually into a single chromosomal site. However, there is a possibility that there will be more than one integration site and that there will be rearrangement or deletion of all or some of the transgene copies during or subsequent to integration. For these reasons, the stability of the transgene through several rounds of germ-line passage (via breeding) should be monitored by appropriate tests such as Southern blots, sequencing or other methods. There should be a stable number of copies of the transgene in a single chromosomal site through several generations. If possible, integration into a single chromosomal site should be confirmed by direct methods at the founder animal stage. If this is not possible for a given species, breeding studies and restriction enzyme analysis of DNA

from multiple progeny could be used to establish single site integration of the transgene. This same approach can be used to establish copy number stability and lack of rearrangement or deletion subsequent to germ-line passage.

b. Stability of expression

The stability of expression of a transgene product can vary depending on interaction of the genetic background of the host animals or imprinting effects due to maternal or paternal inheritance. Instances of decreased expression with increasing germ-line passage have been observed. Therefore, stability in terms of levels of expression of a transgene both within a generation and through several breeding generations should be established for each founder line. Stability of expression of the transgene product should be monitored over the productive life of the transgenic animal. A range of acceptable expression levels should be established and used as a criterion for acceptance in production herds. Where feasible, additional

information should include verification of normal or expected RNA expression of the transgene on the transcriptional level in terms of size, relative abundance of transcript, and tissue or cell lineage where RNA is produced. Methods may include Northern blots, RT-PCR, DNase protection assay or other appropriate techniques. The yields of the desired product and if possible, the expression levels should be monitored in multiple transgenic lineages and animals falling below an established minimum level should not be used for production. The minimum level is determined by considering whether the contribution of each animal is to be used directly or pooled and whether the concentration of the active component in the material to be purified is high enough to assure an adequate purification.

VI. Establishment of a reliable and continuous source of transgenic animals (founder strain)

Since animals cannot be stored indefinitely like cells, it is important to develop approaches to ensure that a desired product

from a useful transgenic founder animal remains available for an extended period of time. This approach should take into account the possibility that genes from the transgenic animal may interact with different genetic backgrounds in the breeding partner, potentially affecting the quantity, quality, and purity of the product produced in each offspring.

A system similar to that used in cell production schemes could be developed to ensure production consistency. The Points to Consider in the Characterization of Cell Lines to Produce Biologicals (4) describes cell banks and the concepts on which this system is based. A tier system is used. The initial tier, the Master Cell Bank (MCB), consists of a homogeneous collection of progenitor cells which are separated from the original cell clone by a low number of generations. The second tier, the Manufacturers Working Cell Bank (MWCB), is derived from a small portion of the MCB and is a low number of generations from the MCB. A substantial number of aliquots of both MCB and MWCB are frozen and constitute an assurance of a consistent source. In analogous fashion, similar tiers could be established for each particular transgenic animal strain. These could be referred to as the Master Transgenic Bank (MTB) and the Manufacturers Working Transgenic Bank (MWTB). If animals are used, these banks could

consist of a limited number of highly characterized transgenic animals derived from a particular founder. If reliable techniques are available, frozen sperm or embryos from a founder and its immediate offspring could be used to establish banks to preserve a valuable transgenic strain. The important feature would be that the animals in the banks could reliably produce offspring that could produce product that is within established acceptance criteria.

Because transgenic animals could be bred in a variety of ways, there may be other plans that meet the goal of ensuring that a desired product from a useful founder transgenic animal remains available for as long as the sponsor may produce the product. The major effort for characterization of the line for product expression and safety should be done on the founder animal (first tier), however, characterization is also expected for subsequent generations of animals (founder strain) from each founder animal. This approach helps to ensure that the source used in the production of a given product is stringently characterized.

VII. Generation and selection of the production herds

Once suitable founder strains have been identified and characterized they can be used for breeding of production

animals. The transgene is transmitted as other genetic traits through traditional breeding with either a nontransgenic or other transgenic animal. Manufacturers should establish criteria for acceptance of transgenic animals into a production herd. This is to ensure that animals reliably produce a product of reasonable quality and safety for the anticipated lifetime of the product. These criteria should be established for each new transgenic strain derived from a particular founder animal and the mating pool of nontransgenic animals (i.e., to include cases where transgenics are bred to nontransgenic animals to produce heterozygous production animals).

