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).

518 *3. Field-grown Plants*
519
520 You must have a permit from APHIS/BRS to grow bioengineered pharmaceutical
521 plants in the field (7 CFR 340.4) and must have control over the growing process
522 from planting through harvesting and over the disposition of remaining crops
523 and/or crop residue and, if required, over the subsequent use of the field if for
524 growth of food or feed or as a pasture during subsequent seasons. All persons
525 involved in field growth of the product should be adequately trained to perform
526 the duties for which they are responsible. Control measures should include an
527 accounting of seed that is transferred from seed bank storage to the field for
528 planting, or for archiving. Documentation of the size and location of all sites
529 where the bioengineered pharmaceutical plants will be grown, of the control of
530 pollen spread, and of the subsequent use of the field and destruction of volunteer
531 plants in subsequent growing seasons should be maintained and provided to the
532 FDA and CVB, as appropriate. Fields should be unambiguously identified, such
533 as by Global Position Satellite (GPS) markers. We recommend that you consider
534 the use of perimeter fencing to help exclude wildlife and escaped livestock. All
535 fields used to grow source bioengineered pharmaceutical plants are subject to
536 inspection by the USDA (7 CFR 340.4; 9 CFR 101-108) and/or by the FDA (42
537 U.S.C. 262; 21 U.S.C. 374).
538
539 *4. Control of Harvested Material*
540
541 APHIS requires that appropriate confinement procedures be in place for transport
542 of the source material from the field or greenhouse to the production facility (7
543 CFR 340.4(b)(10-12)). During transport, containers of harvested material should
544 carry a label that clearly indicates that the material, including but not limited to
545 seeds, leaves, roots, and stems, is not to be used for food or feed or for any
546 purposes in which residual materials could be used for food or feed (such as
547 ethanol production). Reconciliation of the quantities of material leaving the
548 growing facility and arriving at the processing facility should be made. In
549 manufacturing of a regulated product, records must be kept to document control
550 over harvested material in accordance with 21 CFR part 211 subpart J, 21 CFR
551 part 226 subpart E, 7 CFR 340.4, or 9 CFR part 116 and made available for
552 inspection by the FDA or CVB, as appropriate.
553
554 *5. Control at Processing Facilities*
555
556 As stated in section III.C.1., you should implement appropriate procedures to
557 ensure that bioengineered pharmaceutical plants or plant materials do not
558 unintentionally mix with other plant products, particularly those used as food or
559 feed. Source plant materials should not be processed in facilities that also are
560 used for the production of food or feed, such as grain mills, without prior
561 consultation with USDA/APHIS/BRS and FDA.
562

563 *6. Control of Waste Material*
564
565 In-process wastes (e.g., column wash solutions, diafiltration solutions, etc.),
566 rejected in-process material, and residual source plant material from the
567 purification process should be treated to inactivate the regulated product prior to
568 disposal, as appropriate. They should be disposed in a manner to ensure that the
569 material will not enter the human or animal food chain unless you have
570 specifically consulted with FDA for the use of this material in food or feed
571 products. Disposal should conform to local and state regulations. Waste material
572 from the manufacture of human drug and biological products, or animal drugs
573 should be disposed of in a safe and sanitary manner (21 CFR 211.50). Veterinary
574 biologic materials should be disposed of in a manner consistent with 9 CFR
575 114.15, Disposal of Unsatisfactory Products and By-products, following
576 Veterinary Services Memorandum 800.56. If, rather than disposal, the residual
577 material is to be used for a secondary purpose other than a food or feed product,
578 there should be clear procedures in place to verify the disposition of this material
579 and by-products and to document that it will not be used for food or feed.
580
581
582 **IV. MANUFACTURING AND PROCESS-RELATED CONSIDERATIONS**
583
584 **A. General Considerations**
585
586 Facilities and procedures used for the manufacturing of regulated products should be
587 designed to prevent contamination and cross-contamination during harvest and processing
588 of source material. The flow of personnel, material, product, and waste into and out of the
589 facility should be designed to prevent contamination of the product. You should establish
590 written procedures for appropriate cleaning, maintenance, and sanitization of equipment and
591 utensils to prevent malfunctions or contamination that would alter the safety, identity,
592 strength, quality, or purity of the drug products beyond established requirements (21 CFR
593 211.67). In controlled areas with specified air classifications, a program for monitoring the
594 environment for viable and non-viable particulates should be established based on the
595 criticality of the manufacturing process involved and should include active monitoring of
596 critical manufacturing processes as they are performed. For FDA-regulated products,
597 manufacturing controls, including process validation, should be appropriate for the type of
598 product and stage of development. The regulations governing facilities requirements are
599 listed in section IV.C., Applicable FDA and USDA Regulations.
600
601 Because microbiological contaminants can have an adverse effect on product safety, quality,
602 and stability, we recommend that you establish processing steps to decrease bioburden
603 levels as the material moves through the manufacturing process (21 CFR 211.80(b)). The
604 validation activities described in this section should be phased in during the investigational
605 phase, as the clinical studies progress toward submission of a regulated product application.
606 It should be noted however that assurance of sterility or limits on bioburden in the final
607 product may be required as appropriate, depending on the final form and intended use of the
608 product (e.g., parenteral vs. whole fruit or vegetable). (21 CFR 211.80, 211.100-103, and

