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SINGLE-USE TECHNOLOGIES
EXPERT COMMITTEE

Navigating USP <665> and other extractables and leachables standards: Industry strategies for single-use risk assessment and implementation

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Contents

List of figures

List of tables

About BioPhorum

We enable the global biopharmaceutical industry to connect, collaborate and accelerate progress for the benefit of all.

Since its inception in 2004, BioPhorum has become the open and trusted environment where senior leaders of the biopharmaceutical industry come together to openly share and discuss the emerging trends and challenges facing their industry.

Growing from an end-user group in 2008, BioPhorum's membership now comprises top leaders and subject matter experts from global biopharmaceutical manufacturers and suppliers, working in both long-established and new Phorums. They articulate the industry's technology roadmap, define the supply partner practices of the future, and develop and adopt best practices in drug substance, fill finish, process development and manufacturing IT.

In each of these Phorums, BioPhorum facilitators bring leaders together to create future visions, mobilize teams of experts on the opportunities, create partnerships that enable change and provide the quickest route to implementation, so that the industry shares, learns and builds the best solutions together.

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Executive summary

Single-use technologies have greatly enabled innovation in traditional bioprocessing and novel modalities. Their success and growing prominence have brought increased attention to industry-aligned risk assessment strategies regarding how these technologies may affect pharmaceutical manufacturing processes and, ultimately, patient safety. With the growth of single-use technologies, historical standards originally developed for other applications, such as medical devices or container closures, have been adopted to support safety assessments. Over time, these standards have evolved or been replaced by new and, in some cases, multiple standards and best practices more directly applicable to single-use systems (SUS).

The tandem of USP <665> and <1665>, which highlight extractables requirements and implementation guidance for single-use components (SUCs), is one example. As USP <665> will become official on 1 May 2026, there is a need for clarity on how this chapter, together with existing standards and best practice guidance, should be managed. In particular, stakeholders require a clear understanding of:

- The standards needed to support risk assessment of SUCs
- How to ensure the United States Pharmacopeia (USP) standards requirements are met
- Additional supplier information required to support assessment of single-use assemblies
- Examples of how end-users may apply the data to risk assessment models and a cumulative assessment[1] of process equipment related leachables (PERLs)
- Opportunities to enhance alignment with health authority expectations and mitigate unnecessary or low-value testing.

In addition to addressing the above points, this paper also explores:

- How extractables data can be leveraged to support evolving treatment modalities
- Concepts to streamline and strengthen the SUC extractables and leachables (E&L) risk assessment ecosystem.

Throughout this review it is noted that, at the time of publication, there are to our knowledge, no peer reviewed published reports in the available public literature, of leachables from SUCs having been shown to impact patient safety. Early high-profile patient-impact case studies involving leachables from prefilled syringes resulting from a drug formulation change[1] and not considered SUCs, date back more than 25 years, with no similar cases reported since. Based on end-user reviews, most leachables found in final product are associated with final filtration and filling steps, and appear to represent a small segment of the SUC materials landscape. Increased awareness of the potential impact of leachables from manufacturing components on drug manufacturing processes, bioreactor productivity and critical quality attributes, such as aggregation or efficacy, has increased expectations for meaningful component characterization data. The current focus is now on standardization to support a more efficient and consistent risk assessment process. Lastly, it is recognized that implementing new standards in areas where existing practices already exist may require improved communication to support understanding and enable appropriate flexibility that should align with standardization goals while ensuring that leachable risks are addressed efficiently and on a sound scientific basis.

1.0

Extractables standards and guidance

Starting points for safety assessments of materials employed in single-use bioprocessing applications typically start with information provided by the supplier. References to SUCs[2] encompass the scope of fluid-contact polymeric materials from vial thaw for bioreactor seed-train through the final filling step, but do not generally include the final container closure system or containers used for long-term storage.

Generally, these safety assessments take into account:

- Materials and sourcing controls
- Biological reactivity assessments
- Chemical assessments.

