

**DEPARTMENT OF HEALTH AND HUMAN SERVICES****Food and Drug Administration**

[Docket No. 93D-0139]

**International Conference on Harmonisation; Final Guideline on Stability Testing of Biotechnological/Biological Products; Availability****AGENCY:** Food and Drug Administration, HHS.**ACTION:** Notice.

**SUMMARY:** The Food and Drug Administration (FDA) is publishing a final guideline entitled "Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products." The guideline was prepared under the auspices of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). The guideline is intended to provide guidance to applicants regarding the type of stability studies that should be provided in support of marketing applications for biotechnological/biological products.

**DATES:** Effective July 10, 1996. Submit written comments at any time.

**ADDRESSES:** Submit written comments on the guideline to the Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., rm. 1-23, Rockville, MD 20857. Copies of the guideline are available from the Division of Communications Management (HFD-210), Center for Drug Evaluation and Research, Food and Drug Administration, 7500 Standish Pl., Rockville, MD 20855, 301-594-1012. An electronic version of this guideline is also available via Internet by connecting to the CDER file transfer protocol (FTP) server (CDVS2.CDER.FDA.GOV).

**FOR FURTHER INFORMATION CONTACT:**

Regarding the guideline: Nga Y. Nguyen, Center for Biologics Evaluation and Research (HFM-18), Food and Drug Administration, 1401 Rockville Pike, Rockville, MD 20852, 301-402-4996.

Regarding ICH: Janet Jenkins-Showalter, Office of Health Affairs (HFY-1), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, 301-827-0865.

**SUPPLEMENTARY INFORMATION:** In recent years, many important initiatives have been undertaken by regulatory authorities and industry associations to promote international harmonization of

regulatory requirements. FDA has participated in many meetings designed to enhance harmonization and is committed to seeking scientifically based harmonized technical procedures for pharmaceutical development. One of the goals of harmonization is to identify and then reduce differences in technical requirements for drug development among regulatory agencies.

ICH was organized to provide an opportunity for tripartite harmonization initiatives to be developed with input from both regulatory and industry representatives. FDA also seeks input from consumer representatives and others. ICH is concerned with harmonization of technical requirements for the registration of pharmaceutical products among three regions: The European Union, Japan, and the United States. The six ICH sponsors are the European Commission, the European Federation of Pharmaceutical Industries Associations, the Japanese Ministry of Health and Welfare, the Japanese Pharmaceutical Manufacturers Association, the Centers for Drug Evaluation and Research and Biologics Evaluation and Research, FDA, and the Pharmaceutical Research and Manufacturers of America. The ICH Secretariat, which coordinates the preparation of documentation, is provided by the International Federation of Pharmaceutical Manufacturers Associations (IFPMA).

The ICH Steering Committee includes representatives from each of the ICH sponsors and the IFPMA, as well as observers from the World Health Organization, the Canadian Health Protection Branch, and the European Free Trade Area.

In the Federal Register of August 21, 1995 (60 FR 43501), FDA published a draft tripartite guideline entitled "Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products." The notice gave interested persons an opportunity to submit comments by October 5, 1995.

After consideration of the comments received and revisions to the guideline, a final draft of the guideline was submitted to the ICH Steering Committee and endorsed by the three participating regulatory agencies at the ICH meeting held on November 29, 1995.

The guideline is intended to supplement the tripartite ICH guideline entitled "Stability Testing of New Drug Substances and Products" published in the Federal Register of September 22, 1994 (59 FR 48754). Biotechnological/biological products have distinguishing characteristics to which consideration should be given in any well-defined

testing program designed to confirm their stability during the intended storage period. For such products, in which the active components are typically proteins and/or polypeptides, maintenance of molecular conformation and biological activity is dependent on noncovalent as well as covalent forces. The products are particularly sensitive to environmental factors such as temperature changes, oxidation, light, ionic content, shear, and so forth. In order to ensure maintenance of biological activity and to avoid degradation, stringent conditions for their storage are usually necessary. This guideline is intended to assist the applicant in developing appropriate supporting stability data for a biotechnological/biological product.

