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United States  
Department of  
Agriculture

**CENTER FOR VETERINARY BIOLOGICS NOTICE NO. 02-24**

Marketing and  
Regulatory  
Programs

Subject: Guidance Document for Veterinary Biologics Derived from  
Bioengineered Plants

Animal and Plant  
Health Inspection  
Service

To: Biologics Licensees, Permittees, and Applicants  
Directors, Center for Veterinary Biologics

Veterinary Services

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Biologics  
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**I. PURPOSE**

The purpose of this notice is to inform all interested persons of the availability of a draft guideline entitled "Guidance for Industry: Drugs, Biologics, and Medical Devices Derived from Bioengineered Plants for Use in Humans and Animals."

**II. BACKGROUND**

The U.S. Department of Agriculture (USDA), in collaboration with the Food and Drug Administration (FDA), is announcing the availability of a draft guidance document entitled "Guidance for Industry: Drugs, Biologics, and Medical Devices Derived from Bioengineered Plants for Use in Humans and Animals" dated September 12, 2002. The draft guideline outlines important scientific questions and information that should be addressed when applying for a United States Veterinary Biological Product License for veterinary biologics derived from bioengineered plants.

Because the draft guidelines apply to veterinary biologics regulated by the Animal and Plant Health Inspection Service under the Virus-Serum-Toxin Act, we are requesting comments on the scope of the guideline so that we may include any relevant input on the draft in our discussions with the FDA.

This draft guidance document represents the agency's current thinking on this topic. It is being distributed for comment purposes only and is not intended for implementation at this time.

**III. COMMENTS**

Interested persons may submit comments or questions regarding this draft guidance document to Dr. Pat Foley, Center for Veterinary Biologics, 510 South 17th Street, Suite 104, Ames, IA 50010-8197. The deadline for comments on the draft is January 10, 2003.



Veterinary Services – Safeguarding Animal Health  
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Federal Relay Service  
(Voice/TTY/ASCII/Spanish)  
1-800-877-8339

#### IV. DOCUMENT ACCESS

You may request a copy of this document by contacting Ms. Harriet Murphy, Center for Veterinary Biologics, Riverdale, Maryland, at 301-734-8245.

Persons with access to the Internet may obtain the document at one of several locations:

<http://www.aphis.usda.gov/vs/cvb/notices/biop>  
<http://www.fda.gov/cber/guidelines.htm> or  
<http://www.fda.gov/ohrms/dockets/default.htm>.

UPDATE: Guidance document is now appended to the electronic version of this Notice.

/s/ Richard E. Hill, Jr.

Richard E. Hill, Jr.  
Director  
Center for Veterinary Biologics

# 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](mailto: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>*

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## 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

152 humans or animals or as components in clinical diagnostic systems.  
153

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

159  
160 This document is directed at the issues unique to the use of bioengineered pharmaceutical  
161 plants as source material for the production of FDA and/or USDA regulated products.  
162 Therefore, it does not focus on many aspects of regulated products that are shared with other  
163 expression systems. Given the complexity and variety of products, no single document can  
164 anticipate and address all issues. You are encouraged to consult other FDA and USDA  
165 documents for guidance on other specific topics relevant to your product.

166  
167 You should be aware that the Biotechnology Regulatory Services Division (BRS) within  
168 APHIS oversees the importation and interstate movement of bioengineered pharmaceutical  
169 plants and infectious plant vectors as well as the release of these entities into the  
170 environment (i.e., outside of a contained facility, such as a greenhouse, laboratory, or  
171 fermentor). You must receive a permit from APHIS/BRS prior to engaging in these  
172 activities (7 CFR 340). You may obtain guidance on applying for a permit at the  
173 USDA/APHIS website <http://www.aphis.usda.gov/biotech> or by writing to  
174 USDA/APHIS/BRS (see addresses in Appendix A). This document will not describe the  
175 plant permitting process.

## 176 **B. Regulatory Responsibility**

177  
178  
179 The FDA regulates human biologics, and human and animal drugs derived from  
180 bioengineered pharmaceutical plants, intended for therapeutic, preventative, or diagnostic  
181 purposes. Biological products and drugs for use in humans are regulated by the Center for  
182 Biologics Evaluation and Research (CBER) and CDER under authority of the Public Health  
183 Service Act (PHS Act) (42 U.S.C. 262 *et seq.*) and the Federal Food, Drug, and Cosmetic  
184 Act (FD&C Act) (21 U.S.C. 301 *et seq.*). FDA also regulates animal drugs derived from  
185 bioengineered pharmaceutical plants, intended for use in the diagnosis, cure, mitigation,  
186 treatment, or prevention of disease in animals or to alter the structure or function of the  
187 animal. New animal drugs and animal feeds containing new animal drugs are regulated by  
188 the Center for Veterinary Medicine (CVM) under authority of the FD&C Act. The FDA  
189 regulations are found at Title 21 of the Code of Federal Regulations (21 CFR).

190  
191 The USDA regulates veterinary biologics through the Center for Veterinary Biologics  
192 (CVB) within Veterinary Services in APHIS under the authority of the Virus, Serum, and  
193 Toxins Act (21 U.S.C. 151 *et seq.*). The USDA regulations are found at Title 9 of the Code  
194 of Federal Regulations (9 CFR) Parts 101-124.

195  
196 As mentioned above, APHIS/BRS regulates the importation, interstate movement, and  
197 release into the environment (e.g., field testing) of all such bioengineered pharmaceutical

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

200  
201 Appendix A provides a listing of the points of contact at the agencies.

202  
203 To minimize duplication, review of environmental safety issues posed by field growth of the  
204 bioengineered pharmaceutical plants, including National Environmental Policy Act (NEPA)  
205 assessments, will be addressed primarily by APHIS/BRS. Because bioengineered  
206 pharmaceutical plants will be grown under APHIS permit, and because permits enabling  
207 field trials will be obtained prior to submission of a product application, APHIS/BRS will  
208 identify and evaluate the potential environmental effects posed by field growth of such  
209 plants. Environmental concerns posed by use of the regulated product will be addressed in  
210 the NEPA analysis conducted by the regulatory agency responsible for review and/or  
211 approval of the product. These agencies' NEPA analyses will take into account  
212 APHIS/BRS's environmental reviews. Also refer to section III.B. National Environmental  
213 Policy Act.