The regulatory landscape for extractable testing of SUCs has progressed significantly and there is now a variety of historical and new testing approaches (see Table 1). As a result, there is a clear need for industry-aligned consensus on the expected types and levels of supporting test data, as well as expectations for how this data should be used. Until 2014, end-users and SUC suppliers often relied on in-house developed extractable testing methods, USP <661>, USP <381> and other monographs to characterize SUC materials. After publication of the initial BioPhorum extractables protocol in 2014, several iterations of USP <665> were circulated for public comment before the final version was published in 2020, around the same time as an updated version 2.0 of the BioPhorum protocol[12]. Both protocols helped drive strong alignment for the types of data that enable end-users to perform risk assessment and qualify SUCs in moderate and high-risk bioprocessing applications.

Specific definitions for extractables*, PERLS** and leachables*** as used in bioprocessing have been well-defined per USP <1665>. Detailed descriptions of standards and guidances relevant to how extractables, PERLs and leachables are assessed in bioprocessing are summarized in Table 1. In many cases, these standards were originally drafted for primary packaging systems, and in the absence of more directly relevant guidance adopted for SUC.

* Extractable: An organic or inorganic chemical entity that is released from a manufacturing component or system into an extraction solution under laboratory conditions. Depending on the specific purpose of the extraction study, these laboratory conditions (e.g. solvent, temperature, stoichiometry and others) may accelerate or exaggerate the normal conditions of use for a manufacturing component or system.

** Process equipment-related leachable (PERL): A foreign organic or inorganic chemical entity that is present in a process stream because it has leached from a component used in the manufacturing system. A PERL may be present in a manufactured biopharmaceutical drug substance (DS) or pharmaceutical or biopharmaceutical drug product (DP) as a leachable if it persists through the entire manufacturing process.

*** Leachable: A foreign organic or inorganic chemical entity that is present in a manufactured biopharmaceutical DS, or pharmaceutical or biopharmaceutical DP, because it has leached from a component used in the manufacturing system and has persisted through the entire manufacturing process.

Table 1: Overview of standards and guidance impacting the bioprocessing extractables and leachables landscape