In the past, guidelines have generally been issued under § 10.90(b) (21 CFR 10.90(b)), which provides for the use of guidelines to state procedures or standards of general applicability that are not legal requirements but are acceptable to FDA. The agency is now in the process of revising § 10.90(b). Although this guideline does not create or confer any rights for or on any person, and does not operate to bind FDA, it does represent the agency's current thinking on stability testing of biotechnological/biological products.

As with all of FDA's guidelines, the public is encouraged to submit written comments with new data or other new information pertinent to this guideline. The comments in the docket will be periodically review, and, where appropriate, the guideline will be amended. The public will be notified of any such amendments through a notice in the Federal Register.

Interested persons may, at any time, submit written comments on the final guideline to the Dockets Management Branch (address above). Two copies of any comments are to be submitted, except that individuals may submit one copy. Comments are to be identified with the docket number found in brackets in the heading of this document. The guideline and received comments may be seen in the office above between 9 a.m. and 4 p.m., Monday through Friday.

The text of the guideline follows:

Quality of Biotechnological Products:  
Stability Testing of Biotechnological/  
Biological Products

1. Preamble

The guidance stated in the ICH harmonized tripartite guideline entitled "Stability Testing of New Drug Substances and Products" (issued by ICH on October 27, 1993) applies in general to biotechnological/biological products. However, biotechnological/

biological products have distinguishing characteristics to which consideration should be given in any well-defined testing program designed to confirm their stability during the intended storage period. For such products in which the active components are typically proteins and/or polypeptides, maintenance of molecular conformation and, hence, of biological activity, is dependent on noncovalent as well as covalent forces. The products are particularly sensitive to environmental factors such as temperature changes, oxidation, light, ionic content, and shear. To ensure maintenance of biological activity and to avoid degradation, stringent conditions for their storage are usually necessary.

The evaluation of stability may necessitate complex analytical methodologies. Assays for biological activity, where applicable, should be part of the pivotal stability studies. Appropriate physicochemical, biochemical, and immunochemical methods for the analysis of the molecular entity and the quantitative detection of degradation products should also be part of the stability program whenever purity and molecular characteristics of the product permit use of these methodologies.

With these concerns in mind, the applicant should develop the proper supporting stability data for a biotechnological/biological product and consider many external conditions that can affect the product's potency, purity, and quality. Primary data to support a requested storage period for either drug substance or drug product should be based on long-term, real-time, real-condition stability studies. Thus, the development of a proper long-term stability program becomes critical to the successful development of a commercial product. The purpose of this document is to give guidance to applicants regarding the type of stability studies that should be provided in support of marketing applications. It is understood that during the review and evaluation process, continuing updates of initial stability data may occur.

## 2. Scope of the Annex

The guidance stated in this annex to "Stability Testing of New Drug Substances and Products" applies to well-characterized proteins and polypeptides, their derivatives and products of which they are components, and which are isolated from tissues, body fluids, cell cultures, or produced using recombinant deoxyribonucleic acid (r-DNA) technology. Thus, the document covers the generation and submission of stability data for products such as cytokines (interferons, interleukins, colony-stimulating factors, tumor necrosis factors), erythropoietins, plasminogen activators, blood plasma factors, growth hormones and growth factors, insulins, monoclonal antibodies, and vaccines consisting of well-characterized proteins or polypeptides. In addition, the guidance outlined in the following sections may apply to other types of products, such as conventional vaccines, after consultation with the appropriate regulatory authorities. The document does not cover antibiotics, allergenic extracts, heparins, vitamins, whole blood, or cellular blood components.

## 3. Terminology

For the basic terms used in this annex, the reader is referred to the "Glossary" in "Stability Testing of New Drug Substances and Products." However, because manufacturers of biotechnological/biological products sometimes use traditional terminology, traditional terms are specified in parentheses to assist the reader. A supplemental glossary is also included that explains certain terms used in the production of biotechnological/biological products.