A. Animal history and genealogy

Each production animal must be traceable to a particular founder animal. Also, the place and date of birth, use in production, incidence and course of disease and final disposition should be recorded for each production animal.

B. Breeding techniques

Details of the methods that will be used to breed the transgenic production animals should be submitted. Any use of procedures of artificial insemination, embryo transfer,

or semen collection and storage should be documented and appropriate standards applied. If in vitro fertilization is used, the methods and criteria for sperm and oocyte collection and selection should be described. Isolation and implantation procedures for zygotes should be reported. Manufacturers should demonstrate that the animals to be used as recipients of transgenic sperm or embryos are healthy and are free of relevant infectious agents. Pregnancy should be monitored and procedures for delivery of transgenic animals should be described.

C. Adding new animals to the production herd

Criteria for admission to the production herd are important for two reasons: 1) to assure that the quality and levels of the transgene product are acceptable and, 2) to prevent the introduction of an infectious agent into the herd. Specifications for the range of acceptable final yields should be established before considering whether the contribution of the animal is to be used directly or pooled so that the concentration of the active component in the material to be purified will be high enough to assure an adequate purification. To protect against adventitious

agents, sick animals should never be added to the herd, and healthy animals should have met the requirements for entry into the breeding herd and been monitored for a sufficient time period.

VIII. Maintenance of transgenic animals

Detailed plans for maintaining transgenic animals should be submitted. Plans for monitoring the health and housing facilities for transgenic animals as well as plans for removal from production and disposal of the animals or their byproducts should be carefully described in the experimental protocol and approved by the Institutional Animal Care and Use Committee, in accordance with the Animal Welfare Act (7 U.S.C., Sec. 2131 et seq.) and, the Public Health Service Act (42 U.S.C., Sec. 289(d)) where applicable. Consideration should be given to obtaining accreditation by the American Association for Accreditation of Laboratory Animal Care for the facility and the animal care program. Standards for accredited facilities are provided in the NIH Guide for the Care and Use of Laboratory Animals (8).

A. Monitoring the health of transgenic animals

Monitoring plans are important both for maintaining the health of the animals and for preventing product contamination with adventitious agents, pesticides, and animal medications. Submissions should therefore contain a detailed plan describing the monitoring plans. Health records should contain the complete history from birth to death of all animals used in production, including the drugs and vaccines used. Disease episodes should be definitively diagnosed to the extent possible. Sick animals should be removed from production. The plan should include monitoring techniques, endpoints, and methods of reporting results. Methods for recording veterinary care or other preventive measures (e.g., vaccines, vitamin supplements, nutritional additives) should be established. An animal that grazes is not in a controlled environment and therefore may require more rigorous and extensive testing for infectious agents than those raised under barrier conditions.

In some cases, the transgenic animal is itself an in vivo safety test and should be observed for sufficient time to establish effects of constitutive expression of a given product. Deleterious effects may arise from over expression or insertional effects. If feasible, multiple founder

strains should be established so that problems arising due to insertional effects can be differentiated from any problems that may arise due to long-term over expression of the transgene product.

B. Feeding of animals used in production

Transgenic animals should not receive feeds which contain rendered materials derived from species which may contain TSE agents.

Consideration should be given to monitoring feed for pesticide residues. Records of the composition of the feed of the animals and the level of consumption should be kept as part of the animal maintenance record. A change in feed consumption is frequently an early indication of disease in agricultural animals. However, keeping such records may not be possible for grazing animals and other animals not kept in individual pens.

C. Transgenic animal housing facilities

The containment and confinement practices for production operations involving transgenic animals should be in

accordance with applicable portions of the NIH guidelines for Research Involving Recombinant DNA Molecules.

The physical surroundings where the transgenic animals will be maintained should be described in detail, see requirements at 21 CFR 600.11. Information should include herd size, physical isolation and containment, breeding isolation, and biosafety-containment (when appropriate). If the facility is not a single-species-dedicated breeding and maintenance facility, the adventitious agents of the other species must be considered. The surroundings should be capable of containing the animals and of preventing the accidental entry of other animals. Transgenic animals should be neutered after breeding to lessen the chance of escape or inadvertent breeding into the nontransgenic population(s).