## 214 215 216 **II. HOST AND SOURCE PLANT CHARACTERIZATION**

### 217 218 **A. General Considerations**

219  
220 In the development stage, you should give careful consideration to choosing the plant  
221 species that will be used as the source of the desired regulated product. Concerns to be  
222 addressed include: the potential for the plant to express an allergenic or toxic compound; the  
223 method of plant propagation and the measures to ensure confinement; and, if it is a food  
224 crop species engineered to produce non-food material, the measures to ensure that non-food  
225 (or non-feed) material will not get into food or feed. The presence of any such material in  
226 food or feed could render such products adulterated under the FD&C Act (21 U.S.C. 342).

227  
228 You are encouraged to refer to pertinent guidance documents and regulations, and to consult  
229 with the regulatory agencies as early as possible in the development process to ensure that  
230 you are aware of the most current regulatory requirements.

### 231 232 **B. Host Plants**

233  
234 You should provide in your application a thorough description of the host plant biology that  
235 includes information necessary to identify it in the narrowest taxonomic grouping applicable  
236 (e.g., genus, species, subspecies, variety or cultivar, line designation).

237  
238 In order for the agencies to assess the ability of the chosen plant to consistently manufacture  
239 your intended product, you should submit a description of the reproductive biology of the  
240 unmodified plant and production practices with regard to:

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



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

249  
250 In addition, you should provide a description of the host plant including levels of any toxins,  
251 anti-nutrients, and allergens known to be produced by the plant species and whether it is  
252 known to accumulate heavy metals. Please state if the plant is of a species used for food or  
253 feed in a raw or processed form.

## 254 **C. Bioengineered Source Plants**

### 255 *1. General Considerations*

256  
257 The host plant may be bioengineered to increase the expression of an endogenous  
258 gene product or to manipulate the plant to produce a heterologous gene product.  
259 The modifying gene may be transiently added to the plant or it may be inserted in a  
260 stable manner. Regardless of the method of gene expression used, traceable  
261 documentation of the growth and expression phase of the manufacturing process,  
262 including banking of the plant lines and/or vectors should be maintained. Most  
263 importantly, you should include data in your application to demonstrate that the  
264 source plant produces a consistent product.

265  
266 When the bioengineered pharmaceutical plant is from a species that is used for food  
267 or feed, measures should be in place to ensure that there is no inadvertent mixing of  
268 the bioengineered plant material with plant material intended for food or feed use.  
269 The presence of any such material in food or feed could render such products  
270 adulterated under the FD&C Act (21 U.S.C. 342). We strongly recommend that you  
271 have tests available that can detect the presence of the target gene and the protein  
272 product in the raw agricultural commodity.

### 273 *2. Characterization of the Recombinant DNA*

274  
275 In your application, you should provide a full characterization of the recombinant  
276 DNA constructs or viral vectors used to transfer genes, including:

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

290  
291 For the purposes of this document, coding regions include full-length and  
292 truncated sense constructs, antisense constructs, and constructs containing  
293 ribozymes, regardless of whether or not the coding region is designed or expected  
294 to be expressed in the bioengineered pharmaceutical plant.  
295

296 For additional details regarding analysis of r-DNA constructs for human  
297 biologics, please refer to the International Conference on Harmonisation (ICH);  
298 Technical Requirements for Registration of Pharmaceuticals for Human Use –  
299 Guideline Q5B: Quality of Biotechnological Products: Analysis of the Expression  
300 Construct in Cells Used for Production of r-DNA Derived Protein Products (Ref.  
301 1).  
302

### 303 3. *Stable Transformation Systems*

304

305 Before preparing Master Seeds or Master Seed Banks (MSB) and Working Seeds  
306 or Working Seed Banks (WSB), we recommend that you establish a suitable  
307 transformant. For stable transformation systems, you should describe the gene  
308 transfer method in detail and provide relevant references, as appropriate. An  
309 analysis should be performed to determine the number of copies of the gene  
310 inserted, the number of integration sites, and to demonstrate if complete or partial  
311 copies are inserted into the plant's genome. You should determine the nucleotide  
312 sequence of the insert from DNA or mRNA retrieved from the stably-transfected  
313 plants in order to confirm the integrity and fidelity of the DNA insert. When a  
314 fragment of a coding region designed to be expressed in a plant is detected, you  
315 should determine whether a fusion protein could be produced and in which host  
316 tissues it may be located.  
317

318 If the transformation system utilizes a pathogenic organism or nucleic acid  
319 sequences from a pathogen, you should provide a description of the pathogen, the  
320 strain, and the gene(s) involved. If any such pathogenesis-related DNA sequences  
321 were removed or altered prior to transformation, you should describe these  
322 changes in detail. Any helper plasmids or analogous DNA fragments used in the  
323 transformation process should also be described. For example, for  
324 *Agrobacterium*-mediated transformation, provide the strain designation of the  
325 *Agrobacterium* used during the transformation process, indicate how the Ti  
326 plasmid-based vector was disarmed, and indicate whether *Agrobacterium* was  
327 cleared from the transformed tissue.  
328

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

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

340  
341 4. *Transient Transfection Systems:*  
342

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

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

367  
368 You should include relevant literature citations to any of the above information,  
369 as appropriate.

370  
371 5. *Genetic Stability: Seed Banks and Vegetative Propagation*  
372

373 Regardless of whether a transient-transfection system or a stable transformation  
374 system is used, you should prepare a MSB and a WSB to ensure consistent lot-to-  
375 lot growth of the plant and expression of the regulated product. The description  
376 of the MSB in your application should include the identification, the method of  
377 production, the results of analytical tests used to characterize it, the size of the  
378 bank, the storage conditions, and data demonstrating its viability, bioburden  
379 (including speciation of contaminants), uniformity of gene content, and stability.  
380

381 You should submit data demonstrating that bioengineered pharmaceutical plant  
382 lines derived through stable transformation are stable in both phenotype and  
383 genotype. To demonstrate genetic stability, you should include data from a  
384 segregation analysis for the trait of interest, as well as from a molecular  
385 characterization of the genomic insert (e.g., Southern analysis) and from analyses  
386 of expression of the intended product.  
387

388 For plants that are fertile, you should provide data demonstrating the pattern and  
389 stability of inheritance and expression of the new traits over several generations  
390 sufficient to ensure stability over the number of generations that will be used  
391 during manufacture of the regulated product.  
392

