品の臨床応用の可能性

組換えタンパク質性医薬品のヒトに対する免疫原性は、動物を用いた非臨床試験で評価することができず、安全性評価において常に懸念されながらも、市販前に十分な知見を得られないことが多い。植物糖鎖の場合は、ヒトタンパク質に結合している場合の免疫原性は不明ながら、ヒトに対する植物アレルゲンにおけるエピトープとなり得ることが明らかであることから、組換えタンパク質の糖鎖の構成要素としては回避すべきものである。ただし、食物として摂取する植物由来タンパク質にもこれらの糖鎖は含まれているため、組換えタンパク質を経口投与する場合は、これら植物糖鎖に対するIgE を有する患者を除いては問題ない可能性も考えられる。従って、現段階での解釈としては、植物で発現させた糖タンパク質にはヒトに対して免疫原性を示す糖鎖が付加されているものの、経口投与の場合は、食物として摂取していて問題がない患者に対しては許容されるが、非経口投与、特に注射剤としての投与の場合は、ヒト型糖鎖が結合するような製造系を確立する必要があると考えるのが妥当であろう。

最初のバイオ医薬品である組換えインスリンが上市されてから四半世紀を経た現在、トランスジェニック動物で生産された製品やバイオ後続品が欧米で承認されるなど、バイオ医薬品の歴史の中でマイルストーンとなる出来事があり、バイオ医薬品をめぐる環境は大きな転換期を迎えている。新世代バイオ医薬品の一角をなすものとして、トランスジェニック植物を用いて生産された組換えタンパク質性医薬品についても、品質・安全性確保のための方策を確立していく必要がある。

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E. 研究発表

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F. 知的財産権の出願・登録状況

1. 特許取得 なし
2. 実用新案登録 なし
3. その他 なし

Table1 トランスジェニック植物を用いて生産された組換えタンパク質性医薬品の開発動向

組換えタンパク質	適応症	生産方法	収穫性	臨床試験	投与方法
抗*S.mutans*抗体（分泌型IgA/G:CaroRx™）	虫歯	組換えタバコ	種製	Phase Ⅱ	口腔内
インターフェロン アルファ-2b（経皮性製剤Locteron®）	C型肝炎	組換えウキクサ	種製	Phase Ⅱ	皮下**
胃リパーゼ(Merispase®)	急性膵線維症、膵炎	組換えトウモロコシ	種製	Phase Ⅱ*	経口
インスリン(SBS-1000)	糖尿病	組換えベニバナ種子	種製	Phase Ⅰ/Ⅱ	皮下
インターフェロン アルファ-2b（BLX-883）	Locteronの有効成分	組換えウキクサ	種製	Phase Ⅰ	筋肉内**
抗doxorubicin抗体（DoxoRx™）	抗癌剤副作用低減	組換えタバコ	種製	Phase Ⅰ	局所
ラクトフェリン	ドライアイ	組換えトウモロコシ	種製	Phase Ⅰ*	点眼
大腸菌 易熱性腸管毒素B鎖 ワクチン	下痢	組換えトウモロコシ	未種製	Phase Ⅰ	経口
大腸菌 易熱性腸管毒素B鎖 ワクチン	下痢	組換えジャガイモ	未種製	Phase Ⅰ	経口
HBV表面抗原 ワクチン	B型肝炎	組換えジャガイモ	未種製	Phase Ⅰ	経口
HBV表面抗原 ワクチン	B型肝炎	組換えレタス	未種製	Phase Ⅰ	経口
ノーウォークウイルスカプシド ワクチン	ノーウォークウイルス感染	組換えジャガイモ	未種製	Phase Ⅰ	経口
狂犬病ウイルス ワクチン	狂犬病	ホウレンソウ(一過性発現)	未種製	Phase Ⅰ	経口
scFv（ワクチン）	非ホジキンリンパ腫	タバコ(一過性発現)	種製	Phase Ⅰ*	皮下
グルコセレブロシダーゼ(prGCD)	ゴーシェ病	組換えニンジン細胞	種製	Phase Ⅲ	点滴静注