211.113; 21 CFR parts 226, 514, 610, and 820; and 9 CFR part 113.)

You should only use source materials with appropriate quality attributes for manufacture of the product. Each lot of source material should be assessed for the presence of foreign matter. Care should be taken to minimize contaminants (e.g., molds and other agents that may be present in the source material) that could lead to the inadvertent exposure of recipients of regulated products to undesirable impurities or could affect product quality (e.g., microbial proteases).

For veterinary biologics, manufacturing must be in accordance with an Outline of Production filed with CVB as required by 9 CFR 114.8 and 114.9. For all other regulated products, you must document the manufacturing procedure and lot-specific data (21 CFR part 211 subpart F, 226.102, part 514, and 820.184). You should ensure that source material is propagated, harvested, and processed in accordance with written standard operating procedures that will ensure the adequate processing of the plant derived material and specify the acceptable limits and kinds of contaminants that may be present. Specifications should be established regarding the health status of the plants at the time of viral infection and/or harvest.

### B. Special Considerations for Whole Fruit or Vegetable Products

One of the challenges in the use of whole vegetables and/or fruits as the delivery system for edible biologics is the demonstration of batch uniformity and consistency of dose. A homogenization step to produce a uniform bulk drug substance, such as a puree, juice, or milled grain may be necessary. Testing could then be conducted on this homogenized product to demonstrate potency. In addition, if the plant line used for production is known to be allergenic, you should consult with FDA or CVB, as appropriate, to discuss the safety and regulatory issues.

- Packaging for regulated products must comply with applicable regulations. For FDA-regulated products, packaging should be consistent with 21 CFR parts 210, 211, 226, 314, 514, 600, 610, and 820. Packaging for APHIS/CVB-regulated products should comply with 9 CFR part 112. Although edible products for pharmaceutical use in humans, such as whole fruit or vegetable vaccines, are regulated as biologics, not foods, we generally recommend that you package your edible biological products in material that conforms to food packaging regulations (21 CFR 174.5). The plant source must be clearly identified in the label or packaging material for biologics for use in humans (21 CFR 610.61(p)) or animals (9 CFR 112). The plant source should be clearly identified in the labeling of both oral and non-oral prescription drugs (21 CFR 201.57(a)(2); see also 21 CFR 201.100(b)(4) and (5)). For products containing viable seeds, you should consult with FDA or CVB, as appropriate.

### C. Applicable FDA and USDA Regulations

The specific regulations applicable to the manufacture of a regulated product derived from

bioengineered pharmaceutical plants are based on: the intended recipient of the product (i.e., human or animal); the intended use of the product (e.g., biologic, drug, or device); and the intended route of administration (e.g., parenteral vs. oral). The Table below includes, but is not limited to, the following applicable regulations for specific classes of regulated products for use in humans or animals.

| **Planned use** | **Applicable regulations** |
|---|---|
| Human drug or biologic for parenteral administration | 7 CFR part 340, 21 CFR parts 210, 211, 312, 314, 600, 601, 610 |
| Human drug or biologic for oral administration | 7 CFR part 340, 21 CFR 174.5, parts 210, 211, 312, 314, 600, 601, 610 |
| Biologic device for human use | 7 CFR part 340, 21 CFR parts 600, 601, 610, 812, 814, 820 |
| Animal drug: Type A medicated articles and Type B and C medicated feed | 7 CFR part 340, 21 CFR parts 225, 226, 500, 510, 511, 514, 515, 558 |
| Animal drug | 7 CFR part 340, 21 CFR parts 210, 211, 500, 510, 511, 514 |
| Veterinary biologic | 7 CFR part 340, 9 CFR parts 101-118 |