393 For plants that are infertile or for which it is difficult to produce seed (such as  
394 vegetatively propagated male-sterile potatoes), you should provide data to  
395 demonstrate that the trait is stably maintained and expressed during vegetative  
396 propagation over a number of cycles that is appropriate to the crop.  
397

#### 398 *6. Tissue Distribution of Expression Products*

399

400 For all inserted coding regions, you should provide data that demonstrates  
401 whether the protein is or is not produced (describe assay method and indicate  
402 limit of detection) as intended in the expected tissues consistent with the  
403 associated regulatory sequences driving its expression (e.g., if the gene is  
404 inducible, you should determine if the gene is expressed in the expected tissues  
405 under induction conditions). You should provide quantitative data characterizing  
406 the distribution of the product in the major plant tissues (e.g., leaves, roots, stalks,  
407 seeds).  
408  
409

### 410 **III. ENVIRONMENTAL CONSIDERATIONS**

#### 411 **A. General Considerations**

412  
413

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

427  
428 Bioengineered pharmaceutical plants that are grown exclusively in an enclosed building  
429 (e.g., greenhouse) generally will be considered to be confined during the growing period  
430 if there are control measures in place to eliminate the spread of pollen or seeds outside of  
431 the facility. Growing plants in such an enclosed building does not require a  
432 USDA/APHIS/BRS permit, however, the importation or interstate movement of  
433 bioengineered pharmaceutical plants would require a permit (7 CFR 340.4).

434  
435 **B. National Environmental Policy Act (NEPA)**

436  
437 You should be aware of NEPA requirements for both the FDA (21 CFR part 25) and the  
438 USDA (7 CFR part 372). You should consider the potential environmental impact of all  
439 aspects of the manufacturing process, including but not limited to transport of seeds and  
440 plants, planting, growing, harvesting, processing, purifying, packaging, storage, and  
441 disposal. If you believe that your activities are categorically excluded by 7 CFR  
442 372.5(c), 21 CFR 25.31, or 25.33 from the requirement to submit an environmental  
443 assessment, you should state this in your application. You are encouraged to consult  
444 available guidance documents (Refs. 2, 3) and to talk directly with the USDA and the  
445 FDA regarding NEPA requirements. A copy of the letter from APHIS/BRS granting  
446 your permit should be submitted in your application for the regulated product in support  
447 of the environmental assessment (21 CFR 25.15 and 25.40) or the claim of categorical  
448 exclusion (21 CFR 25.31, 25.33 or 7 CFR 372.5(c)). FDA and CVB intend to take  
449 APHIS/BRS evaluations and determinations into account in doing their own NEPA  
450 assessments.

451  
452 **C. Confinement Measures**

453  
454 *1. General Considerations*

455  
456 Regardless of whether the bioengineered pharmaceutical plants are grown and/or  
457 processed by you or on a contractual basis by other persons, manufacturing  
458 controls are your responsibility and should be documented clearly in standard  
459 operating procedures (SOPs), Outlines of Production, or other records, as  
460 appropriate (see section IV.C., Applicable FDA and USDA Regulations). For  
461 FDA regulated products, refer to 21 CFR 200.10, parts 210 and 211, 514.1, and  
462 820.50; see also FDA's Draft Guidance for Industry: Cooperative Manufacturing  
463 Arrangements for Licensed Biologics (Ref. 4) once it is finalized.

464  
465 In developing a bioengineered pharmaceutical plant, you should implement  
466 procedures to ensure that such a plant line is used only for its intended purpose as  
467 a source material for a regulated product. As described in 7 CFR 340.4, 340.7,  
468 and 340.8, a permit from USDA/APHIS/BRS is required for the interstate  
469 transport of bioengineered pharmaceutical plants or seeds for such plants, and you  
470 must keep records documenting the handling and transfer of such materials.  
471 Shipment of bioengineered pharmaceutical plants for veterinary biologics requires  
472 permission from USDA/APHIS/BRS. When manufacturing firms are shipping

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

477  
478 When a plant species that is used for food or feed is bioengineered to produce a  
479 regulated product, you should consider the use of strategies that allow the  
480 bioengineered pharmaceutical plant line to be readily distinguished from its food  
481 or feed counterpart. Such strategies might include the use of genetic markers that  
482 alter the physical appearance of the plant (e.g., a novel color or leaf pattern), or  
483 change the conditions under which a plant will grow (e.g., the use of an  
484 auxotrophic marker gene). You should also consider strategies to reduce the  
485 likelihood of unintended exposure to a regulated product by restricting the  
486 expression of the bioengineered pharmaceutical product to a few specific plant  
487 tissues (e.g., the use of tissue specific promoters) or by restricting the conditions  
488 under which the product will be expressed (e.g., use of an inducible promoter).  
489 For such plants that outcross, you may want to consider growing them in regions  
490 of the country where little or none of its food/feed counterparts are grown.

491  
492 Measures should be in place to ensure that there is no inadvertent mixing of the  
493 bioengineered pharmaceutical plant with plant material intended for food or feed  
494 (including inadvertent mixing with seeds for food or feed crops). During the  
495 development of your overall production process (from the farm through the final  
496 product), you should determine where in the process inadvertent mixing could  
497 occur and establish appropriate control measures. We strongly recommend that  
498 you have tests available that can detect the presence of the target gene and the  
499 protein product in the raw agricultural commodity. The presence of the target  
500 gene or gene product in food or feed could render such products adulterated under  
501 the FD&C Act (21 U.S.C. 342). You may wish to consult with FDA’s Center for  
502 Food Safety and Applied Nutrition (CFSAN) or with CVM about the legal  
503 implications of any such material getting into food or feed.

## 504 505 2. *Control of Seed Stocks*

506  
507 You should maintain careful control over the inventory and disposition of viable  
508 seeds to preclude the possibility that such seeds will be used to produce material  
509 that could be used for food or feed production. When seed stocks are produced,  
510 there should be an accounting of the total yield of seed (e.g., by weight or by  
511 volume). Seed stocks should be stored in aliquots of appropriate volume to allow  
512 reasonably accurate accounting of use and disposition. A record of the amount  
513 and disposition of any withdrawals from the seed bank should be made (7 CFR  
514 340.4(b)(12)). Seed stocks should be prominently labeled in accordance with the  
515 permit issued by APHIS/BRS for field growth or interstate shipment of  
516 bioengineered seeds (7 CFR 340.7).