* 開発企業の財産等により、開発が中止された可能性がある。
**投与方法が明らかでないため、対照薬の投与方法を記載した。

Spoek A. et al. Trends in Biotech 26, 505, 2008 をもとに作成

Table2 ガイドライン案でコメントが求められていた点に対して寄せられた意見と、それに対する回答

	寄せられた案	回答
4.1:遺伝子組換え体の作製 植物の種類に関する問題について	T0…最初の形質転換体 T1, T2, T3…T0に続く世代 Tp…生産に用いられる形質転換体	本記述の例として、寄せられた案を採用する。
	Elite line: 生産性向上のために形質転換体との交配に用いられる優れた特徴を備えた植物体	寄せられたコメントを考慮して、必要に応じて修正して用いる。
4.1.5:遺伝子組換え体のバンキングシステム 適切なバンキングシステムについて	バンキングに関する記載は一般的であることが望ましい。植物は非常に多種である。種子の保存についても、長期に可能なものから短期に限られるものがある。また、植物体でのみ無限なものもあり、そのような場合は植物体での維持が必要となる。 医薬品生産に用いられる植物が新たに出現すれば、それに応じたガイダンスが必要になる。	有性繁殖するものだけでなく、植物体で繁殖する植物についても同様にバンキングシステムを構築できると考えられる。 ガイドライン本文では、トランスジェニック植物による生産系の多様性を述べた上で、可能であれば、バッチ間の一定性を確保するための戦略にバンキングシステムをあてこと、と記載する。
	農作物の品種改良においてはバンキングシステムが既に確立されているため、それを応用するのがよい。	本ガイドラインは農作物に関するものではない。
4.2.1:一般的な製造方法 GMPが適用できない初期工程を含む場合について	ガイドライン案で、GACP(Good Agricultural Collection System)を考慮することとされているのはよい提案である。 GACPは、GMP対応施設としての適合性が示されるのと同様、変異や適合性確認についてもまむと考えられる。	GACPは非組換え体の栽培植物の栽培に関するものでありトランスジェニック植物の管理にはそれだけでは十分ではない。
	出発原料が精製工程に入った段階から速やかにGMPによる品質管理を実施する。	GMPとnon-GMP操作の区別についてはガイドライン案でも言及しているが、より明確にするため、バンク、一次加工、下流の加工工程、に関する品質および製造管理システムの記載を希望する。
4.2.2:栽培、収穫、初期加工 適切な工程管理の方策について	GACPの適用が望ましい。	品質システムの構築の過程でGACPを参照することはできるが、医薬品生産の場合はGACPに準拠するとすべきではない。 植物種や栽培法の多様性を考慮し、個別のケースに応じて品質システムを構築するべきである。

Table3 トランスジェニック植物を用いた組換えタンパク質生産系における遺伝子発現構成体に関連する評価

試験試料	解析事項
遺伝子発現構成体（遺伝子導入ベクター）	・複製起点、選択マーカー、レポーター遺伝子、プロモーター、エンハンサー、リーダー／ターゲット配列などの各要素の起源と機能 ・プラスミドの全塩基配列情報 （構成要素の詳細なマップと注釈を付ける） ・目的遺伝子のコーディング領域とベクターに挿入されているコーディング領域近傍の塩基配列解析結果 （ベクターとの境界部位より上流を含む） ・プラスミドにコードされる他の発現タンパク質に関する情報 ・宿主植物の特性を制御あるいは改変するために導入あるいは改変される目的遺伝子以外の遺伝子 （例：細胞移酵素の発現や阻害に影響する因子、増殖性に影響する因子） アグロバクテリウムのような微生物による遺伝子導入法を用いた場合は、用いたシステムの起源、履歴、生物学的特性を詳細に記載する。
最初の形質転換体 Primary transformant (*)	・目的の配列、挿入された部位および数、単純反復配列、逆方向反復配列、インサート配列、インサート近傍領域、挿入の連結部位 ・形質転換の工程での残存物に関する情報 （例：アグロバクテリウムの感染後の運命）
マスター・トランスジェニック・バンク作製に用いられる植物材料 Final transformant	・導入遺伝子 （例：配列、完全性、挿入部位、コピー数、マーカー配列の運命） ・遺伝子発現（組織／器官特異性、調節、発現量） ・植物のジーンサイレンシング効果 ・他のタンパク質の過剰発現 ・倍数性 ・核型
規定された生産用の栽培期間を経た世代	・遺伝子発現（目的タンパク質発現の確認） ・導入遺伝子の保持

(*) 最初の形質転換体における遺伝子発現構成体の解析は、挙げられた項目を参考にして、形質転換体の選択に役立つ項目の解析を必要に応じて実施することでよいと考えられる。