We encourage you to refer to FDA and CVB guidance documents for additional information and recommendations specific to the product class. Any exceptions to the regulatory requirements must be obtained as provided by regulation. For example, the general safety, sterility, and mycoplasma tests prescribed in 21 CFR 610.11-12 and 610.30 (for biologics for use in humans) or 9 CFR 113.26-28 (for veterinary biologics) may be inappropriate for some products (e.g., edible plant material intended for use as an oral dosage form) and modifications or alternative, but equivalent, methods of demonstrating a product's safety and sterility may be permitted in accordance with 21 CFR 610.9 or the product may be exempted in accordance with 9 CFR 113.4 (see Table, above).

## D. Product Manufacturing Procedures

### *1. General Considerations*

Your application should include a description of each step of the purification process including analytical tests to demonstrate identity, purity, and concentration, and the levels of product related and non-product related impurities. This is particularly important if the impurities are determined to be toxins, allergens, teratogens, or carcinogens. For each process that is not intended to be sterile, you should describe the procedures to be followed to control extraneous bioburden and the in-process testing used to monitor the level of bioburden (see, 21 CFR 211.113, 226.102, 312.23, 314.50(d), 514.1(b), 820.70, 820.181, and 820.184). A summary of the manufacturing, including propagation of the source material, should be available at the site where the manufacturing occurred (21 CFR 211 subpart J). You should consult with the appropriate agency regarding the applicability of these considerations to device components.

689 *2. Growth Conditions*
690
691 The Chemistry, Manufacturing, and Controls (CMC) section or the Outline of
692 Production should include information regarding the location of source plant
693 propagation. For greenhouse-grown material, you should include in the
694 description the types of containers, the soil mix composition and qualification
695 criteria, and the greenhouse growth conditions. For field grown material, the
696 description should include the previous uses of the land (e.g., agricultural and/or
697 industrial use). We recommend that you establish specification/acceptance
698 criteria/limits for the soil composition and potential soil contaminants that may
699 affect the source material. In addition, you should describe the agricultural
700 methods utilized during crop growth, including specifications regarding the use of
701 chemicals and limits on specific agricultural practices (e.g., the use of specified
702 fertilizers, pesticides, or herbicides, and irrigation practices relative to a specified
703 harvest time frame, etc.). You should provide in your application a list of
704 expected pests that will require control during the growth of the bioengineered
705 pharmaceutical plants. All pest-control measures implemented should be in
706 accordance with good agricultural practices for the growth of food crops in the
707 United States. The Pesticide Product Information System (Ref. 5) contains
708 information concerning all pesticide products registered in the United States. In
709 order to evaluate the purity of the product, all pest-control interventions should be
710 described in appropriate SOPs and should be documented in the Batch Record
711 (for FDA-regulated products) or Outline of Production (for veterinary biologics).
712 We recommend that you follow current Good Agricultural Practices (e.g., Ref. 6).
713 If product expression is induced, either chemically or physically, you should
714 establish criteria to ensure that induction is performed consistently from batch to
715 batch. (See generally, 21 CFR parts 210, 211, 226, 312, 314, 514, 601, 610, and
716 820; see e.g., 21 CFR 211.84, 211.186, 312.23(a)(7), 314.50(d), 514.1, 820.50,
717 and 9 CFR parts 101-118.)
718
719 *3. Harvest*
720
721 You should describe the method of harvesting the source material in written
722 procedures and document the process in production records. You should have
723 procedures for determining when the harvest will occur in order to ensure lot-to-
724 lot consistency of the source material. You should establish specifications for the
725 harvested material with regard to the levels of active component, process-derived
726 contaminants, significant endogenous impurities, and adventitious agents. For
727 example, you should describe agricultural practices and training of harvesting
728 personnel regarding plant source material quality (e.g., assessment of the disease
729 status of plant for manual harvesting operations, etc.) (21 CFR part 211 subpart
730 B). You should have written procedures for establishing the necessary training of
731 personnel engaged in harvesting plants to ensure the quality of the harvested
732 material (21 CFR 211.25). We recommend the use of dedicated equipment. We
733 recommend that equipment-cleaning procedures be developed and that cleaning
734 agents used on harvesting equipment be described (21 FR 211.67). In addition,

you should consider measures to prevent the contamination of the harvested source material with equipment lubricants during processing. (21 CFR part 211 subparts F and J; 21 CFR part 226; 21 CFR 314.50(d)(1), 514.(b)(5); 21 CFR part 814 subpart B; 21 CFR 820.70, 820.75, 820.181, 820.250; and 9 CFR parts 101-118).

