IPAC-RS Comments on Pharmacopoeial Forum *Chapter ‹87› Biological Reactivity Tests, In Vitro*

General Comments

1. The method for interpretation of results remains a subjective graded assessment of "reactivity". Has consideration been given for an evaluation of high throughput public available assays which are being used as part of TOX21 program (https://tripod.nih.gov/tox/assays) these list at least six method assays for cytotoxicity …
2. With inclusion of methods for genotoxicity, it is unclear how the validity of the output can be shown since both a positive or a negative result may be a reflection of the sensitivity of the assay to a given genotoxic substance. Genotoxicity measurements in general are applied to single component substances of known concentration, rather than in this format where there is a complex mixture with many substances of varying concentrations (See Rainer, B. et al. Direct comparison of the lowest effect concentrations of mutagenic reference substances in two Ames test formats. *Toxics* 9, 1–17 (2021).)
3. Would this chapter, once finalized, be retroactive? What about for drug products already approved and marketed in US?
4. Will USP<661.1> & <661.2> be updated and changed accordingly (before the application date planned in December 2025)?
5. Please ensure that the USP<87> is aligned with equivalent parts described in ISO10993?

Specific Comments:

Page, Line or Section	Original Language	Proposed Changed Language	Justification of Proposed Change
1.0 Scope	This chapter describes several in vitro biological reactivity tests designed to evaluate biocompatibility, including tests for cytotoxicity and genotoxicity.	This chapter describes a number of in vitro biological reactivity tests designed to evaluate biocompatibility with respect to cytotoxicity and genotoxicity	Tests included cover only cytotoxicity and genotoxicity
2.1 Preparation of extracts Extraction	Sodium Chloride Injection containing 0.9% of sodium chloride (0.9% saline), serum-free mammalian cell culture medium, or serum-	The following extraction system may be used: • Serum-Supplemented Mammalian Cell Culture	Improve readability as a list. Given in rough order of preference. There seems to be an expressed preference for extraction in cell culture. Perhaps more

Solvents	supplemented mammalian cell culture medium may be used. Serum-supplemented mammalian cell culture medium is used when extraction is done at 37 ± 1°. Different types of serum (e.g., fetal, bovine/calf serum, newborn calf serum) may be used; the choice of the serum is dependent on the cell type. Culture medium with serum is preferred for extraction because of its ability to support cellular growth and well as extract both polar and nonpolar substances. Other extraction solvents include Purified Water and dimethyl sulfoxide (DMSO). It is important to recognize that serum/proteins are known to bind extractables. The extraction solvent and extraction conditions (e.g., temperature or time) should be selected so as to not impact the physical state of the sample such as dissolution, fusion, or melting of the material pieces. A slight adherence of the pieces may occur and can be tolerated. [Note—DMSO is cytotoxic in selected assay systems at greater than 0.5% (volume fraction). The cellular exposure concentration of extractables in DMSO will be lower due to the greater dilution as	Medium • Sodium Chloride Injection containing 0.9% of sodium chloride (0.9% saline), • Purified Water • 0.5 % DMSO in Water It should be noted Serum-supplemented mammalian cell culture medium is used when extraction is done at 37 ± 1°. Different types of serum (e.g., fetal, bovine/calf serum, newborn calf serum) may be used; the choice of the serum is dependent on the cell type. Culture medium with serum is preferred for extraction because of its ability to support cellular growth and well as extract both polar and nonpolar substances It is important to recognize that serum/proteins are known to bind extractables. The extraction solvent and extraction conditions (e.g., temperature or time) should be selected so as to not impact the physical state of the sample such as dissolution, fusion, or melting of the material pieces. A slight adherence of the pieces may occur and can be tolerated. Additionally, DMSO is cytotoxic in selected assay systems at greater than 0.5% (volume	language should be added to support the preference and other extraction systems only used if cell culture is determined to be unsuitable (with justification)

	compared with extraction in culture medium with serum.]	fraction). The cellular exposure concentration of extractables in DMSO will be lower due to the greater dilution as compared with extraction in culture medium with serum.	
Page 4, 2.1 Preparation of extracts Extraction Procedure	Whole Section "Extraction Procedure"	Move Extraction Procedure to Become Section 2.2 Extraction Procedure Place a properly prepared sample to be tested in an extraction container and add 20 mL of the selected extraction medium If other volumes of extraction media are used, a proportional amount of sample should be used so that the surface area to volume or weight to volume ratio does not change. Repeat these directions for each extraction medium required for testing. Also, prepare one 20-mL blank of each respective extraction medium for concurrent extraction, testing, and comparison with samples. Extractions in serum-supplemented mammalian cell culture medium should be	Extraction Procedure is separation from preparation of Materials and Apparatus. It also avoids confusion with Autoclave setup description Creation of a list of extraction conditions improves readability considerably

		performed at: • 37 ± 1° for 24 ± 2 h and may be prolonged to 72 ± 2 h Other extraction solvents: • In an incubator at 37 ± 1° for 24 ± 2 h, or • In an oven at 50 ± 2° for 72 ± 2 h, or • In an oven at 70 ± 2° for 24 ± 2 h, or In an autoclave at 121 ± 2° for 1 ± 0.2 h	Under what circumstances would the extraction be increased to 72 hrs?
2.2 Test Controls		Positive control: A material known to give a positive cytotoxicity result when tested in the conditions outlined in this standard Negative control: A material known to give a negative cytotoxicity result when tested in the conditions outlined in this standard	Current standard is very limiting in its suggested test material. Consider allowing materials which have a demonstrated cytotoxicity result (positive or negative) as alternatives
2.3 Test Selection		Suggest moving Table 2 (Cytotoxicity Test Selection) from USP 1031 into standard Would also suggest adding an order of preference while	Adds clarity to selection of appropriate test. Could consider addition of Fluorometric assays in addition to NRU or others?

		advantages and disadvantages are useful there is surely a consensus on "best method" for a given system. It would seem Neutral Red uptake is typically best choice in most situations, so perhaps should be recommended by default unless justification made for alternative	
3.1 Extraction solvents	….Both polar and non-polar solvents should be used…		It is unclear why there is a suggestion to use both polar and non-polar solvents, since the extraction media listed in Section 2 would suggest only polar extractions are feasible.