Standard or guidance	Description and application	Relevance to SUCs
USP <661>	**Plastic Packaging Systems* and their Materials of Construction.** Although intended for plastic drug containers, this was also used historically for characterization of some single-use components. Moving forward, this chapter is specific to containers and packaging systems. *(Updated revision to be official 01 DEC 2025)*	N/A[4] (historical use)
USP <661.1>	**Plastic Materials of Construction.** Establishes testing approach to characterize plastics to be used in final drug product packaging systems. Although it may be used to support characterization or selection of polymers, it is specific to packaging systems and not to single-use components. *(Updated revision to be official 01 DEC 2025)*	N/A**
USP <661.2>	**Plastic Packaging Systems* for Pharmaceutical Use.** *(To be official 01 DEC 2025)*	N/A
USP <662>	**Metallic Packaging Systems* and their Materials and Components.** Applicable to packaging systems such as aerosols, blister packs, canisters, etc. but not generally applicable to SUCs. *(Last draft published in USP PF 50[4], not official)*	N/A
USP <665>	**Plastic Components and Systems Used to Manufacture Pharmaceutical Drug Products and Biopharmaceutical Drug Substances and Products.** Applicable to fluid-contact, plastic bioprocess components used to manufacture biopharmaceutical drug substances and drug products. Manufacturing components used in production of active pharmaceutical ingredients (APIs) are out of scope for traditional small molecule drug substances, and evaluated on a case-by case basis for APIs used as precursors.	Yes
USP <232>, ICH Q3D	**Elemental Impurities – Limits.** Specifies limits for the amounts of elemental impurities in drug products and may be relevant for assessment in drug substances and excipients.	Informational guidance
USP <381>	**Elastomeric Components in Injectable Pharmaceutical Product Packaging*/Delivery Systems.** Although not a requirement for single-use elastomeric components, it is sometimes used for single-use elastomers in the absence of other directly applicable standards. *(Updated revision to be official 01 DEC 2026)*	Protocol can be used to support assessment
USP <383>	**Cured Silicone Elastomers for Pharmaceutical Packaging and Manufacturing Components.** Specifically addresses silicone manufacturing components and is generally aligned with EP 3.1.9. Per USP General Notices 6.30, alternative and harmonized methods and procedures are permitted, and as many single-use plastic bioprocess components also contain silicone materials of construction, USP <665> may also be relevant. *(To be official 01 DEC 2027)*	Protocol can be used to support assessment
USP <467>, ICH Q3C	**Residual Solvents.** Defines acceptable amounts of residual solvents in pharmaceutical drug products.	Informational reference values
USP <87> and <88>	**Biological Reactivity Tests, in vitro (<87>[5]) and in vivo (<88>[6]).** Historically used as a baseline requirement for materials in bioprocessing. Forthcoming changes strengthen expectations for *in vitro* (<87>) testing, while sunsetting most *in vivo* (<88>) requirements for SUCs.	USP <87>[5] may continue to serve as baseline materials assessment
ISO 10993-18	**Biological evaluation of medical devices.** Part 18: Chemical characterization of medical device materials within a risk management process. Although specific to medical devices, and not bioprocess components, this standard has been used for cross-over applications, including with cell therapies. The standard does not specify prescriptive solvents for materials but does provide guidance on solvent selection for different applications.	This ISO standard does not mandate any specific industry-standard extraction conditions and hence does not support an industry-aligned approach for SUCs
ICH Q3E	Pending globally harmonized guideline for extractables and leachables. *(Step 2)*	N/A (draft)
ICH M7[11]	**Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk – Scientific guideline.** Provides guidance for classifying impurities into five risk classes and setting acceptable exposure limits taking into consideration the intended conditions of human use.	Informational guidance
Ph. Eur. 3.1	**Materials used for the manufacture of containers.** Establishes requirements for materials used in final container materials. Compliance with these materials standards may facilitate risk assessment of many materials (includes sub-sections).	Protocol can be used to support assessment

* Packaging systems contain or are intended to contain a drug product and provide a means for storing and distributing drug products[4]. This is generally different from components of single-use bioprocess assemblies.

** USP <661> and <661.1> are specific to packaging requirements for drug product and are beyond the scope of most SUCs. Historical <661> pass/fail test requirements for plastic containers have been revised with material-specific testing requirements specifically for final drug product containers. During the revision process, the new USP <665> chapter was created to focus specifically on plastic manufacturing components.

Table 1: Overview of standards and guidance impacting the bioprocessing extractables and leachables landscape (continued)

Standard or guidance	Description and application	Title
BioPhorum protocol v2	**Disposables: Extractables testing of polymeric single-use components used in biopharmaceutical manufacturing (2020)[12].** Provides extractables testing recommendations (four solvents, two time points) for various SUCs to facilitate end-user risk assessment. Largely overlaps the time points and solvents required per USP <665>, with the exception that the high-pH solvent (0.5N NaOH; pH ~13.5) is more extreme and may not satisfy or may require justification for the high-pH requirement of USP <665> (pH 10).	Yes
<1663>, <1665>, PQRI	<1663> **Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems.** <1665> **Characterization and Qualification of Plastics Components and Systems Used to Manufacture Pharmaceutical Drug Products and Biopharmaceutical Drug Substances and Products.** Guidance chapters related to risk assessment and testing per USP <665>, or design of studies to bracket a specific drug manufacturing product and process, typically referred to as simulation studies (see <1663>, Product Quality Research Institute (PQRI)).	Informational guidance
<1664> and BioPhorum leachables best practice	<1664> **Assessment of drug product leachables associated with pharmaceutical packaging/delivery systems (includes additional sub-chapters).** BioPhorum Leachables: Best practices guide for evaluating leachables risk from polymeric single-use systems.	Informational guidance
NMPA (China) Annex 2 (2020)	**Technical Guidelines for Compatibility Studies of Plastic Component Systems Used in the Production of Chemical Injections (Trial Implementation).** Annex 2 includes extractables testing guidance for plastic components aligned with a 2019 draft of USP <665> (PF 45[2]). This draft also included requirements for USP <87> and <88>, which are not covered by the final USP <665> chapter.	N/A
EP 2.4.35, USP <1031>	**Pharmaceutical plastics.** A relatively new term increasing in use with USP <1031> (The biocompatibility of pharmaceutical packaging systems and their materials of construction), and EP 2.4.35 (Extractable elements in plastic materials for pharmaceutical use) that is specific to container closures.	N/A
ASTM E3231-19[13]	**Standard Guide for Cell Culture Growth Assessment of Single Use Material.** Provides a testing framework to assess cell growth following contact with SUCs.	Protocol can be used to support assessment

