

Summary of Planned Revisions to *Design and Analysis of Biological Assays* <111>

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ABSTRACT An ad hoc Advisory Panel to the USP Statistics Expert Committee has been charged with revising *USP* General Chapter *Design and Analysis of Biological Assays* <111>. This revision is nearing readiness for publication in *Pharmacopeial Forum* as an In-Process Revision that constitutes a complete rewrite of this Chapter and is substantially different in style and content from the current <111>. The Advisory Panel anticipates that these sweeping changes may have both foreseen and unforeseen consequences for industry. For example, companies that have relied on <111> may need to review and revise SOPs that are related to this Chapter. The purpose of the current *Stimuli* article is to describe the substantive differences between the revision and the current Chapter. The revisions are summarized in *Table 1*, and most are also described below. Stakeholders are invited to comment on these changes.

CHANGES RELATED TO GENERAL STRUCTURE

<111Rev>[†] is envisioned as less prescriptive than <111>. The revision focuses on concepts and considerations that influence the design and analysis of bioassays rather than on formulas. The Chapter's authors assume that bioassay practitioners have access to sound and validated computer software that reliably performs requisite calculations. The revised chapter will include several worked examples that illustrate particular methods of analysis but will not prescribe specific instructions about how to calculate potency or confidence intervals for specific drug substances or products.

The changes in general approach and style in <111Rev> are sufficiently large that the Advisory Panel has recommended to the Statistics Expert Committee that the chapter be renumbered above 1000. As specified in the *USP General Notices*, articles in *USP* must comply with General Chapters numbered below 1000. In contrast, General Chapters numbered above 1000 are considered interpretive and are intended to provide information about or descriptive of a particular subject. Chapters numbered above 1000 contain no official requirements applicable to any pharmacopeial article unless specifically referenced in a monograph or elsewhere in the pharmacopeia or otherwise required by law or regulation.

CHANGES INTERNAL TO *USP-NF*

General Chapter <111> is linked to *USP* monographs or other General Chapters in two ways: either a monograph or General Chapter contains reference to <111> for methods of analysis, or <111> refers directly to a drug monograph or General Chapter. The presence of either of these links for a particular drug, product, or Chapter does not imply the presence or absence of a link in the other direction.

Currently, nine monographs and seven General Chapters contain references to <111> and would need to be revised by *USP* to correspond to <111Rev>. These are: chorionic gonadotropin; corticotropin; digitalis; glucagons; heparin sodium; iron dextran injection; menotropins; sincalide for injection; oil- and water-soluble vitamins with mineral tablets; *Antibiotics—Microbial Assays* <81>; *Calcium Pantothenate Assay* <91>; *Insulin Assays* <121>; *Vitamin B12 Activity Assay* <171>; *Vitamin D Assay* <581>; *Analytical Data—Interpretation and Treatment* <1010>; and *Biotechnology-Derived Articles* <1045>.

General Chapter <111> contains specific information for a total of 12 Monographs and General Chapters. General Chapter <111Rev> will not contain monograph-specific methods or methods specific to other General Chapters. Specific methods of analysis for *USP* Monographs and General Chapters that are present in <111> will be moved into the Monographs and General Chapters.

SPECIFIC CHANGES

The revised General Chapter will focus on the analysis of bioassays. Design considerations will be moved to a new chapter tentatively titled *Design and Development of Biological Assays* <1032>. Additionally, bioassay validation issues will be addressed in Chapter <1033>, tentatively titled *Validation of Biological Assays*.

General Chapter <111Rev> will not contain procedures for outliers. Instead, it will discuss approaches to outlier identification and rejection and will refer to <1010> for statistical methods to determine whether a data set contains outliers.

The section *Combination of Independent Assays* will stress that the preferred method is to assume that the relative potencies differ by assay and that they will be averaged unweighted to determine potency and the confidence interval. This differs from the current method of <111> that assumes homogeneity of relative potencies and weights the individual assays according to variance. The method that assumes homogeneity will be included with a description of the required justification.

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† Note: In this *Stimuli* article <111> denotes the current official chapter in *USP*, and <111Rev> denotes the planned draft revision.

In <111Rev> testing for parallelism and linearity will be based on equivalence testing approaches. The current method is to test the null hypothesis of no difference—i.e., detecting no significant difference in slopes leads to the conclusion of parallelism. The current approach will no longer be acceptable for the determination of similarity in <111Rev>. The procedure in the current version of <111> often fails bioassays that have very little variance and minor differences in slope and passes assays that have large amounts of variation and considerable differences in slope. This proposed change has been presented at a number of conferences and in the literature (*1*, and references therein).

General Chapter <111> was written more than 50 years ago, before the widespread availability of computer-assisted computation. General Chapter <111Rev> will contain more modern statistical methods, such as nonlinear mixed effects models, and will describe their application as effective analytical alter-

natives. Also, <111Rev> includes the four- and five-parameter logistic (nonlinear) models in addition to the linear models in <111>. Given that most bioassay analysis currently is carried out by computers and dedicated software, <111Rev> will contain data sets that may help validate software. Analysis of these data sets will give some indication that the software is performing correctly in the determination of similarity, potency, and confidence intervals.

CONCLUSION

Table 1 provides a summary of some important changes between <111> and <111Rev>. USP and the ad hoc Advisory Panel believe that the proposed changes will improve the General Chapter and make it more suitable for modern bioassay applications. As always, USP welcomes public comments on the proposed changes.

Topic	<111>	<111Rev>
Focus	Algebraic manipulation of data	Concepts and considerations
Placement in <i>USP–NF</i>	Less than 1000	Greater than 1000
Links to monographs and General Chapters	Included	Not included
Bioassay design	Some included	Moved to new <1032>
Similarity	Hypothesis test for linear case only	Equivalence test for both linear and nonlinear case
Standard curve fit	Linear only	Linear and nonlinear (4- and 5-parameter logistic)
Computations	No examples; formulas set up for hand calculations	Fewer formulas; numerous examples stressing use of validated computer software
Outliers	Discussed in chapter with methods	Discussed in chapter, but reference to <1010> for methods
Data imputation	Discussed as viable means to handle missing or unbalanced data	Not discussed; stress use of modern statistical methods (e.g., nonlinear mixed effects models) instead
Combinations of independent assays	Only method provided assumes homogeneous relative potencies	Two methods given: preferred method assumes relative potencies differ by assay
Validation data sets	Not included	Many of the data sets used as examples will be made available on the USP Web site for use in checking software determination of similarity, potency, and the confidence intervals

REFERENCES

1. Hauck WW, et al. Assessing parallelism prior to determining relative potency. *PDA J Pharm Sci Tech.* 2005;59(2):127–137.