The description of the harvesting process in the CMC section or Outline of Production should include specifications regarding acceptable conditions of the plants and a listing of equipment used to harvest the source material, including power equipment, hand tools, and transport equipment (see Table, above, for applicable regulations and refer to applicable FDA and CVB guidance documents). If the equipment is not dedicated to harvesting only the source material, other uses should be documented.

*4. Transfer and Storage Conditions*

Of special concern is the transfer of source material from the field or greenhouse to the manufacturing facility (see section III.C.1., Confinement Measures; for authorities concerning the movement of plant materials). The source material should be transported in such a way as to exclude introduction of insects, vermin, or potential surface contaminants, which may be carried from the farm field or greenhouse environment, and to ensure that plant material remains confined within the container during transport. We recommend that during transport, containers of regulated product material should carry a label that clearly indicates that the material is not to be used for food or feed.

If the harvested source material is to be stored prior to further processing, the storage conditions (e.g., temperature, humidity, volume, density, storage time, etc.) should be fully described in your application. The material to be stored should be characterized and all properties that may be reasonably expected to affect product quality should be identified and appropriate controls should be specified (e.g., stability of the product, ability to support growth of microorganisms, residual soil content, presence of foreign material, insects, vermin). Source material should be stored under appropriate conditions to ensure that decomposition processes do not increase the concentration of contaminants above specified levels or adversely affect the desired active pharmaceutical ingredient. (21 CFR parts 211, 226, 314, 514, 601, and 820, and 9 CFR parts 101-118).

*5. Initial Processing of Source Material*

Procedures used to process harvested material should be validated. Harvested material may be processed to lower bioburden or viability, improve its handling characteristics, bulk consistency, and/or its extractability using various procedures, including washing, sanitizing, milling of grain, shredding of leaves, and homogenization of source plant material, fruits or vegetables. The material

produced by these processes may be intended for further processing or for use as the final product (e.g., as an oral vaccine). (21 CFR 211.110, 211.186, 226.40, and 820.75, and 9 CFR parts 101-118).

*6. Extraction*

The extraction process should be designed to efficiently concentrate the active component or separate it from the rest of the plant material. As with any purification procedure, the extraction method should not introduce contaminants into the process intermediate. Acceptance criteria for relevant parameters (e.g., product concentration, total protein concentration) should be established in order to verify lot-to-lot consistency. If the drug or biologic is extracted into a soluble form, it is advisable to implement sterilizing filtration procedures early in the process. (See generally, 21 CFR 226.40, 312.23(a)(7), 314.50, 514.1(b)(5)(iv), 820.75 and 9 CFR parts 101-118.)

*7. Aseptic Processing*

For those products for which sterility is required, sterility of protein products is usually achieved through the use of appropriately validated filtration steps. However, for products for which sterile filtration is not feasible, we recommend that you use a validated aseptic process. For FDA-regulated products, refer to 21 CFR 211.113, 610.12(g)(4), and 820.75, and current guidance, such as the Guideline on Sterile Drug Products Produced by Aseptic Processing (Ref. 7) and Guidance for Industry: For the Submission of Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products (Ref. 8). For veterinary biologics, refer to 9 CFR 113.26 and 113.28 for further information.

*8. Changeover Procedures*

Changeover procedures designed to prevent contamination between harvests of source material should be in place and documented (21 CFR 211.67, 226.30, and 820.75). These procedures should include clearance of all materials and waste from the receiving area and plant material processing equipment, and cleaning/sanitization of surfaces. Pieces of equipment used for harvesting (e.g., scythe bars, harvested material transportation vehicles) and initial source material processing (e.g., maceration equipment) are of particular concern in terms of cross-contamination. We recommend that only one lot of source material be processed at a time. If multiple lots of source material are to be processed at one time, segregation procedures should be developed and implemented. Integrity of processing equipment should be demonstrated or closed systems employed, when possible. Product contact equipment should be sufficiently cleaned between each lot operation to prevent product carry-over contamination of subsequent lots.