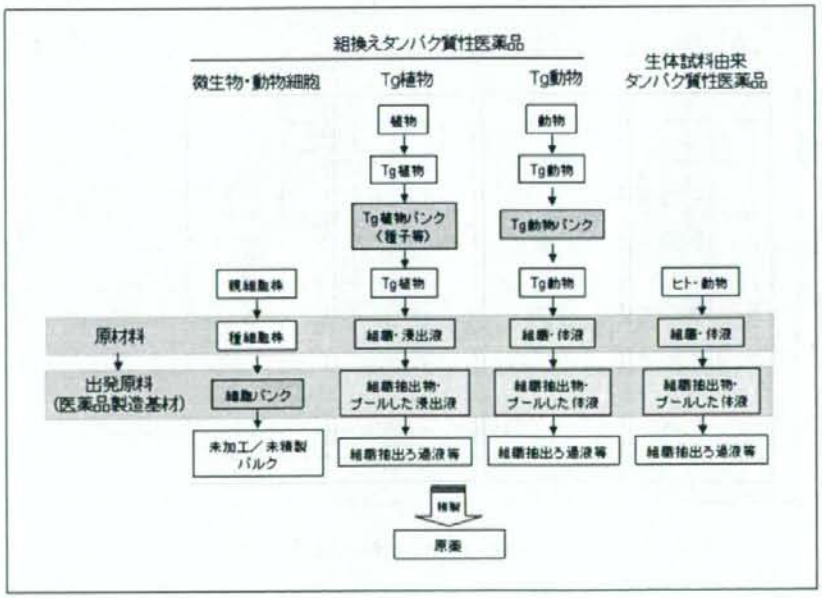

Fig.1. タンパク質性医薬品の原材料と出発原料

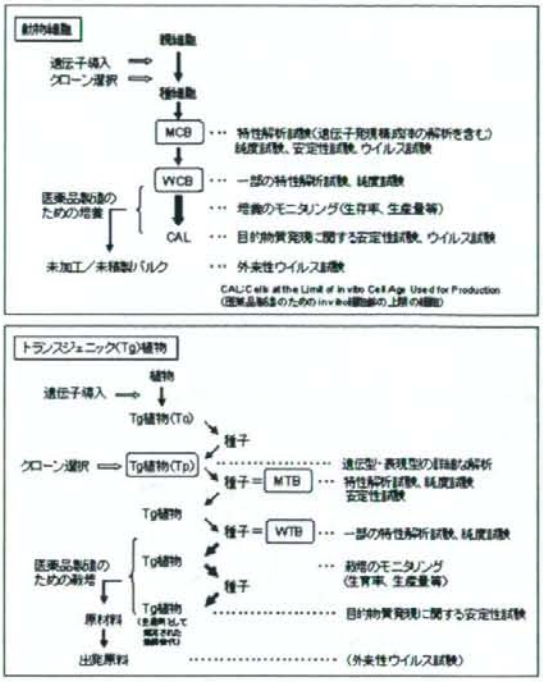

Fig.2. 動物細胞あるいはトランスジェニック植物を用いた組換えタンパク質性医薬品の製造工程

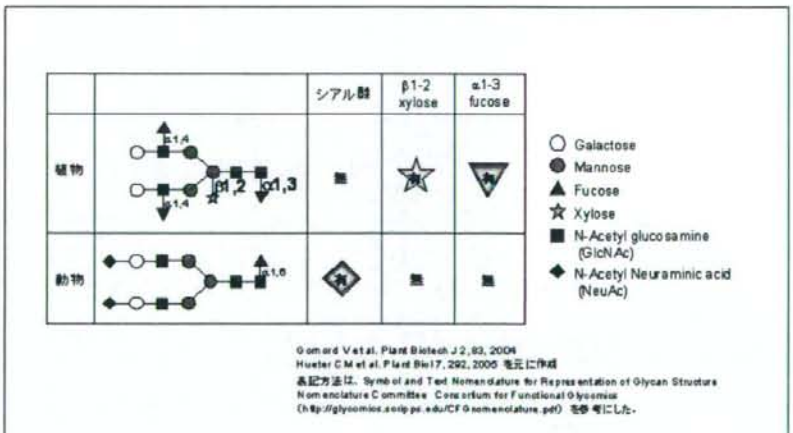

Fig.3 植物由来N結合複合型糖鎖の特徴

Guidance for Industry

Drugs, Biologics, and Medical Devices Derived from Bioengineered Plants for Use in Humans and Animals

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Submit comments and suggestions regarding this draft document by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit comments to Dockets Management Branch (HFA-305), Food and Drug Administration, 5600 Fishers Lane, rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions on the content of this draft document as it relates to FDA-regulated products, contact Keith Webber, Ph.D., at 301-827-0850 (CBER), Yuan Yuan Chiu, Ph.D., at 301-827-5918 (CDER), Wendelyn Jones Warren, Ph.D., at 301-827-6978 (CVM). For questions regarding veterinary biological products, contact Patricia L. Foley, D.V.M., Ph.D., at 515-232-5785 (USDA/APHIS/CVB).