* Packaging systems contain or are intended to contain a drug product and provide a means for storing and distributing drug products[4]. This is generally different from components of single-use bioprocess assemblies.

** USP <661> and <661.1> are specific to packaging requirements for drug product and are beyond the scope of most SUCs. Historical <661> pass/fail test requirements for plastic containers have been revised with material-specific testing requirements specifically for final drug product containers. During the revision process, the new USP <665> chapter was created to focus specifically on plastic manufacturing components.

Materials and sourcing controls. A starting point for a material assessment may include a general understanding of the type of polymeric material and its supply chain. Many common polymers such as polypropylene, polyethylene, polyether sulfone and silicone are used quite versatilely in SUCs. In some cases, the risk of a specific compound may be related to the type of material used or its manufacturing process. For example, bisphenol A(BPA) may be a specific compound of interest primarily for polycarbonate, epoxy and, in some cases, polysulfone materials. Risk assessment strategies for other compounds, such as nitrosamines, start with whether the type of material would be expected to contain secondary/tertiary amines or nitrosating agents, as extractables screening methods may not have the required sensitivity or be well suited for these types of compounds.

Elastomers versus plastics. Whereas the BioPhorum extractables protocol[12] applies equally to elastomeric components (e.g. tubing, gaskets, seals) and plastics, USP has a history of segregating chapters associated with plastics (USP <661>) and elastomers (USP <381>) for final container closures. This separation has continued with a new USP chapter for cured silicone elastomers (USP <383>) and separate 600-level chapters for plastic containers (USP <661.1>, USP <661.2>) and plastic SUCs (USP <665>). In many cases, a SUC may contain both plastic and elastomeric materials (e.g. filters with O-rings, sensors), and in these cases testing aligned to USP <665> or BioPhorum v2.0 is considered appropriate. In addition, USP General Notices section 6.30 allows for alternative and harmonized methods and procedures in several circumstances, which would appear to be applicable here.

Regulatory requirements for SUC extractables.
SUCs are used in a wide range of biopharmaceutical manufacturing applications posing different risks to the drug manufacturing process and final drug product. The most critical single-use bioprocess applications tend to focus on those components from the final clearance/purification step (e.g. ultrafiltration/diafiltration (UF/DF)) to final filling (see Section 2). SUCs used in bioreactors are often considered critical due to their potential impact on biologics production, the high costs associated with this stage of the process, and the potential risk of drug shortages. These higher-risk applications are expected to be prioritized for component extractables data availability and USP <665> reporting and are most likely to be subject to increased regulatory scrutiny.

At the time of writing, there is neither a clearly stated regulatory requirement specifically for USP <665> or BioPhorum data for all bioprocess components, nor is there a stated requirement for when USP <665> becomes official on 1 May 2026. In addition, the USP has previously stated: "As currently published, there are no requirements that are mandatory for compendial compliance purposes in this chapter, … and that a chapter below <1000> does not become an applicable general chapter unless referenced as such in General Notices, a monograph, or another applicable chapter numbered below 1000)"[14].