826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871

*9. Process Validation*

All processes used to manufacture the product should be validated prior to marketing the regulated product. Laboratory studies may help to establish appropriate operating and process parameters and may be used in support of the formal validation study. You should include information and data from validation protocols and executed validation studies in your application. (21 CFR 211.110, 211.165, 211.194(a)(2), and 226.40)

**E. Characterization of the Product**

You should provide a complete characterization of the regulated product. For purified drug substances and drug products provide a characterization sufficient to ensure its identity, strength, quality, and purity (21 CFR 211.160-165, 211.186, 226.58, 312.23(a)(7), 314.50(d)(1)(i), 601.2(a), 820.60, 820.70, 820.75, 820.80-86, and 820.181). You should include both physicochemical as well as functional assessments. For purified protein products, the physicochemical description should also include molecular weight, subunit composition, isoelectric point, post-translational modifications, impurity profile, and other relevant parameters. Functional assays should evaluate clinically relevant activities of the product. You should provide a description of the potency assay for the active component. You should submit information on the sensitivity, specificity, and variability of all assays, including the data from the material used to prepare clinical/pre-clinical lots and prelicense serials that were used to set the acceptance limits for the assay.

In your application, you should provide specifications for the product, including identity, purity, potency, physicochemical measurements, and measures of stability (21 CFR 211.160(b) or 9 CFR 114.9). If test results are reported for final release of the product, you should establish estimates of variability and upper and lower limits for each specification. If the purified drug substance is held prior to further processing, a description of the storage conditions and verification of its stability under the conditions described should be included (see section V.). For FDA-regulated biological products, you are encouraged to consult related guidance documents for general product characterization guidance (Refs. 9, 10). For new animal drugs, consult with CVM and for veterinary biologics, CVB.

You should give special consideration to the characterization of edible plant biologics as noted above (section IV.B.) especially for measurements of identity of the active drug or biologic, bioburden limits, dose considerations and final presentation of the product (e.g., juice, puree, whole fruit, etc.).

**F. Product Stability**

Your application should include a stability protocol containing, but not limited to, testing for:

- potency;
- physicochemical measurements that are stability-indicating;
- moisture, if lyophilized;

- pH, if appropriate;
- sterility or control of bioburden;
- pyrogenicity, if applicable; and
- general safety, if applicable.

For products intended for use in humans and for new animal drugs, you should submit information on the stability of the final product and any in-process material at each holding step (21 CFR 211.166, 226.58(d), 312.23(a)(7)(iv), 314.50(d)(1), 601.2(a), and 820.75). Additional information for human drugs and biologics can be found in ICH and FDA guidance documents (Refs. 9, 10), in 21 CFR part 514, and a CVM specific guidance document for new animal drugs (Ref. 11). FDA has also published a draft guidance document issued for public comment and an ICH document on human drug and biological product stability (Refs. 12, 13). For veterinary biologics, you should establish the stability of the product prior to licensure.

You should propose an expiration dating period for the final product and designate the recommended storage conditions. Also, you should define the procedure for determining the date from which the expiration dating period begins.

A plan for an ongoing stability program should be provided in your application. This should include the protocol to be used, number of final lots/serials to be entered into the stability protocol each year, and how such lots/serials will be selected.

## V. PRE-CLINICAL CONSIDERATIONS FOR BIOENGINEERED PHARMACEUTICAL PLANT-DERIVED PRODUCTS FOR USE IN HUMANS

### A. General Considerations

This section does not attempt to delineate acceptable practices or testing procedures for each specific technology or particular class of products, but rather is to provide a general approach to pre-clinical testing of bioengineered pharmaceutical plant-derived products for use in humans. You should consult with the appropriate reviewing division of the appropriate agency for pre-clinical requirements for a specific product class.

The extent of pre-clinical testing will be determined by the known attributes of the product, the donor genetic material, the host plant, and the extent of structurally and pharmacologically comparable products for which there is clinical experience. Guidance for the pre-clinical testing of various biological products is available (Refs. 14-16). Additional consideration given to pre-clinical testing of the bioengineered pharmaceutical plant source material includes the presence and identity of potentially harmful constituents such as: toxicants, pathogens, pesticides, herbicides, fungicides, heavy metals, anti-nutrients, and allergens. Both in vitro and in vivo studies may contribute to this characterization.