## 4. Selection of Batches

### 3.1 Drug Substance (Bulk Material)

Where bulk material is to be stored after manufacture, but before formulation and final manufacturing, stability data should be provided on at least three batches for which manufacture and storage are representative of the manufacturing scale of production. A minimum of 6 months stability data at the time of submission should be submitted in cases where storage periods greater than 6 months are requested. For drug substances with storage periods of less than 6 months, the minimum amount of stability data in the initial submission should be determined on a case-by-case basis. Data from pilot-plant scale batches of drug substance produced at a reduced scale of fermentation and purification may be provided at the time the dossier is submitted to the regulatory agencies with a commitment to place the first three manufacturing scale batches into the long-term stability program after approval.

The quality of the batches of drug substance placed into the stability program should be representative of the quality of the material used in preclinical and clinical studies and of the quality of the material to be made at manufacturing scale. In addition, the drug substance (bulk material) made at pilot-plant scale should be produced by a process and stored under conditions representative of that used for the manufacturing scale. The drug substance entered into the stability program should be stored in containers that properly represent the actual holding containers used during manufacture. Containers of reduced size may be acceptable for drug substance stability testing provided that they are constructed of the same material and use the same type of container/closure system that is intended to be used during manufacture.

### 3.2 Intermediates

During manufacture of biotechnological/biological products, the quality and control of certain intermediates may be critical to the production of the final product. In general, the manufacturer should identify intermediates and generate in-house data and process limits that assure their stability within the bounds of the developed process. Although the use of pilot-plant scale data is permissible, the manufacturer should establish the suitability of such data using the manufacturing scale process.

### 3.3 Drug Product (Final Container Product)

Stability information should be provided on at least three batches of final container product representative of that which will be used at manufacturing scale. Where possible,

batches of final container product included in stability testing should be derived from different batches of bulk material. A minimum of 6 months data at the time of submission should be submitted in cases where storage periods greater than 6 months are requested. For drug products with storage periods of less than 6 months, the minimum amount of stability data in the initial submission should be determined on a case-by-case basis. Product expiration dating should be based upon the actual data submitted in support of the application. Because dating is based upon the real-time/real-temperature data submitted for review, continuing updates of initial stability data should occur during the review and evaluation process. The quality of the final container product placed on stability studies should be representative of the quality of the material used in the preclinical and clinical studies. Data from pilot-plant scale batches of drug product may be provided at the time the dossier is submitted to the regulatory agencies with a commitment to place the first three manufacturing scale batches into the long-term stability program after approval. Where pilot-plant scale batches were submitted to establish the dating for a product and, in the event that the product produced at manufacturing scale does not meet those long-term stability specifications throughout the dating period or is not representative of the material used in preclinical and clinical studies, the applicant should notify the appropriate regulatory authorities to determine a suitable course of action.

## 4.4 Sample Selection

Where one product is distributed in batches differing in fill volume (e.g., 1 milliliter (mL), 2 mL, or 10 mL), unitage (e.g., 10 units, 20 units, or 50 units), or mass (e.g., 1 milligram (mg), 2 mg, or 5 mg), samples to be entered into the stability program may be selected on the basis of a matrix system and/or by bracketing.

Matrixing, i.e., the statistical design of a stability study in which different fractions of samples are tested at different sampling points, should only be applied when appropriate documentation is provided that confirms that the stability of the samples tested represents the stability of all samples. The differences in the samples for the same drug product should be identified as, for example, covering different batches, different strengths, different sizes of the same closure, and, possibly, in some cases, different container/closure systems. Matrixing should not be applied to samples with differences that may affect stability, such as different strengths and different containers/closures, where it cannot be confirmed that the products respond similarly under storage conditions.

Where the same strength and exact container/closure system is used for three or more fill contents, the manufacturer may elect to place only the smallest and largest container size into the stability program, i.e., bracketing. The design of a protocol that incorporates bracketing assumes that the stability of the intermediate condition samples are represented by those at the

extremes. In certain cases, data may be needed to demonstrate that all samples are properly represented by data collected for the extremes.