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3. *Field-grown Plants*

You must have a permit from APHIS/BRS to grow bioengineered pharmaceutical plants in the field (7 CFR 340.4) and must have control over the growing process from planting through harvesting and over the disposition of remaining crops and/or crop residue and, if required, over the subsequent use of the field if for growth of food or feed or as a pasture during subsequent seasons. All persons involved in field growth of the product should be adequately trained to perform the duties for which they are responsible. Control measures should include an accounting of seed that is transferred from seed bank storage to the field for planting, or for archiving. Documentation of the size and location of all sites where the bioengineered pharmaceutical plants will be grown, of the control of pollen spread, and of the subsequent use of the field and destruction of volunteer plants in subsequent growing seasons should be maintained and provided to the FDA and CVB, as appropriate. Fields should be unambiguously identified, such as by Global Position Satellite (GPS) markers. We recommend that you consider the use of perimeter fencing to help exclude wildlife and escaped livestock. All fields used to grow source bioengineered pharmaceutical plants are subject to inspection by the USDA (7 CFR 340.4; 9 CFR 101-108) and/or by the FDA (42 U.S.C. 262; 21 U.S.C. 374).

4. *Control of Harvested Material*

APHIS requires that appropriate confinement procedures be in place for transport of the source material from the field or greenhouse to the production facility (7 CFR 340.4(b)(10-12)). During transport, containers of harvested material should carry a label that clearly indicates that the material, including but not limited to seeds, leaves, roots, and stems, is not to be used for food or feed or for any purposes in which residual materials could be used for food or feed (such as ethanol production). Reconciliation of the quantities of material leaving the growing facility and arriving at the processing facility should be made. In manufacturing of a regulated product, records must be kept to document control over harvested material in accordance with 21 CFR part 211 subpart J, 21 CFR part 226 subpart E, 7 CFR 340.4, or 9 CFR part 116 and made available for inspection by the FDA or CVB, as appropriate.

5. *Control at Processing Facilities*

As stated in section III.C.1., you should implement appropriate procedures to ensure that bioengineered pharmaceutical plants or plant materials do not unintentionally mix with other plant products, particularly those used as food or feed. Source plant materials should not be processed in facilities that also are used for the production of food or feed, such as grain mills, without prior consultation with USDA/APHIS/BRS and FDA.

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

655 bioengineered pharmaceutical plants are based on: the intended recipient of the product (i.e.,  
656 human or animal); the intended use of the product (e.g., biologic, drug, or device); and the  
657 intended route of administration (e.g., parenteral vs. oral). The Table below includes, but is  
658 not limited to, the following applicable regulations for specific classes of regulated products  
659 for use in humans or animals.  
660

| <b>Planned use</b>   | <b>Applicable regulations</b>   |
|--|---|
| 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                                   |

661 We encourage you to refer to FDA and CVB guidance documents for additional information  
662 and recommendations specific to the product class. Any exceptions to the regulatory  
663 requirements must be obtained as provided by regulation. For example, the general safety,  
664 sterility, and mycoplasma tests prescribed in 21 CFR 610.11-12 and 610.30 (for biologics  
665 for use in humans) or 9 CFR 113.26-28 (for veterinary biologics) may be inappropriate for  
666 some products (e.g., edible plant material intended for use as an oral dosage form) and  
667 modifications or alternative, but equivalent, methods of demonstrating a product's safety and  
668 sterility may be permitted in accordance with 21 CFR 610.9 or the product may be  
669 exempted in accordance with 9 CFR 113.4 (see Table, above).  
670  
671

## 672 **D. Product Manufacturing Procedures**

### 673 *1. General Considerations*

674 Your application should include a description of each step of the purification  
675 process including analytical tests to demonstrate identity, purity, and  
676 concentration, and the levels of product related and non-product related  
677 impurities. This is particularly important if the impurities are determined to be  
678 toxins, allergens, teratogens, or carcinogens. For each process that is not intended  
679 to be sterile, you should describe the procedures to be followed to control  
680 extraneous bioburden and the in-process testing used to monitor the level of  
681 bioburden (see, 21 CFR 211.113, 226.102, 312.23, 314.50(d), 514.1(b), 820.70,  
682 820.181, and 820.184). A summary of the manufacturing, including propagation  
683 of the source material, should be available at the site where the manufacturing  
684 occurred (21 CFR 211 subpart J). You should consult with the appropriate  
685 agency regarding the applicability of these considerations to device components.  
686  
687  
688

2. *Growth Conditions*

The Chemistry, Manufacturing, and Controls (CMC) section or the Outline of Production should include information regarding the location of source plant propagation. For greenhouse-grown material, you should include in the description the types of containers, the soil mix composition and qualification criteria, and the greenhouse growth conditions. For field grown material, the description should include the previous uses of the land (e.g., agricultural and/or industrial use). We recommend that you establish specification/acceptance criteria/limits for the soil composition and potential soil contaminants that may affect the source material. In addition, you should describe the agricultural methods utilized during crop growth, including specifications regarding the use of chemicals and limits on specific agricultural practices (e.g., the use of specified fertilizers, pesticides, or herbicides, and irrigation practices relative to a specified harvest time frame, etc.). You should provide in your application a list of expected pests that will require control during the growth of the bioengineered pharmaceutical plants. All pest-control measures implemented should be in accordance with good agricultural practices for the growth of food crops in the United States. The Pesticide Product Information System (Ref. 5) contains information concerning all pesticide products registered in the United States. In order to evaluate the purity of the product, all pest-control interventions should be described in appropriate SOPs and should be documented in the Batch Record (for FDA-regulated products) or Outline of Production (for veterinary biologics). We recommend that you follow current Good Agricultural Practices (e.g., Ref. 6). If product expression is induced, either chemically or physically, you should establish criteria to ensure that induction is performed consistently from batch to batch. (See generally, 21 CFR parts 210, 211, 226, 312, 314, 514, 601, 610, and 820; see e.g., 21 CFR 211.84, 211.186, 312.23(a)(7), 314.50(d), 514.1, 820.50, and 9 CFR parts 101-118.)