D. Removal of animals from production

Criteria that will result in the temporary or permanent removal of animals from production should be described in detail. This could include illness, cessation of production, the appearance of an adventitious agent (even without clinical signs), injury, or other conditions. If

illness is a temporary cause of removal from production, the veterinary care given to animals removed from production and results of the treatment should be documented. Regardless of the reason for temporary removal, stringent criteria for readmission to the production herd should be proposed.

E. Disposal of transgenic animals or use of byproducts

In general, disposal of transgenic animals, including retired or dead animals, should be in accordance with the applicable portion of the NIH guidelines for Research Involving Recombinant DNA Molecules (7).

Nontransgenic animals associated with the herd or animals in which the attempt to insert new genetic material has failed may be used in the food supply in accordance with FSIS regulations (9 CFR 301.17 and 381.75). If the manufacturer wishes to slaughter transgenic animals for human or pet food, or render transgenic animals for livestock feed, CBER or CDER will refer the manufacturer to CVM for a food safety assessment. Use of medications (and the residues that may remain in edible tissues) may affect the ability of the sponsor to subsequently use transgenic animals for human

food products. Careful consideration should be given to the selection of medications for transgenic animals if food for humans is expected to be harvested later. CVM regulates new animal drugs and animal feeds, and evalutates the safety of these products in food producing animals. In addition, CVM establishes conditions for the release of animals to the USDA for inspection by the FSIS. This includes animals which have been treated with investigational drugs and are to be slaughtered for human food; see Federal Register notice March 17, 1994, (59 FR 12582). Some products derived from animals are not inspected by USDA and are regulated instead under FDA authority. These include, but are not limited to, milk, eggs and seafood. CVM and CFSAN will coordinate the appropriate food safety assessment of these products, when there is a desire to harvest the food products (in addition to the therapeutic product) from transgenic animals. Such requests will be referred through CBER or CDER.

F. Animal sentinel program

When feasible, a sentinel animal program that will allow periodic health evaluations should be considered. Such sentinel animals should be infertile, of the same species of

origin, and should be maintained with the transgenic production herd.

IX. Purification and characterization of the transgenic product

A. Recovery of the product from transgenic animals

Recovery may begin with a variety of procedures including milking, exsanguination (See 21 CFR 600.11 (f) (4)), extirpation of tissues, or other steps. The recovery procedure should be designed to maximize the safety, sterility, potency, and purity of the product. In general, where large numbers of animals are involved, the recovery of the biologic under sterile conditions may not be practical. The collection facilities should be as clean as possible.

B. Defining a þproduction lotþ

Monitoring the quality of the product and the manufacturing processes require the purity of the product to be determined at multiple points during the production process. The tests found to be most sensitive to changes in product structure and/or potency should be validated and then incorporated as part of the lot release protocol. Conditions and criteria

by which a þproduction lotþ is defined (e.g., animals used, lot size, collection storage times, the time before sterile filtering and acceptance criteria for source material and product pooling) should be provided.

C. Endogenous and adventitious agents

1. The host animal

Many animal species have been proposed as hosts for production of therapeutic products. The lack of experience with many of these hosts raises potential safety concerns about adventitious agents which will be considered on a case-by-case basis as submissions are received by the FDA.

It is possible that adventitious agents or chemical contaminants could enter the animals and be concentrated in the product by the purification procedure without signaling their presence by altering the health of the herd. Therefore, health monitoring is necessary, but not sufficient to guarantee absence of these contaminants.

As with most biological products, contamination by adventitious agents should be carefully considered. Furthermore, the production process may need to be validated to eliminate and/or inactivate adventitious infectious agents. The rigor of infection control in the animal host and validation of elimination of adventitious agents from the product will depend on several factors including: the intended use of the product; the tissue from which the product is derived; how the product is collected; the purification process; and animal husbandry practices used during production of the founder and production animals. For example, products derived from milk, blood, or urine will have a different set of concerns than products derived from an aseptically collected organ. Manufacturers should consult veterinary experts to determine what infectious agents may be of concern and methods to reduce or eliminate their presence. Zoonotic diseases are of particular concern. If validation of manufacturing processes for elimination or inactivation of adventitious agents is needed, Section VI, Validation of Viral Elimination, in the Points to Consider in the Characterization of Cell Lines Used to Produce

Biologicals (4) may provide useful information.