For plant lines derived from a host plant or related species having a known potential to produce toxins, anti-nutrients, or allergens, you should perform sensitive tests early in

product development to demonstrate whether the levels of these components have changed in the bioengineered source plant. If the donor of the DNA is known to be a source of allergens or toxicants, then you should perform appropriate allergenicity or toxicity testing.

## B. Evaluation of Impurities

Impurities and contaminants include: source-plant-derived impurities, pesticides, herbicides, fungicides, bacterial or fungal-derived impurities, and downstream processing-derived impurities. Product-related impurities include degradation products, aggregates, or other modified forms of the desired product (e.g., deamidated, isomerized, mismatched disulfide-linked, oxidized, or altered conjugated forms). You should give special attention to post-translational modifications unique to plant expression systems, for example the presence of xylose in glycoproteins.

Further information on this topic is provided in the ICH; Technical Requirements for Registration of Pharmaceuticals for Human Use - Guideline Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products (Ref. 9).

### *1. Toxicants*

If the host species is known to contain toxicants (e.g., protease inhibitors, hemolytic agents, neurotoxins), analytical tests, animal tests, or validation of removal may be appropriate to establish that the toxicant levels are in a safe range in the final product. Consult with FDA for further guidance.

### *2. Evaluation of Pesticide, Herbicide, and Fungicide Levels*

You should use only pesticides, herbicides and/or fungicides registered by the Environmental Protection Agency (EPA) for use on the crop you are using. With regard to the final pharmaceutical product, you should specify the maximum amount of any pesticide, herbicide, and/or fungicide residues anticipated to be present, justify the safety of those amounts under conditions of anticipated use of the pharmaceutical, and demonstrate that the final product does not exceed those limits. A developer who has a new plant that expresses both a bioengineered biologic product and a bioengineered pesticide should consult with EPA regarding the safety of the pesticide. In some instances, validation of removal of the pesticide from the preparation may be an acceptable alternative to final product safety tests. This document only addresses FDA and USDA guidance; if you have questions regarding the use or safety of pesticides, you should contact EPA.

### *3. Evaluation of Metal Toxicants*

You should evaluate both the presence and levels of toxic heavy metals. Consideration should be given to the host plant and whether it stores or accumulates these metals.

### C. Allergenicity

As part of the pre-clinical evaluation, you should consider the allergenicity or immunogenicity of the intended biological product or drug. Appropriate testing protocols depend upon the intended effect of the product, the intended use (route of administration of the product), and the purity of the product. You should assess the need for allergenicity testing for each product on an individual basis and take into account production methods that might introduce allergens into the final product (e.g., from inadvertent contamination by mold, animal dander, animal excrement, or dust mite due to field or storage conditions), in addition to the potential allergenicity of the bioengineered pharmaceutical plant, itself. Consult with FDA for further guidance.

If the source plant producing the product is allergenic or immunogenic, you should test the product for those substances. Consideration should be given to plant-specific modifications, such as altered glycosylation (e.g., xylose), with regard to potential effects on immunogenic and allergenic responses to the intended product.

You should evaluate the final product for allergenic determinants, such as N-glycans. Specific serum screening of the expressed protein could be evaluated using sera derived from patients allergic to the source material. Any positive outcome from specific serum screening would define the product as likely to be allergenic.

### D. Immunogenicity

You should evaluate your product for plant specific modifications that may contribute to unintended immunogenicity. Standard immunogenicity testing for these products should be performed according to existing guidance (Refs. 14, 15) and consultation with FDA.

## VI. CLINICAL TESTING FOR FDA-REGULATED PRODUCTS AND PRE-LICENSE TESTING FOR USDA-REGULATED PRODUCTS

We recommend that you refer to existing guidance(s) for conduct of clinical studies for drugs and biologics for humans and contact CDER or CBER, respectively if you have further questions. The potential residues of animal drugs (derived from bioengineered plants) in edible food animal tissues may be of concern, and you should contact CVM directly for guidance. You should contact CVM or CVB before animal drugs or veterinary biologics are tested on non-laboratory animals.