3. *Harvest*

You should describe the method of harvesting the source material in written procedures and document the process in production records. You should have procedures for determining when the harvest will occur in order to ensure lot-to-lot consistency of the source material. You should establish specifications for the harvested material with regard to the levels of active component, process-derived contaminants, significant endogenous impurities, and adventitious agents. For example, you should describe agricultural practices and training of harvesting personnel regarding plant source material quality (e.g., assessment of the disease status of plant for manual harvesting operations, etc.) (21 CFR part 211 subpart B). You should have written procedures for establishing the necessary training of personnel engaged in harvesting plants to ensure the quality of the harvested material (21 CFR 211.25). We recommend the use of dedicated equipment. We recommend that equipment-cleaning procedures be developed and that cleaning agents used on harvesting equipment be described (21 FR 211.67). In addition,

735 you should consider measures to prevent the contamination of the harvested  
736 source material with equipment lubricants during processing. (21 CFR part 211  
737 subparts F and J; 21 CFR part 226; 21 CFR 314.50(d)(1), 514.(b)(5); 21 CFR part  
738 814 subpart B; 21 CFR 820.70, 820.75, 820.181, 820.250; and 9 CFR parts 101-  
739 118).

740  
741 The description of the harvesting process in the CMC section or Outline of  
742 Production should include specifications regarding acceptable conditions of the  
743 plants and a listing of equipment used to harvest the source material, including  
744 power equipment, hand tools, and transport equipment (see Table, above, for  
745 applicable regulations and refer to applicable FDA and CVB guidance  
746 documents). If the equipment is not dedicated to harvesting only the source  
747 material, other uses should be documented.

#### 748 749 *4. Transfer and Storage Conditions*

750  
751 Of special concern is the transfer of source material from the field or greenhouse  
752 to the manufacturing facility (see section III.C.1., Confinement Measures; for  
753 authorities concerning the movement of plant materials). The source material  
754 should be transported in such a way as to exclude introduction of insects, vermin,  
755 or potential surface contaminants, which may be carried from the farm field or  
756 greenhouse environment, and to ensure that plant material remains confined  
757 within the container during transport. We recommend that during transport,  
758 containers of regulated product material should carry a label that clearly indicates  
759 that the material is not to be used for food or feed.

760  
761 If the harvested source material is to be stored prior to further processing, the  
762 storage conditions (e.g., temperature, humidity, volume, density, storage time,  
763 etc.) should be fully described in your application. The material to be stored  
764 should be characterized and all properties that may be reasonably expected to  
765 affect product quality should be identified and appropriate controls should be  
766 specified (e.g., stability of the product, ability to support growth of  
767 microorganisms, residual soil content, presence of foreign material, insects,  
768 vermin). Source material should be stored under appropriate conditions to ensure  
769 that decomposition processes do not increase the concentration of contaminants  
770 above specified levels or adversely affect the desired active pharmaceutical  
771 ingredient. (21 CFR parts 211, 226, 314, 514, 601, and 820, and 9 CFR parts  
772 101-118).

#### 773 774 *5. Initial Processing of Source Material*

775  
776 Procedures used to process harvested material should be validated. Harvested  
777 material may be processed to lower bioburden or viability, improve its handling  
778 characteristics, bulk consistency, and/or its extractability using various  
779 procedures, including washing, sanitizing, milling of grain, shredding of leaves,  
780 and homogenization of source plant material, fruits or vegetables. The material

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

784  
785 *6. Extraction*

786  
787 The extraction process should be designed to efficiently concentrate the active  
788 component or separate it from the rest of the plant material. As with any  
789 purification procedure, the extraction method should not introduce contaminants  
790 into the process intermediate. Acceptance criteria for relevant parameters (e.g.,  
791 product concentration, total protein concentration) should be established in order  
792 to verify lot-to-lot consistency. If the drug or biologic is extracted into a soluble  
793 form, it is advisable to implement sterilizing filtration procedures early in the  
794 process. (See generally, 21 CFR 226.40, 312.23(a)(7), 314.50, 514.1(b)(5)(iv),  
795 820.75 and 9 CFR parts 101-118.)

796  
797 *7. Aseptic Processing*

798  
799 For those products for which sterility is required, sterility of protein products is  
800 usually achieved through the use of appropriately validated filtration steps.  
801 However, for products for which sterile filtration is not feasible, we recommend  
802 that you use a validated aseptic process. For FDA-regulated products, refer to 21  
803 CFR 211.113, 610.12(g)(4), and 820.75, and current guidance, such as the  
804 Guideline on Sterile Drug Products Produced by Aseptic Processing (Ref. 7) and  
805 Guidance for Industry: For the Submission of Documentation for Sterilization  
806 Process Validation in Applications for Human and Veterinary Drug Products  
807 (Ref. 8). For veterinary biologics, refer to 9 CFR 113.26 and 113.28 for further  
808 information.

809  
810 *8. Changeover Procedures*

811  
812 Changeover procedures designed to prevent contamination between harvests of  
813 source material should be in place and documented (21 CFR 211.67, 226.30, and  
814 820.75). These procedures should include clearance of all materials and waste  
815 from the receiving area and plant material processing equipment, and  
816 cleaning/sanitization of surfaces. Pieces of equipment used for harvesting (e.g.,  
817 scythe bars, harvested material transportation vehicles) and initial source material  
818 processing (e.g., maceration equipment) are of particular concern in terms of  
819 cross-contamination. We recommend that only one lot of source material be  
820 processed at a time. If multiple lots of source material are to be processed at one  
821 time, segregation procedures should be developed and implemented. Integrity of  
822 processing equipment should be demonstrated or closed systems employed, when  
823 possible. Product contact equipment should be sufficiently cleaned between each  
824 lot operation to prevent product carry-over contamination of subsequent lots.  
825

826 9. *Process Validation*

827  
828 All processes used to manufacture the product should be validated prior to  
829 marketing the regulated product. Laboratory studies may help to establish  
830 appropriate operating and process parameters and may be used in support of the  
831 formal validation study. You should include information and data from validation  
832 protocols and executed validation studies in your application. (21 CFR 211.110,  
833 211.165, 211.194(a)(2), and 226.40)

834  
835 **E. Characterization of the Product**

836  
837 You should provide a complete characterization of the regulated product. For purified drug  
838 substances and drug products provide a characterization sufficient to ensure its identity,  
839 strength, quality, and purity (21 CFR 211.160-165, 211.186, 226.58, 312.23(a)(7),  
840 314.50(d)(1)(i), 601.2(a), 820.60, 820.70, 820.75, 820.80-86, and 820.181). You should  
841 include both physicochemical as well as functional assessments. For purified protein  
842 products, the physicochemical description should also include molecular weight, subunit  
843 composition, isoelectric point, post-translational modifications, impurity profile, and other  
844 relevant parameters. Functional assays should evaluate clinically relevant activities of the  
845 product. You should provide a description of the potency assay for the active component.  
846 You should submit information on the sensitivity, specificity, and variability of all assays,  
847 including the data from the material used to prepare clinical/pre-clinical lots and prelicense  
848 serials that were used to set the acceptance limits for the assay.