#### 5. Stability-Indicating Profile

On the whole, there is no single stability-indicating assay or parameter that profiles the stability characteristics of a biotechnological/biological product. Consequently, the manufacturer should propose a stability-indicating profile that provides assurance that changes in the identity, purity, and potency of the product will be detected.

At the time of submission, applicants should have validated the methods that comprise the stability-indicating profile, and the data should be available for review. The determination of which tests should be included will be product-specific. The items emphasized in the following subsections are not intended to be all-inclusive, but represent product characteristics that should typically be documented to demonstrate product stability adequately.

##### 5.1 Protocol

The dossier accompanying the application for marketing authorization should include a detailed protocol for the assessment of the stability of both drug substance and drug product in support of the proposed storage conditions and expiration dating periods. The protocol should include all necessary information that demonstrates the stability of the biotechnological/biological product throughout the proposed expiration dating period including, for example, well-defined specifications and test intervals. The statistical methods that should be used are described in the tripartite guideline on stability.

##### 5.2 Potency

When the intended use of a product is linked to a definable and measurable biological activity, testing for potency should be part of the stability studies. For the purpose of stability testing of the products described in this guideline, potency is the specific ability or capacity of a product to achieve its intended effect. It is based on the measurement of some attribute of the product and is determined by a suitable *in vivo* or *in vitro* quantitative method. In general, potencies of biotechnological/biological products tested by different laboratories can be compared in a meaningful way only if expressed in relation to that of an appropriate reference material. For that purpose, a reference material calibrated directly or indirectly against the corresponding national or international reference material should be included in the assay.

Potency studies should be performed at appropriate intervals as defined in the stability protocol and the results should be reported in units of biological activity calibrated, whenever possible, against nationally or internationally recognized standards. Where no national or international reference standards exist, the assay results may be reported in *in-house* derived units using a characterized reference material.

In some biotechnological/biological products, potency is dependent upon the

conjugation of the active ingredient(s) to a second moiety or binding to an adjuvant. Dissociation of the active ingredient(s) from the carrier used in conjugates or adjuvants should be examined in real-time/real-temperature studies (including conditions encountered during shipment). The assessment of the stability of such products may be difficult because, in some cases, *in vitro* tests for biological activity and physicochemical characterization are impractical or provide inaccurate results. Appropriate strategies (e.g., testing the product before conjugation/binding, assessing the release of the active compound from the second moiety, *in vivo* assays) or the use of an appropriate surrogate test should be considered to overcome the inadequacies of *in vitro* testing.

##### 5.3 Purity and Molecular Characterization

For the purpose of stability testing of the products described in this guideline, purity is a relative term. Because of the effect of glycosylation, deamidation, or other heterogeneities, the absolute purity of a biotechnological/biological product is extremely difficult to determine. Thus, the purity of a biotechnological/biological product should be typically assessed by more than one method and the purity value derived is method-dependent. For the purpose of stability testing, tests for purity should focus on methods for determination of degradation products.

The degree of purity, as well as the individual and total amounts of degradation products of the biotechnological/biological product entered into the stability studies, should be reported and documented whenever possible. Limits of acceptable degradation should be derived from the analytical profiles of batches of the drug substance and drug product used in the preclinical and clinical studies.

The use of relevant physicochemical, biochemical, and immunochemical analytical methodologies should permit a comprehensive characterization of the drug substance and/or drug product (e.g., molecular size, charge, hydrophobicity) and the accurate detection of degradation changes that may result from deamidation, oxidation, sulfoxidation, aggregation, or fragmentation during storage. As examples, methods that may contribute to this include electrophoresis (SDS-PAGE, immunoelectrophoresis, Western blot, isoelectrofocusing), high-resolution chromatography (e.g., reversed-phase chromatography, gel filtration, ion exchange, affinity chromatography), and peptide mapping.