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research (CBER)
Center for Drug Evaluation and Research (CDER)
Center for Food Safety and Applied Nutrition (CFSAN)
Center for Devices and Radiological Health (CDRH)
Center for Veterinary Medicine (CVM)
U.S. Department of Agriculture
Animal and Plant Health Inspection Service (APHIS)
Center for Veterinary Biologics (CVB)
Biotechnology Regulatory Services (BRS)
September 2002

Guidance for Industry

Drugs, Biologics, and Medical Devices Derived from Bioengineered Plants for Use in Humans and Animals

Additional copies of this guidance are available from:
Office of Communication, Training, and Manufacturers Assistance, HFM-40
Center for Biologics Evaluation and Research
Food and Drug Administration
1401 Rockville Pike, Rockville, MD 20852-1448
Phone: 301-827-4573
Internet: http://www.fda.gov/cber/guidelines.htm
Mail: The Voice Information System at 800-835-4709 or 301-827-1800
or
Office of Training and Communication
Division of Communications Management Drug Information Branch, HFD-210
Center for Drug Evaluation and Research
Food and Drug Administration
5600 Fishers Lane, Rockville, MD 20857
Phone: 301-827-4573
Internet: http://www.fda.gov/cder/guidance/index.htm
or
Division of Small Manufacturers, International, and Consumer Assistance (DSMICA), HFZ-220
Center for Devices and Radiological Health
Food and Drug Administration
1350 Piccard Drive, Rockville, MD 20850
800-638-2041 or 301-443-6597
Internet: http://www.fda.gov/cdrh
Email: DSMICA@cdrh.fda.gov
Facts-On-Demand (faxback): 301-827-0111
or
Communications Staff, HFV-12
Center for Veterinary Medicine (CVM)
Food and Drug Administration
7500 Standish Place
Rockville, MD 20855
Phone: 301-594-1755
Internet :http://www.fda.gov/cvm

77 **Table of Contents**
78

108

Guidance for Industry

Drugs, Biologics, and Medical Devices Derived from Bioengineered Plants for Use in Humans and Animals

This guidance document represents the agencies' current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA, USDA, or the public. An alternative approach may be used if such approach satisfies the requirements of applicable statutes and regulations.

I. INTRODUCTION

A. Purpose and Scope

This document is the result of a combined effort by the U.S. Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA) to provide guidance with regard to the use of bioengineered plants or plant materials to produce biological products, including intermediates, protein drugs, medical devices, new animal drugs, and veterinary biologics regulated by FDA or USDA (hereafter referred to as "regulated products"). This document does not address non-protein drugs, botanicals, or allergenic products (21 CFR 680.1) for human use. It should be noted, however, that if a bioengineered pharmaceutical plant is used to produce a non-protein drug product, the principles described in this document regarding the host and source plant characterization and the environmental considerations would be applicable. If you are planning to produce a non-protein drug product for human use in a bioengineered pharmaceutical plant, consultation with FDA's Center for Drug Evaluation and Research (CDER) early in the drug development process is encouraged. For the purposes of this document, the term "bioengineered pharmaceutical plant" means any plant manipulated by recombinant DNA technology to express a gene encoding a biological or drug product.

Within this document, "you" refers collectively to sponsors, manufacturers, licensees, and applicants; "we" refers to FDA and/or USDA/Animal and Plant Health Inspection Service (APHIS)/Center for Veterinary Biologics (CVB).

This document outlines important scientific questions and information that you should address during the investigation of a new animal drug and preparation of an Investigational New Drug (IND) application, Investigational Device Exemptions (IDE), Biologic License Application (BLA), New Drug Application (NDA), New Animal Drug Application (NADA), Premarket Approval (PMA), or 510(k) to the FDA, or a United States Veterinary Biological Product License Application (VBPLA) to the USDA (hereafter referred to as "your application"). This document presents points that you should consider to demonstrate the safety and effectiveness of products from bioengineered pharmaceutical plants for use in

humans or animals or as components in clinical diagnostic systems.