2. Source tissue

Current efforts to produce therapeutic products in transgenic animals utilize systems in which the product will be isolated from bodily fluids such as milk, blood, or urine. Unlike production in continuous cell cultures, considerable lot-to-lot variation in both microbiological and virological loads may arise in unpurified product from transgenic animals. The extent of such variability should be documented for each product and downstream manufacturing procedures should be shown to have sufficient capability for providing a safe and consistent product.

3. Pathogen testing and elimination

Transgenic animal source materials have the potential to contain a variety of human pathogens (e.g., viruses, bacteria, mycoplasma, transmissible spongiform encephalopathy agents). For this reason each manufacturer should develop analytical methods and purification processes that can ensure the safety of

the product. The microbiological and virological issues associated with different animals and/or product isolated from different tissues within the same animal will vary considerably. Following consultation with veterinarians and other experts in the field, a specific list of pathogenic agents and accepted testing methods should be prepared and prioritized with regard to the risk of human infection. The submission should present the methods for pathogens testing and/or elimination by adherence to the appropriate GMPs, State and Federal regulations or other available guidelines as appropriate.

Manufacturers should provide information on tests that will be performed on the material from each animal before pooling fluids. Pooling schema should be justified (e.g., one animal vertically or multiple animals horizontally). The necessity of performing these studies before initiating a clinical study will be determined by analysis of the contaminant levels in samples of source material, the sensitivity of the analytical tests for a contaminant, the potential toxicity of the contaminant and the clinical issues of

proposed patient population, dose and dose regimen. Appropriate model systems (e.g., enveloped and non-enveloped model viruses) may be used when necessary. For a more complete discussion of process validation issues please refer to the validation section in the Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (4). At a minimum, tests for bacteria, viruses, fungi, and mycoplasma should be performed. The criteria for permitting source material pooling should be considered. These criteria should be consistent with standard veterinary practices and supported by the results of the tests performed to evaluate the lot-to-lot consistency.

Clearly, the extent of testing required before pooling of material will vary depending on the particular case. Factors to consider in defining these tests include the volume of source material or the amount of product that can be obtained from a single animal at a single time. Since adventitious agent tests have the greatest sensitivity when performed on source material before processing or pooling, such tests are ideally performed at the single animal level; however, this may not

always be feasible. Manufacturers should clearly define the number of animals that may contribute to a production þlot.þ

In addition to species-specific pathogens, sponsors should consider the value of testing the pre-processed bulk for antibiotics or other medications, bioburden, mycoplasma, fungi, and possibly prion protein. The presence of other human pathogens should also be tested by in vitro inoculation of the "pooled" pre-purified bulk onto human and primate cell lines. Tests for milk allergens, toxic metals and herbicides or other potential contaminants may also be considered at the end of the production process if contaminants are expected to be concentrated in the final product. In cases where recombinant DNA was introduced by direct plasmid injection, viral vector administration, or other similar methods, the possibility of transgene DNA in the product should be monitored. The likelihood of this type of contamination and the methods for preventing and detecting its occurrence should be considered and discussed.

D. Analysis of product identity and purity

Before initiating studies in humans, the product from the transgenic source material should be characterized with respect to safety, identity, purity, and potency. Additional studies that define product similarities (e.g., potency, pharmacokinetics) and differences (e.g., glycosylation, antigenicity) with well characterized natural or recombinant molecule(s) should also be performed when possible. The manufacturer should describe all tests performed for in-process control and final product acceptability (1-5).

Characterization of the transgenic production process should initially focus on: 1) the presence of known and potential human pathogens, (both adventitious and endogenous agents) in the source material, 2) the amount of immunogenic and toxic materials in the final product, and 3) the lot-to-lot consistency of the productþs physicochemical characterization and product potency. Manufacturers should include details of the biochemical, physical and biological methods used. The stages of production where purity will be assessed should also be described.