Wherever significant qualitative or quantitative changes indicative of degradation product formation are detected during long-term, accelerated, and/or stress stability studies, consideration should be given to potential hazards and to the need for characterization and quantification of degradation products within the long-term stability program. Acceptable limits should be proposed and justified, taking into account the levels observed in material used in preclinical and clinical studies.

For substances that cannot be properly characterized or products for which an exact

analysis of the purity cannot be determined through routine analytical methods, the applicant should propose and justify alternative testing procedures.

##### 5.4 Other Product Characteristics

The following product characteristics, though not specifically relating to biotechnological/biological products, should be monitored and reported for the drug product in its final container:

Visual appearance of the product (color and opacity for solutions/suspensions; color, texture, and dissolution time for powders), visible particulates in solutions or after the reconstitution of powders or lyophilized cakes, pH, and moisture level of powders and lyophilized products.

Sterility testing or alternatives (e.g., container/closure integrity testing) should be performed at a minimum initially and at the end of the proposed shelf life.

Additives (e.g., stabilizers, preservatives) or excipients may degrade during the dating period of the drug product. If there is any indication during preliminary stability studies that reaction or degradation of such materials adversely affect the quality of the drug product, these items may need to be monitored during the stability program.

The container/closure has the potential to affect the product adversely and should be carefully evaluated (see below).

#### 6. Storage Conditions

##### 6.1 Temperature

Because most finished biotechnological/biological products need precisely defined storage temperatures, the storage conditions for the real-time/real-temperature stability studies may be confined to the proposed storage temperature.

##### 6.2 Humidity

Biotechnological/biological products are generally distributed in containers protecting them against humidity. Therefore, where it can be demonstrated that the proposed containers (and conditions of storage) afford sufficient protection against high and low humidity, stability tests at different relative humidities can usually be omitted. Where humidity-protecting containers are not used, appropriate stability data should be provided.

##### 6.3 Accelerated and Stress Conditions

As previously noted, the expiration dating should be based on real-time/real-temperature data. However, it is strongly suggested that studies be conducted on the drug substance and drug product under accelerated and stress conditions. Studies under accelerated conditions may provide useful support data for establishing the expiration date, provide product stability information or future product development (e.g., preliminary assessment of proposed manufacturing changes such as change in formulation, scale-up), assist in validation of analytical methods for the stability program, or generate information that may help elucidate the degradation profile of the drug substance or drug product. Studies under stress conditions may be useful in determining whether accidental exposures to

conditions other than those proposed (e.g., during transportation) are deleterious to the product and also for evaluating which specific test parameters may be the best indicators of product stability. Studies of the exposure of the drug substance or drug product to extreme conditions may help to reveal patterns of degradation; if so, such changes should be monitored under proposed storage conditions. Although the tripartite guideline on stability describes the conditions of the accelerated and stress study, the applicant should note that those conditions may not be appropriate for biotechnological/biological products. Conditions should be carefully selected on a case-by-case basis.

#### 6.4 Light

Applicants should consult the appropriate regulatory authorities on a case-by-case basis to determine guidance for testing.

#### 6.5 Container/Closure

Changes in the quality of the product may occur due to the interactions between the formulated biotechnological/biological product and container/closure. Where the lack of interactions cannot be excluded in liquid products (other than sealed ampules), stability studies should include samples maintained in the inverted or horizontal position (i.e., in contact with the closure), as well as in the upright position, to determine the effects of the closure on product quality. Data should be supplied for all different container/closure combinations that will be marketed.

In addition to the standard data necessary for a conventional single-use vial, the applicant should demonstrate that the closure used with a multiple-dose vial is capable of withstanding the conditions of repeated insertions and withdrawals so that the product retains its full potency, purity, and quality for the maximum period specified in the instructions-for-use on containers, packages, and/or package inserts. Such labeling should be in accordance with relevant national/regional requirements.

#### 6.6 Stability after Reconstitution of Freeze-Dried Product

The stability of freeze-dried products after their reconstitution should be demonstrated for the conditions and the maximum storage period specified on containers, packages, and/or package inserts. Such labeling should be in accordance with relevant national/regional requirements.