In addition, this document presents points you should consider in addressing environmental issues as well as confinement measures that should be an integral part of the manufacturing process for all pharmaceutical products produced in bioengineered pharmaceutical plants or plants infected with engineered vectors containing genetic material for the expression of regulated products.

This document is directed at the issues unique to the use of bioengineered pharmaceutical plants as source material for the production of FDA and/or USDA regulated products. Therefore, it does not focus on many aspects of regulated products that are shared with other expression systems. Given the complexity and variety of products, no single document can anticipate and address all issues. You are encouraged to consult other FDA and USDA documents for guidance on other specific topics relevant to your product.

You should be aware that the Biotechnology Regulatory Services Division (BRS) within APHIS oversees the importation and interstate movement of bioengineered pharmaceutical plants and infectious plant vectors as well as the release of these entities into the environment (i.e., outside of a contained facility, such as a greenhouse, laboratory, or fermentor). You must receive a permit from APHIS/BRS prior to engaging in these activities (7 CFR 340). You may obtain guidance on applying for a permit at the USDA/APHIS website http://www.aphis.usda.gov/biotech or by writing to USDA/APHIS/BRS (see addresses in Appendix A). This document will not describe the plant permitting process.

B. Regulatory Responsibility

The FDA regulates human biologics, and human and animal drugs derived from bioengineered pharmaceutical plants, intended for therapeutic, preventative, or diagnostic purposes. Biological products and drugs for use in humans are regulated by the Center for Biologics Evaluation and Research (CBER) and CDER under authority of the Public Health Service Act (PHS Act) (42 U.S.C. 262 *et seq.*) and the Federal Food, Drug, and Cosmetic Act (FD&C Act) (21 U.S.C. 301 *et seq.*). FDA also regulates animal drugs derived from bioengineered pharmaceutical plants, intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in animals or to alter the structure or function of the animal. New animal drugs and animal feeds containing new animal drugs are regulated by the Center for Veterinary Medicine (CVM) under authority of the FD&C Act. The FDA regulations are found at Title 21 of the Code of Federal Regulations (21 CFR).

The USDA regulates veterinary biologics through the Center for Veterinary Biologics (CVB) within Veterinary Services in APHIS under the authority of the Virus, Serum, and Toxins Act (21 U.S.C. 151 *et seq.*). The USDA regulations are found at Title 9 of the Code of Federal Regulations (9 CFR) Parts 101-124.

As mentioned above, APHIS/BRS regulates the importation, interstate movement, and release into the environment (e.g., field testing) of all such bioengineered pharmaceutical

plants, under the Plant Protection Act (7 U.S.C. 7701-7772). The APHIS/BRS regulations are found at Title 7 of the Code of Federal Regulations (7 CFR), in particular 7 CFR 340.

Appendix A provides a listing of the points of contact at the agencies.

To minimize duplication, review of environmental safety issues posed by field growth of the bioengineered pharmaceutical plants, including National Environmental Policy Act (NEPA) assessments, will be addressed primarily by APHIS/BRS. Because bioengineered pharmaceutical plants will be grown under APHIS permit, and because permits enabling field trials will be obtained prior to submission of a product application, APHIS/BRS will identify and evaluate the potential environmental effects posed by field growth of such plants. Environmental concerns posed by use of the regulated product will be addressed in the NEPA analysis conducted by the regulatory agency responsible for review and/or approval of the product. These agencies' NEPA analyses will take into account APHIS/BRS's environmental reviews. Also refer to section III.B. National Environmental Policy Act.

II. HOST AND SOURCE PLANT CHARACTERIZATION

A. General Considerations

In the development stage, you should give careful consideration to choosing the plant species that will be used as the source of the desired regulated product. Concerns to be addressed include: the potential for the plant to express an allergenic or toxic compound; the method of plant propagation and the measures to ensure confinement; and, if it is a food crop species engineered to produce non-food material, the measures to ensure that non-food (or non-feed) material will not get into food or feed. The presence of any such material in food or feed could render such products adulterated under the FD&C Act (21 U.S.C. 342).

You are encouraged to refer to pertinent guidance documents and regulations, and to consult with the regulatory agencies as early as possible in the development process to ensure that you are aware of the most current regulatory requirements.

B. Host Plants

You should provide in your application a thorough description of the host plant biology that includes information necessary to identify it in the narrowest taxonomic grouping applicable (e.g., genus, species, subspecies, variety or cultivar, line designation).