1. Biochemical identity, purity and potency

Physicochemical, immunological and biological characterizations should be carried out on the transgene product to assess its identity (to include, for example, chemical structure, amino acid composition, sequence disulfide linkages, etc.), purity and potency. The anatomical site of production in the transgenic animal may differ from the natural site of production and result in biochemical modifications that affect the ability to purify a given biological product with the same activity as the naturally occurring molecule. Efforts should be made to eliminate product-related contaminants. In addition limits should be set for contaminants (including immunogenic proteins and polynucleotides) which may copurify with the product. Ideally manufacturers should provide information on tests that will be performed on the material from each animal before pooling fluids. Again, manufactures should clearly define the number of animals that may contribute to a production þlot.þ In specific instances, treatment of the source material may be

necessary to provide unambiguous results. In cases where data illustrating that analytical techniques are sufficiently sensitive to provide adequate assurance of safety have been supplied, tests for contaminants may be performed on processed pooled source material.

2. Product heterogeneity

Once analytical techniques sensitive to changes in product structure, purity and potency are identified, variations in final product activity should be examined with respect to variables associated with maintaining the transgenic herd and generating source material. For example, does the product structure, purity or potency change when the source material is obtained: a) from different animals, b) during different seasons, c) from animals facing other sources of stress, d) from source material that has been stored for extended periods of time, or e) during different times in a particular lactation cycle or during a different lactation cycle.

E. Lot release testing of the final product

The purified product prior to final formulation should be characterized in a manner similar to other recombinant products. Thus the potency of the final product is best determined with an assay(s) that reflects the proposed clinical use of the product. Contaminant levels in the final product that are acceptable for product release will be determined by preclinical studies, the dose and route of product administration, frequency and duration of product administration and the proposed patient population for the clinical study.

For requirements relating to in-process and final product tests for sterility, purity, pyrogenicity, identity, stability, general safety and potency, refer to 21 CFR Part 610.

F. Special concerns for transgenic animals intended as tissue donors

The potential exists to use transgenic animals as hosts to produce tissues that could be used as replacements for human tissue transplants. It is conceivable that some of these

tissues could be stored before use in transplantation so that there would be sufficient time to assess crucial safety issues such as the presence of adventitious agents in the animal-derived tissue. In cases where delay between removal of animal tissue and transplantation to human recipients is not possible, the level of monitoring of the donor animals must be sufficient to ensure safety, especially in terms of sterility and freedom from adventitious agents in the transplanted tissue.

Preclinical safety testing of candidate transplant tissue will be very important in assessing its safety, sterility, and freedom from other adventitious agents. Animal models may be especially useful in assessing the safety of tissues derived from transgenic animals.

Because of the high level of concern regarding adventitious agents, details of housing, veterinary care, and drug and biologic treatment of tissue donor animals would be crucial in evaluation of the potential use of genetically engineered tissue from transgenic animals.

X. Preclinical safety evaluation

The physical and biological characterizations are used to determine the similarity of the product to naturally occurring molecules. As with other products derived through recombinant DNA technology, similar products, especially if well characterized as natural substances, may require limited toxicology studies.

Proposed preclinical safety studies should be presented. The extent of testing will be based upon the product and the proposed clinical study (e.g., route of administration, dose, frequency and duration). In vitro studies and in vivo animal studies in normal or in animal models of disease may be used to examine product efficacy and safety. Dose ranges should include and exceed the doses to be used in humans.

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9. þGuide for the Care and Use of Laboratory Animals,þ Office for the Protection from Research Risks, 9000 Rockville Pike, Building 31, Room 4B09, Bethesda, MD 20892, 301- 496 - 7005.
10. þPoints to Consider for the Evaluation of Transgenic Animals from Transgenic Animal Research, þ 1994, USDA, FSIS (this document may be obtained from Dr. Pat Basu, Director, Technology Transfer and Coordination Staff, Science and Technology, Food Safety and Inspection Service, USDA, Washington, DC 20250, 202-720-8623.
11. þFinal Policy Statement for Research and Regulation of Biotechnology Processes and Products, þ USDA, Federal Register notice, June 26, 1986, (51 FR 23336).
12. þLivestock and Poultry Connected with Biotechnology Research,þ USDA, Federal Register notice, December 27, 1991, (56 FR 67054).
13. þUpdate on Livestock and Poultry Connected with Biotechnology Research, þ USDA, Federal Register notice, March 17, 1994, (59 FR 12582).

Note: All documents denoted with an þ*þ can be obtained by writing to FDA, CBER, Congressional and Consumer Affairs Branch, (HFM-12), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or calling 301-594-1800.