#### 7. Testing Frequency

The shelf lives of biotechnological/biological products may vary from days to several years. Thus, it is difficult to draft uniform guidelines regarding the stability study duration and testing frequency that would be applicable to all types of biotechnological/biological products. With only a few exceptions, however, the shelf

lives for existing products and potential future products will be within the range of 0.5 to 5 years. Therefore, the guidance is based upon expected shelf lives in that range. This takes into account the fact that degradation of biotechnological/biological products may not be governed by the same factors during different intervals of a long storage period.

When shelf lives of 1 year or less are proposed, the real-time stability studies should be conducted monthly for the first 3 months and at 3 month intervals thereafter.

For products with proposed shelf lives of greater than 1 year, the studies should be conducted every 3 months during the first year of storage, every 6 months during the second year, and annually thereafter.

While the testing intervals listed above may be appropriate in the preapproval or prelicense stage, reduced testing may be appropriate after approval or licensure where data are available that demonstrate adequate stability. Where data exist that indicate the stability of a product is not compromised, the applicant is encouraged to submit a protocol that supports elimination of specific test intervals (e.g., 9-month testing) for postapproval/postlicensure, long-term studies.

#### 8. Specifications

Although biotechnological/biological products may be subject to significant losses of activity, physicochemical changes, or degradation during storage, international and national regulations have provided little guidance with respect to distinct release and end of shelf life specifications. Recommendations for maximum acceptable losses of activity, limits for physicochemical changes, or degradation during the proposed shelf life have not been developed for individual types or groups of biotechnological/biological products but are considered on a case-by-case basis. Each product should retain its specifications within established limits for safety, purity, and potency throughout its proposed shelf life. These specifications and limits should be derived from all available information using the appropriate statistical methods. The use of different specifications for release and expiration should be supported by sufficient data to demonstrate that the clinical performance is not affected, as discussed in the tripartite guideline on stability.

#### 9. Labeling

For most biotechnological/biological drug substances and drug products, precisely defined storage temperatures are recommended. Specific recommendations should be stated, particularly for drug substances and drug products that cannot tolerate freezing. These conditions, and where appropriate, recommendations for protection against light and/or humidity, should appear on containers, packages, and/

or package inserts. Such labeling should be in accordance with relevant national and regional requirements.

#### 10. Glossary

##### *Conjugated Product*

A conjugated product is made up of an active ingredient (e.g., peptide, carbohydrate) bound covalently or noncovalently to a carrier (e.g., protein, peptide, inorganic mineral) with the objective of improving the efficacy or stability of the product.

##### *Degradation Product*

A molecule resulting from a change in the drug substance (bulk material) brought about over time. For the purpose of stability testing of the products described in this guideline, such changes could occur as a result of processing or storage (e.g., by deamidation, oxidation, aggregation, proteolysis). For biotechnological/biological products, some degradation products may be active.

##### *Impurity*

Any component of the drug substance (bulk material) or drug product (final container product) that is not the chemical entity defined as the drug substance, an excipient, or other additives to the drug product.

##### *Intermediate*

For biotechnological/biological products, a material produced during a manufacturing process that is not the drug substance or the drug product but for which manufacture is critical to the successful production of the drug substance or the drug product. Generally, an intermediate will be quantifiable and specifications will be established to determine the successful completion of the manufacturing step before continuation of the manufacturing process. This includes material that may undergo further molecular modification or be held for an extended period before further processing.

##### *Manufacturing Scale Production*

Manufacture at the scale typically encountered in a facility intended for product production for marketing.

##### *Pilot-Plant Scale*

The production of the drug substance or drug product by a procedure fully representative of and simulating that to be applied at manufacturing scale. The methods of cell expansion, harvest, and product purification should be identical except for the scale of production.

Dated: July 1, 1996.  
William K. Hubbard,  
Associate Commissioner for Policy  
Coordination.  
[FR Doc. 96-17471 Filed 7-9-96; 8:45 am]  
BILLING CODE 4160-01-F