In order for the agencies to assess the ability of the chosen plant to consistently manufacture your intended product, you should submit a description of the reproductive biology of the unmodified plant and production practices with regard to:

- growth habitat as an annual, perennial, or biennial;
- timing of sexual maturity and duration of flowering;
- seed production and harvesting;

- recognized practices for maintaining seed stock purity;
- conditions of growth;
- timing of harvest;
- method of harvesting; and
- transporting, storage and sorting of harvested materials.

In addition, you should provide a description of the host plant including levels of any toxins, anti-nutrients, and allergens known to be produced by the plant species and whether it is known to accumulate heavy metals. Please state if the plant is of a species used for food or feed in a raw or processed form.

C. Bioengineered Source Plants

1. General Considerations

The host plant may be bioengineered to increase the expression of an endogenous gene product or to manipulate the plant to produce a heterologous gene product. The modifying gene may be transiently added to the plant or it may be inserted in a stable manner. Regardless of the method of gene expression used, traceable documentation of the growth and expression phase of the manufacturing process, including banking of the plant lines and/or vectors should be maintained. Most importantly, you should include data in your application to demonstrate that the source plant produces a consistent product.

When the bioengineered pharmaceutical plant is from a species that is used for food or feed, measures should be in place to ensure that there is no inadvertent mixing of the bioengineered plant material with plant material intended for food or feed use. The presence of any such material in food or feed could render such products adulterated under the FD&C Act (21 U.S.C. 342). We strongly recommend that you have tests available that can detect the presence of the target gene and the protein product in the raw agricultural commodity.

2. Characterization of the Recombinant DNA

In your application, you should provide a full characterization of the recombinant DNA constructs or viral vectors used to transfer genes, including:

- the origin and function of all component parts of the construct, including coding regions, antibiotic- or herbicide-resistance genes, origins of replication, promoters, and enhancers;
- physical map of the construct(s) illustrating the position of each functional component;
- method used for plasmid propagation;
- any sequences required for bacterial expression of plasmid constructs;
- the nucleotide sequence of the intended insert up to and including the junctions at the 5'- and 3'- ends; and
- any changes in codons to reflect more acceptable codon usage in plants.

For the purposes of this document, coding regions include full-length and truncated sense constructs, antisense constructs, and constructs containing ribozymes, regardless of whether or not the coding region is designed or expected to be expressed in the bioengineered pharmaceutical plant.

For additional details regarding analysis of r-DNA constructs for human biologics, please refer to the International Conference on Harmonisation (ICH); Technical Requirements for Registration of Pharmaceuticals for Human Use – Guideline Q5B: Quality of Biotechnological Products: Analysis of the Expression Construct in Cells Used for Production of r-DNA Derived Protein Products (Ref. 1).

3. Stable Transformation Systems

Before preparing Master Seeds or Master Seed Banks (MSB) and Working Seeds or Working Seed Banks (WSB), we recommend that you establish a suitable transformant. For stable transformation systems, you should describe the gene transfer method in detail and provide relevant references, as appropriate. An analysis should be performed to determine the number of copies of the gene inserted, the number of integration sites, and to demonstrate if complete or partial copies are inserted into the plant's genome. You should determine the nucleotide sequence of the insert from DNA or mRNA retrieved from the stably-transfected plants in order to confirm the integrity and fidelity of the DNA insert. When a fragment of a coding region designed to be expressed in a plant is detected, you should determine whether a fusion protein could be produced and in which host tissues it may be located.

If the transformation system utilizes a pathogenic organism or nucleic acid sequences from a pathogen, you should provide a description of the pathogen, the strain, and the gene(s) involved. If any such pathogenesis-related DNA sequences were removed or altered prior to transformation, you should describe these changes in detail. Any helper plasmids or analogous DNA fragments used in the transformation process should also be described. For example, for *Agrobacterium*-mediated transformation, provide the strain designation of the *Agrobacterium* used during the transformation process, indicate how the Ti plasmid-based vector was disarmed, and indicate whether *Agrobacterium* was cleared from the transformed tissue.

You should submit a complete description of the process, including selection methods for the final transformant. You should include the source of and the methods used to prepare the recipient tissue or cells and, if the tissues or cells are cultured or pre-treated in any way, you should provide a complete description of the reagents used and composition of the culture medium. For direct transformation methods, you also should provide a thorough description of the transforming DNA preparation: including amount and concentration of transgenic

DNA; the nature, source, and concentration of any carrier DNA; the composition and source of carrier particles; and the source and concentration of any other excipients. In addition, you should describe in detail any tests used to evaluate the transformations process and provide the results.