849  
850 In your application, you should provide specifications for the product, including identity,  
851 purity, potency, physicochemical measurements, and measures of stability (21 CFR  
852 211.160(b) or 9 CFR 114.9). If test results are reported for final release of the product, you  
853 should establish estimates of variability and upper and lower limits for each specification. If  
854 the purified drug substance is held prior to further processing, a description of the storage  
855 conditions and verification of its stability under the conditions described should be included  
856 (see section V.). For FDA-regulated biological products, you are encouraged to consult  
857 related guidance documents for general product characterization guidance (Refs. 9, 10). For  
858 new animal drugs, consult with CVM and for veterinary biologics, CVB.

859  
860 You should give special consideration to the characterization of edible plant biologics as  
861 noted above (section IV.B.) especially for measurements of identity of the active drug or  
862 biologic, bioburden limits, dose considerations and final presentation of the product (e.g.,  
863 juice, puree, whole fruit, etc.).

864  
865 **F. Product Stability**

866  
867 Your application should include a stability protocol containing, but not limited to, testing  
868 for:

- 869 • potency;
- 870 • physicochemical measurements that are stability-indicating;
- 871 • moisture, if lyophilized;

- 872 • pH, if appropriate;
- 873 • sterility or control of bioburden;
- 874 • pyrogenicity, if applicable; and
- 875 • general safety, if applicable.

876  
877 For products intended for use in humans and for new animal drugs, you should submit  
878 information on the stability of the final product and any in-process material at each holding  
879 step (21 CFR 211.166, 226.58(d), 312.23(a)(7)(iv), 314.50(d)(1), 601.2(a), and 820.75).  
880 Additional information for human drugs and biologics can be found in ICH and FDA  
881 guidance documents (Refs. 9, 10), in 21 CFR part 514, and a CVM specific guidance  
882 document for new animal drugs (Ref. 11). FDA has also published a draft guidance  
883 document issued for public comment and an ICH document on human drug and biological  
884 product stability (Refs. 12, 13). For veterinary biologics, you should establish the stability  
885 of the product prior to licensure.

886  
887 You should propose an expiration dating period for the final product and designate the  
888 recommended storage conditions. Also, you should define the procedure for determining  
889 the date from which the expiration dating period begins.

890  
891 A plan for an ongoing stability program should be provided in your application. This should  
892 include the protocol to be used, number of final lots/serials to be entered into the stability  
893 protocol each year, and how such lots/serials will be selected.

894  
895  
896 **V. PRE-CLINICAL CONSIDERATIONS FOR BIOENGINEERED**  
897 **PHARMACEUTICAL PLANT-DERIVED PRODUCTS FOR USE IN HUMANS**

898  
899 **A. General Considerations**

900  
901 This section does not attempt to delineate acceptable practices or testing procedures for each  
902 specific technology or particular class of products, but rather is to provide a general  
903 approach to pre-clinical testing of bioengineered pharmaceutical plant-derived products for  
904 use in humans. You should consult with the appropriate reviewing division of the  
905 appropriate agency for pre-clinical requirements for a specific product class.

906  
907 The extent of pre-clinical testing will be determined by the known attributes of the product,  
908 the donor genetic material, the host plant, and the extent of structurally and  
909 pharmacologically comparable products for which there is clinical experience. Guidance for  
910 the pre-clinical testing of various biological products is available (Refs. 14-16). Additional  
911 consideration given to pre-clinical testing of the bioengineered pharmaceutical plant source  
912 material includes the presence and identity of potentially harmful constituents such as:  
913 toxicants, pathogens, pesticides, herbicides, fungicides, heavy metals, anti-nutrients, and  
914 allergens. Both in vitro and in vivo studies may contribute to this characterization.

915  
916 For plant lines derived from a host plant or related species having a known potential to  
917 produce toxins, anti-nutrients, or allergens, you should perform sensitive tests early in

918 product development to demonstrate whether the levels of these components have changed  
919 in the bioengineered source plant. If the donor of the DNA is known to be a source of  
920 allergens or toxicants, then you should perform appropriate allergenicity or toxicity testing.  
921

## 922 **B. Evaluation of Impurities**

923 Impurities and contaminants include: source-plant-derived impurities, pesticides, herbicides,  
924 fungicides, bacterial or fungal-derived impurities, and downstream processing-derived  
925 impurities. Product-related impurities include degradation products, aggregates, or other  
926 modified forms of the desired product (e.g., deamidated, isomerized, mismatched disulfide-  
927 linked, oxidized, or altered conjugated forms). You should give special attention to post-  
928 translational modifications unique to plant expression systems, for example the presence of  
929 xylose in glycoproteins.  
930

931 Further information on this topic is provided in the ICH; Technical Requirements for  
932 Registration of Pharmaceuticals for Human Use - Guideline Q6B Specifications: Test  
933 Procedures and Acceptance Criteria for Biotechnological/Biological Products (Ref. 9).  
934

### 935 *1. Toxicants*

936 If the host species is known to contain toxicants (e.g., protease inhibitors,  
937 hemolytic agents, neurotoxins), analytical tests, animal tests, or validation of  
938 removal may be appropriate to establish that the toxicant levels are in a safe range  
939 in the final product. Consult with FDA for further guidance.  
940

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

942 You should use only pesticides, herbicides and/or fungicides registered by the  
943 Environmental Protection Agency (EPA) for use on the crop you are using. With  
944 regard to the final pharmaceutical product, you should specify the maximum  
945 amount of any pesticide, herbicide, and/or fungicide residues anticipated to be  
946 present, justify the safety of those amounts under conditions of anticipated use of  
947 the pharmaceutical, and demonstrate that the final product does not exceed those  
948 limits. A developer who has a new plant that expresses both a bioengineered  
949 biologic product and a bioengineered pesticide should consult with EPA regarding  
950 the safety of the pesticide. In some instances, validation of removal of the  
951 pesticide from the preparation may be an acceptable alternative to final product  
952 safety tests. This document only addresses FDA and USDA guidance; if you  
953 have questions regarding the use or safety of pesticides, you should contact EPA.  
954