## VII. DEFINITIONS

**APHIS** – Animal and Plant Health Inspection Service of the USDA.

**Batch** – a specific quantity of a drug or other material that is intended to have uniform character and quality, within specified limits, and is produced according to a single manufacturing order during the same cycle of manufacture.

**Bioengineered pharmaceutical plant** – a plant manipulated by recombinant DNA technology to express a gene encoding a biologic or drug product.

**BLA** – Biologics License Application.

**BRS** – Biotechnology Regulatory Services Division of the USDA/APHIS.

**CFR** – Code of Federal Regulations.

**Coding region** – protein coding regions contain an open reading frame which can be transcribed into messenger RNA to direct the synthesis of a protein product.

**Confinement** – measures implemented to control the co-mingling of bioengineered pharmaceutical plants with non-bioengineered plants or to limit the distribution of an introduced gene to a defined area.

**Construct** – an engineered DNA fragment that contains, but is not limited to, the DNA sequences to be integrated into a target plant's genome.

**CBER** – Center for Biologics Evaluation and Research of the FDA.

**CDER** – Center for Drug Evaluation and Research of the FDA.

**CDRH** – Center for Devices and Radiological Health of the FDA.

**CFSAN** – Center for Food Safety and Applied Nutrition of the FDA

**CVB** – Center for Veterinary Biologics of the USDA/APHIS.

**CVM** – Center for Veterinary Medicine of the FDA.

**Direct delivery systems** – gene delivery systems that do not use biological agents to introduce foreign genes into plants. Examples include electroporation, the chemical polyethylene glycol, microprojectile bombardment, and injection via a capillary tube or pipette.

**Drug** – human protein drug and new animal drug.

**FDA** – United States Food and Drug Administration.

**Genetic stability** – the ability of the introduced DNA to be inherited in a predictable fashion and the introduced trait to be expressed in the transformed plant line and plant lines derived therefrom in a consistent, reliable, and predictable manner.

**Host Plant** – the parent plant prior to insertion of the gene encoding the regulated product.

**ICH** – International Conference on Harmonisation.

**IDE** – Investigational Device Exemption.

**INAD** – notice of claimed investigational exemption for a New Animal Drug that must be submitted prior to shipment of a new animal drug for clinical tests; establishes an Investigational New Animal Drug file, if one has not already been established for the new animal drug.

**IND** – Investigational New Drug Application.

**Indirect delivery systems** – indirect delivery systems use a biologic agent to introduce the foreign genes into the plant's genome.

**Lot** – a batch, or a specific identified portion of a batch, having uniform character and quality within specified limits; or, in the case of a process, it is a specific identified amount produced in a unit of time or quantity in a manner that assures its having a uniform character and quality within specified limits.

**Marketing application** – a BLA, NDA, NADA, PMA, 510(k), or VBPLA.

**MSB** – Master Seed Bank (or Master Seed for veterinary biologics).

**NADA** – New Animal Drug Application.

**NDA** – New Drug Application.

**NEPA** – National Environmental Policy Act.

**New animal drug** – are articles other than food intended for therapeutic, preventative, mitigation or diagnostic purposes OR alter the structure and function of the animal.

**Non-coding region** – DNA sequences that lie outside of an open reading frame and which are not translated to become part of a protein. These might include scaffold attachment regions, promoters, leader sequences, enhancers, introns, terminators, and any other sequences that are used for gene expression either in the plant or other hosts.

**Outline of Production** – a detailed protocol of methods of manufacture to be followed in the preparation of a veterinary biological product.

**Raw agricultural commodity** – any food in its raw or natural state, including all unprocessed fruits, vegetables, nuts, and grains.

**Regulated products** – FDA- or CVB-regulated intermediates, and biological products, vaccines, and drugs, intended for human or animal use and/or animal feed.

**Serials** – consecutive lots or batches in support of a CVB product license application.

**Source material** – plant biomass from which the regulated product is produced.

**Source plant** – bioengineered host plant.

**Source plant material** – any biomass, including seeds, from a source plant.

**Target gene** – the gene encoding the regulated product, including any linked regulatory elements and selectable markers.

**Trait(s)** – the phenotypic characteristic(s) conferred to the recipient plant by the introduced DNA.

**Transfection system** – a method for transitory gene expression using a plant virus.

**Transformation event** – the introduction into an organism of genetic material that has been manipulated in vitro. For the purpose of this document, 'organism' refers to plants.