4. Transient Transfection Systems:

Virus-mediated transient transfection systems, in their simplest form, employ two components: a recombinant virus vector and a host plant. Characterization of the host plant should include the information outlined in section II. B., above. The information you provide regarding the recombinant virus vector should include the following:

- the taxonomic name of the virus, including family, genus, and strain designation, including any synonyms;
- the type of nucleic acid contained in the virus (DNA or RNA);
- whether the virus is associated with any satellite or helper viruses;
- the natural host range of the virus;
- how the virus is transmitted;
- if the virus is transmitted by a vector, the identity of the vector including mode of transmission (e.g., persistent or non-persistent);
- the identity of the viral gene(s) (if known) involved in vector transmission;
- whether any synergistic or transcapsidation interactions with other viruses under field situations have been reported in the literature;
- the protocol for purification of the virus;
- the protocol for cloning of recombinant virus;
- a description of the preparation of the Master Plasmid Bank (MPB), if one is used;
- the storage conditions and data demonstrating stability of the MPB;
- the protocol for the preparation of infectious nucleic acid from plasmid; and
- data characterizing the infectious nucleic acid with respect to its identity with the parental genome.

You should include relevant literature citations to any of the above information, as appropriate.

5. Genetic Stability: Seed Banks and Vegetative Propagation

Regardless of whether a transient-transfection system or a stable transformation system is used, you should prepare a MSB and a WSB to ensure consistent lot-to-lot growth of the plant and expression of the regulated product. The description of the MSB in your application should include the identification, the method of production, the results of analytical tests used to characterize it, the size of the bank, the storage conditions, and data demonstrating its viability, bioburden (including speciation of contaminants), uniformity of gene content, and stability.

You should submit data demonstrating that bioengineered pharmaceutical plant lines derived through stable transformation are stable in both phenotype and genotype. To demonstrate genetic stability, you should include data from a segregation analysis for the trait of interest, as well as from a molecular characterization of the genomic insert (e.g., Southern analysis) and from analyses of expression of the intended product.

For plants that are fertile, you should provide data demonstrating the pattern and stability of inheritance and expression of the new traits over several generations sufficient to ensure stability over the number of generations that will be used during manufacture of the regulated product.

For plants that are infertile or for which it is difficult to produce seed (such as vegetatively propagated male-sterile potatoes), you should provide data to demonstrate that the trait is stably maintained and expressed during vegetative propagation over a number of cycles that is appropriate to the crop.

6. Tissue Distribution of Expression Products

For all inserted coding regions, you should provide data that demonstrates whether the protein is or is not produced (describe assay method and indicate limit of detection) as intended in the expected tissues consistent with the associated regulatory sequences driving its expression (e.g., if the gene is inducible, you should determine if the gene is expressed in the expected tissues under induction conditions). You should provide quantitative data characterizing the distribution of the product in the major plant tissues (e.g., leaves, roots, stalks, seeds).

III. ENVIRONMENTAL CONSIDERATIONS

A. General Considerations

Using bioengineered pharmaceutical plants to produce regulated products for use in animals or humans raises a number of environmental concerns that you should address, including confinement measures that may be needed to control the spread of the bioengineered pharmaceutical plants and to keep them from entering the food or feed supply. We encourage you to consult with the regulatory agencies as early as possible in the development process to ensure that you are aware of the most current regulatory requirements. For example, you should contact APHIS/BRS for more information on regulations governing the plants while in the field or in transport. APHIS/BRS authorization is required for the interstate movement, importation, and field release of plants addressed by this guidance (7 CFR 340). For most initial experiments and commercial uses of these plants, a USDA/APHIS/BRS permit will be needed. Refer to USDA regulations (7 CFR 340) that can be found at APHIS's home page http://www.aphis.usda.gov/biotech.

Bioengineered pharmaceutical plants that are grown exclusively in an enclosed building (e.g., greenhouse) generally will be considered to be confined during the growing period if there are control measures in place to eliminate the spread of pollen or seeds outside of the facility. Growing plants in such an enclosed building does not require a USDA/APHIS/BRS permit, however, the importation or interstate movement of bioengineered pharmaceutical plants would require a permit (7 CFR 340.4).