### 955 *3. Evaluation of Metal Toxicants*

956 You should evaluate both the presence and levels of toxic heavy metals.  
957 Consideration should be given to the host plant and whether it stores or  
958 accumulates these metals.  
959



964 **C. Allergenicity**

965  
966 As part of the pre-clinical evaluation, you should consider the allergenicity or  
967 immunogenicity of the intended biological product or drug. Appropriate testing protocols  
968 depend upon the intended effect of the product, the intended use (route of administration of  
969 the product), and the purity of the product. You should assess the need for allergenicity  
970 testing for each product on an individual basis and take into account production methods  
971 that might introduce allergens into the final product (e.g., from inadvertent contamination by  
972 mold, animal dander, animal excrement, or dust mite due to field or storage conditions), in  
973 addition to the potential allergenicity of the bioengineered pharmaceutical plant, itself.  
974 Consult with FDA for further guidance.  
975

976 If the source plant producing the product is allergenic or immunogenic, you should test the  
977 product for those substances. Consideration should be given to plant-specific modifications,  
978 such as altered glycosylation (e.g., xylose), with regard to potential effects on immunogenic  
979 and allergenic responses to the intended product.  
980

981 You should evaluate the final product for allergenic determinants, such as N-glycans.  
982 Specific serum screening of the expressed protein could be evaluated using sera derived  
983 from patients allergic to the source material. Any positive outcome from specific serum  
984 screening would define the product as likely to be allergenic.  
985

986 **D. Immunogenicity**

987  
988 You should evaluate your product for plant specific modifications that may contribute to  
989 unintended immunogenicity. Standard immunogenicity testing for these products should be  
990 performed according to existing guidance (Refs. 14, 15) and consultation with FDA.  
991  
992

993 **VI. CLINICAL TESTING FOR FDA-REGULATED PRODUCTS AND PRE-**  
994 **LICENSE TESTING FOR USDA-REGULATED PRODUCTS**

995  
996 We recommend that you refer to existing guidance(s) for conduct of clinical studies for drugs  
997 and biologics for humans and contact CDER or CBER, respectively if you have further  
998 questions. The potential residues of animal drugs (derived from bioengineered plants) in edible  
999 food animal tissues may be of concern, and you should contact CVM directly for guidance. You  
1000 should contact CVM or CVB before animal drugs or veterinary biologics are tested on non-  
1001 laboratory animals.  
1002  
1003

1003 **VII. DEFINITIONS**

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**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.

1049  
1050 **Genetic stability** – the ability of the introduced DNA to be inherited in a predictable fashion and  
1051 the introduced trait to be expressed in the transformed plant line and plant lines derived  
1052 therefrom in a consistent, reliable, and predictable manner.  
1053  
1054 **Host Plant** – the parent plant prior to insertion of the gene encoding the regulated product.  
1055  
1056 **ICH** – International Conference on Harmonisation.  
1057  
1058 **IDE** – Investigational Device Exemption.  
1059  
1060 **INAD** – notice of claimed investigational exemption for a New Animal Drug that must be  
1061 submitted prior to shipment of a new animal drug for clinical tests; establishes an Investigational  
1062 New Animal Drug file, if one has not already been established for the new animal drug.  
1063  
1064 **IND** – Investigational New Drug Application.  
1065  
1066 **Indirect delivery systems** – indirect delivery systems use a biologic agent to introduce the  
1067 foreign genes into the plant's genome.  
1068  
1069 **Lot** – a batch, or a specific identified portion of a batch, having uniform character and quality  
1070 within specified limits; or, in the case of a process, it is a specific identified amount produced in  
1071 a unit of time or quantity in a manner that assures its having a uniform character and quality  
1072 within specified limits.  
1073  
1074 **Marketing application** – a BLA, NDA, NADA, PMA, 510(k), or VBPLA.  
1075  
1076 **MSB** – Master Seed Bank (or Master Seed for veterinary biologics).  
1077  
1078 **NADA** – New Animal Drug Application.  
1079  
1080 **NDA** – New Drug Application.  
1081  
1082 **NEPA** – National Environmental Policy Act.  
1083  
1084 **New animal drug** – are articles other than food intended for therapeutic, preventative,  
1085 mitigation or diagnostic purposes OR alter the structure and function of the animal.  
1086  
1087 **Non-coding region** – DNA sequences that lie outside of an open reading frame and which are  
1088 not translated to become part of a protein. These might include scaffold attachment regions,  
1089 promoters, leader sequences, enhancers, introns, terminators, and any other sequences that are  
1090 used for gene expression either in the plant or other hosts.  
1091  
1092 **Outline of Production** – a detailed protocol of methods of manufacture to be followed in the  
1093 preparation of a veterinary biological product.  
1094

- 1095 **Raw agricultural commodity** – any food in its raw or natural state, including all unprocessed  
1096 fruits, vegetables, nuts, and grains.  
1097
- 1098 **Regulated products** – FDA- or CVB-regulated intermediates, and biological products, vaccines,  
1099 and drugs, intended for human or animal use and/or animal feed.  
1100
- 1101 **Serials** – consecutive lots or batches in support of a CVB product license application.  
1102
- 1103 **Source material** – plant biomass from which the regulated product is produced.  
1104
- 1105 **Source plant** – bioengineered host plant.  
1106
- 1107 **Source plant material** – any biomass, including seeds, from a source plant.  
1108
- 1109 **Target gene** – the gene encoding the regulated product, including any linked regulatory elements  
1110 and selectable markers.  
1111
- 1112 **Trait(s)** – the phenotypic characteristic(s) conferred to the recipient plant by the introduced  
1113 DNA.  
1114
- 1115 **Transfection system** – a method for transitory gene expression using a plant virus.  
1116
- 1117 **Transformation event** – the introduction into an organism of genetic material that has been  
1118 manipulated in vitro. For the purpose of this document, ‘organism’ refers to plants.  
1119
- 1120 **Transformation system** – a method for introducing new genes into plants by either direct or  
1121 indirect delivery systems.  
1122
- 1123 **USDA** – United States Department of Agriculture.  
1124
- 1125 **VBPLA** – United States Veterinary Biological Product License Application.  
1126
- 1127 **Vector** – an autonomously replicating DNA molecule into which foreign DNA is inserted and  
1128 then propagated in a host cell.  
1129
- 1130 **Veterinary biologic** - all viruses, serums, toxins, or analogous products at any stage of  
1131 production, shipment, distribution, or sale, which are intended for the use in the treatment of  
1132 animals and which act primarily through the direct stimulation, supplementation, enhancement, or  
1133 modulation of the immune system or immune response.  
1134
- 1135 **Viral vector** – a virus that has been modified to contain foreign genes.  
1136
- 1137 **Virus** – infectious agents containing only nucleic acid and a protein coat that can enter and  
1138 replicate in a cell.  
1139
- 1140 **WSB** – Working Seed Bank (or Working Seed for veterinary biologics).  
1141