**Transformation system** – a method for introducing new genes into plants by either direct or indirect delivery systems.

**USDA** – United States Department of Agriculture.

**VBPLA** – United States Veterinary Biological Product License Application.

**Vector** – an autonomously replicating DNA molecule into which foreign DNA is inserted and then propagated in a host cell.

**Veterinary biologic** - all viruses, serums, toxins, or analogous products at any stage of production, shipment, distribution, or sale, which are intended for the use in the treatment of animals and which act primarily trough the direct stimulation, supplementation, enhancement, or modulation of the immune system or immune response.

**Viral vector** – a virus that has been modified to contain foreign genes.

**Virus** – infectious agents containing only nucleic acid and a protein coat that can enter and replicate in a cell.

**WSB** – Working Seed Bank (or Working Seed for veterinary biologics).

## VIII. REFERENCES

1. 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 – (1996).

2. FDA Guidance for Industry: Environmental Assessment of Human Drug and Biologics Applications – (1998).

3. FDA Guidance for Industry: Environmental Impact Assessments (EIA's) for Veterinary Medicinal Products (VMP's) - Phase I – (2001).

4. FDA Draft Guidance for Industry: Cooperative Manufacturing Arrangements for Licensed Biologics – (1999).

5. The Pesticide Product Information System contains information concerning all pesticide products registered in the United States. (http://www.epa.gov/opppmsd1/PPISdata/index.html).

6. Guide to minimize microbial food safety hazards for fresh fruits and vegetables, http://www.cfsan.fda.gov/~dms/guidance.html or http://www.foodsafety.gov/~dms/prodguid.html

7. FDA Guideline on Sterile Drug Products Produced by Aseptic Processing – (1987).

8. FDA Guidance for Industry: For the Submission of Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products – (1994).

9. ICH; Technical Requirements for Registration of Pharmaceuticals for Human Use - Guideline Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products – (1999).

10. FDA Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use – (1997).

11. FDA Guidance for Industry: Stability Guidelines. CVM Guidance #5. (1990).

12. FDA Draft Guidance for Industry: Stability Testing of Drug Substances and Drug Products – (1998).

13. ICH; Technical Requirements for Registration of Pharmaceuticals for Human Use - Guideline Q1A(R): Stability Testing of New Drug Substances and Products – (2001).

14. ICH; Technical Requirements for Registration of Pharmaceuticals for Human Use - Guideline S5B: Detection of Toxicity to Reproduction for Medicinal Products – (2000).

15. ICH; Technical Requirements for Registration of Pharmaceuticals for Human Use - Guideline S6: Pre-Clinical Testing of Biotechnology-Derived Pharmaceuticals – (1997).

16. ICH; Technical Requirements for Registration of Pharmaceuticals for Human Use - Guideline M3: Nonclinical Safety Studies for the Conduct of Human Clinical Trials for Pharmaceuticals – (2000).

**APPENDIX A**

CONTACTS:

To apply for a permit for importation, interstate movement, and field testing of bioengineered plants and plant viruses:
James White, Ph.D.
U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology Regulatory Services, Unit 147
4700 River Road
Riverdale, MD 20737
Ph. # (301) 734-5940
http://www.aphis.usda.gov/ppq/biotech

For permission to ship experimental veterinary biological products (9 CFR 103.3 authorization) or for information regarding veterinary biologics:
U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Center for Veterinary Biologics
Licensing and Policy Development
510 S. 17th St., Suite 104
Ames, Iowa 50010
Ph. # (515) 232-5785; Fax # (515) 232-7120
http://www.aphis.usda.gov/vs/cvb/

For permission to import veterinary biological products:
U.S. Department of Agriculture
Animal and Plant Health Inspection Service
Center for Veterinary Biologics
4700 River Road, Unit 148
Riverdale, MD 20737
Ph. # (301) 734-8245; Fax # (301) 734-4314
http://www.aphis.usda.gov/vs/cvb/

For information regarding therapeutic or diagnostic biologics for use in humans:
U.S. Food and Drug Administration
Center for Biologics Evaluation and Research
Office of Therapeutics Research and Review
1401 Rockville Pike
Rockville, MD 20852
Ph. # (301) 827-5101; Fax # (301) 827-5397
www.fda.gov/cber

For information regarding vaccines for use in humans:
U.S. Food and Drug Administration