B. National Environmental Policy Act (NEPA)

You should be aware of NEPA requirements for both the FDA (21 CFR part 25) and the USDA (7 CFR part 372). You should consider the potential environmental impact of all aspects of the manufacturing process, including but not limited to transport of seeds and plants, planting, growing, harvesting, processing, purifying, packaging, storage, and disposal. If you believe that your activities are categorically excluded by 7 CFR 372.5(c), 21 CFR 25.31, or 25.33 from the requirement to submit an environmental assessment, you should state this in your application. You are encouraged to consult available guidance documents (Refs. 2, 3) and to talk directly with the USDA and the FDA regarding NEPA requirements. A copy of the letter from APHIS/BRS granting your permit should be submitted in your application for the regulated product in support of the environmental assessment (21 CFR 25.15 and 25.40) or the claim of categorical exclusion (21 CFR 25.31, 25.33 or 7 CFR 372.5(c)). FDA and CVB intend to take APHIS/BRS evaluations and determinations into account in doing their own NEPA assessments.

C. Confinement Measures

1. General Considerations

Regardless of whether the bioengineered pharmaceutical plants are grown and/or processed by you or on a contractual basis by other persons, manufacturing controls are your responsibility and should be documented clearly in standard operating procedures (SOPs), Outlines of Production, or other records, as appropriate (see section IV.C., Applicable FDA and USDA Regulations). For FDA regulated products, refer to 21 CFR 200.10, parts 210 and 211, 514.1, and 820.50; see also FDA's Draft Guidance for Industry: Cooperative Manufacturing Arrangements for Licensed Biologics (Ref. 4) once it is finalized.

In developing a bioengineered pharmaceutical plant, you should implement procedures to ensure that such a plant line is used only for its intended purpose as a source material for a regulated product. As described in 7 CFR 340.4, 340.7, and 340.8, a permit from USDA/APHIS/BRS is required for the interstate transport of bioengineered pharmaceutical plants or seeds for such plants, and you must keep records documenting the handling and transfer of such materials. Shipment of bioengineered pharmaceutical plants for veterinary biologics requires permission from USDA/APHIS/BRS. When manufacturing firms are shipping

veterinary biological products at any stage of production, shipment must be authorized by CVB and is regulated under 9 CFR 103.3. Such controlled transfer of source materials helps ensure that these plants are not diverted to unintended uses.

When a plant species that is used for food or feed is bioengineered to produce a regulated product, you should consider the use of strategies that allow the bioengineered pharmaceutical plant line to be readily distinguished from its food or feed counterpart. Such strategies might include the use of genetic markers that alter the physical appearance of the plant (e.g., a novel color or leaf pattern), or change the conditions under which a plant will grow (e.g., the use of an auxotrophic marker gene). You should also consider strategies to reduce the likelihood of unintended exposure to a regulated product by restricting the expression of the bioengineered pharmaceutical product to a few specific plant tissues (e.g., the use of tissue specific promoters) or by restricting the conditions under which the product will be expressed (e.g., use of an inducible promoter). For such plants that outcross, you may want to consider growing them in regions of the country where little or none of its food/feed counterparts are grown.

Measures should be in place to ensure that there is no inadvertent mixing of the bioengineered pharmaceutical plant with plant material intended for food or feed (including inadvertent mixing with seeds for food or feed crops). During the development of your overall production process (from the farm through the final product), you should determine where in the process inadvertent mixing could occur and establish appropriate control measures. We strongly recommend that you have tests available that can detect the presence of the target gene and the protein product in the raw agricultural commodity. The presence of the target gene or gene product in food or feed could render such products adulterated under the FD&C Act (21 U.S.C. 342). You may wish to consult with FDA's Center for Food Safety and Applied Nutrition (CFSAN) or with CVM about the legal implications of any such material getting into food or feed.

2. Control of Seed Stocks

You should maintain careful control over the inventory and disposition of viable seeds to preclude the possibility that such seeds will be used to produce material that could be used for food or feed production. When seed stocks are produced, there should be an accounting of the total yield of seed (e.g., by weight or by volume). Seed stocks should be stored in aliquots of appropriate volume to allow reasonably accurate accounting of use and disposition. A record of the amount and disposition of any withdrawals from the seed bank should be made (7 CFR 340.4(b)(12)). Seed stocks should be prominently labeled in accordance with the permit issued by APHIS/BRS for field growth or interstate shipment of bioengineered seeds (7 CFR 340.7).