1141 **VIII. REFERENCES**  
1142

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1145 for Production of r-DNA Derived Protein Products – (1996).
- 1146  
1147 2. FDA Guidance for Industry: Environmental Assessment of Human Drug and Biologics  
1148 Applications – (1998).  
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- 1150 3. FDA Guidance for Industry: Environmental Impact Assessments (EIA's) for Veterinary  
1151 Medicinal Products (VMP's) - Phase I – (2001).  
1152
- 1153 4. FDA Draft Guidance for Industry: Cooperative Manufacturing Arrangements for Licensed  
1154 Biologics – (1999).  
1155
- 1156 5. The Pesticide Product Information System contains information concerning all pesticide  
1157 products registered in the United States. (<http://www.epa.gov/opppmsd1/PPISdata/index.html>).  
1158
- 1159 6. Guide to minimize microbial food safety hazards for fresh fruits and vegetables,  
1160 <http://www.cfsan.fda.gov/~dms/guidance.html> or  
1161 <http://www.foodsafety.gov/~dms/prodguid.html>  
1162
- 1163 7. FDA Guideline on Sterile Drug Products Produced by Aseptic Processing – (1987).  
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1166 Validation in Applications for Human and Veterinary Drug Products – (1994).  
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1169 Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological  
1170 Products – (1999).  
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1178 (1998).  
1179
- 1180 13. ICH; Technical Requirements for Registration of Pharmaceuticals for Human Use -  
1181 Guideline Q1A(R): Stability Testing of New Drug Substances and Products – (2001).  
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1184 Guideline S5B: Detection of Toxicity to Reproduction for Medicinal Products – (2000).  
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1187 Guideline S6: Pre-Clinical Testing of Biotechnology-Derived Pharmaceuticals – (1997).  
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1191 Pharmaceuticals – (2000).  
1192  
1193

1193 **APPENDIX A**

1194

1195 **CONTACTS:**

1196

1197 To apply for a permit for importation, interstate movement, and field testing of bioengineered  
1198 plants and plant viruses:

1199 James White, Ph.D.

1200 U.S. Department of Agriculture

1201 Animal and Plant Health Inspection Service

1202 Biotechnology Regulatory Services, Unit 147

1203 4700 River Road

1204 Riverdale, MD 20737

1205 Ph. # (301) 734-5940

1206 <http://www.aphis.usda.gov/ppq/biotech>

1207

1208 For permission to ship experimental veterinary biological products (9 CFR 103.3 authorization)  
1209 or for information regarding veterinary biologics:

1210 U.S. Department of Agriculture

1211 Animal and Plant Health Inspection Service

1212 Center for Veterinary Biologics

1213 Licensing and Policy Development

1214 510 S. 17th St., Suite 104

1215 Ames, Iowa 50010

1216 Ph. # (515) 232-5785; Fax # (515) 232-7120

1217 <http://www.aphis.usda.gov/vs/cvb/>

1218

1219 For permission to import veterinary biological products:

1220 U.S. Department of Agriculture

1221 Animal and Plant Health Inspection Service

1222 Center for Veterinary Biologics

1223 4700 River Road, Unit 148

1224 Riverdale, MD 20737

1225 Ph. # (301) 734-8245; Fax # (301) 734-4314

1226 <http://www.aphis.usda.gov/vs/cvb/>

1227

1228 For information regarding therapeutic or diagnostic biologics for use in humans:

1229 U.S. Food and Drug Administration

1230 Center for Biologics Evaluation and Research

1231 Office of Therapeutics Research and Review

1232 1401 Rockville Pike

1233 Rockville, MD 20852

1234 Ph. # (301) 827-5101; Fax # (301) 827-5397

1235 [www.fda.gov/cber](http://www.fda.gov/cber)

1236

1237 For information regarding vaccines for use in humans:

1238 U.S. Food and Drug Administration

*Draft – Not for Implementation*

1239 Center for Biologics Evaluation and Research  
1240 Office of Vaccines Research and Review  
1241 1401 Rockville Pike  
1242 Rockville, MD 20852  
1243 Ph. # (301) 827-3070; Fax # (301) 827-3532  
1244 [www.fda.gov/cber](http://www.fda.gov/cber)  
1245  
1246 For information regarding animal feeds and animal drugs:  
1247 U.S. Food and Drug Administration  
1248 Center for Veterinary Medicine  
1249 HFV-200, 7500 Standish Place  
1250 Rockville, MD 20855  
1251 Ph. # (301) 827-6652; Fax # (301) 827-1484  
1252 [www.fda.gov/cvm](http://www.fda.gov/cvm)  
1253  
1254 For consultation on issues related to human food:  
1255 U.S. Food and Drug Administration  
1256 Center for Food Safety and Applied Nutrition  
1257 HFS-013, 5100 Paint Branch Parkway  
1258 College Park, MD 20740-3835  
1259 Ph # (301) 436-1715; Fax # (301) 436-2637  
1260 [www.cfsan.fda.gov](http://www.cfsan.fda.gov)  
1261  
1262 For information regarding drugs for use in humans:  
1263 U.S. Food and Drug Administration  
1264 Center for Drug Evaluation and Research  
1265 5600 Fishers Lane  
1266 Rockville, MD 20857  
1267 Ph.# (301) 827-4573; Fax # (301) 827-3056  
1268 [www.fda.gov/cder](http://www.fda.gov/cder)  
1269  
1270 For information regarding medical devices:  
1271 U.S. Food and Drug Administration  
1272 Center for Devices and Radiological Health  
1273 Division of Small Manufacturers Assistance  
1274 1350 Piccard Drive  
1275 Rockville, MD 20850  
1276 Ph.# (301) 443-6597; Fax # (800) 638-2041  
1277 [www.fda.gov/cdrh](http://www.fda.gov/cdrh)