It is, however, expected that all plastic components are risk assessed and, based on a 2024 BioPhorum survey, it is understood that many regulators are already asking how SUCs are being assessed. Sections 4 and 5 provide more detail on navigating risk assessment approaches and regulatory expectations.

2.0

What is expected for USP <665> and BioPhorum 2.0 data?

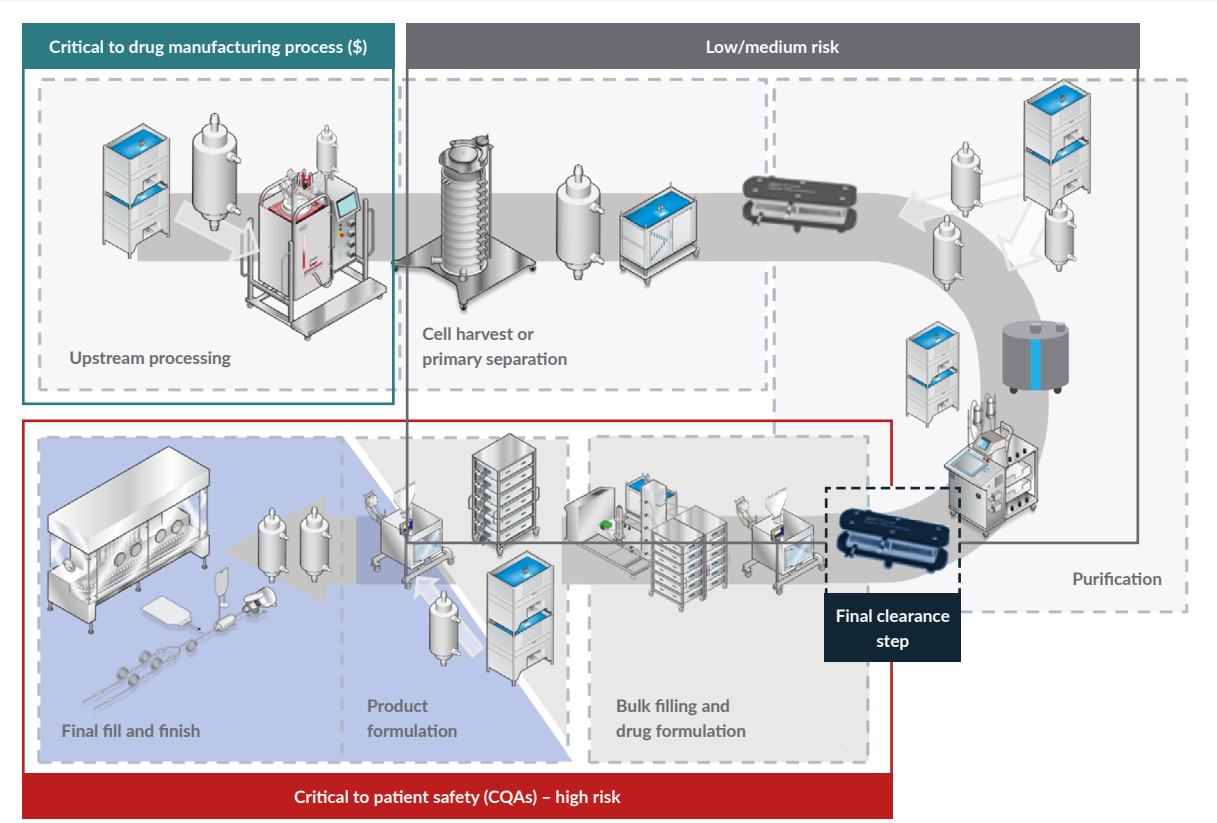
The new USP <665> chapter seeks to establish a baseline for risk-based assessment and testing requirements for the qualification of plastic components used in manufacturing. End-users are expected to qualify components and systems for their intended use, determining suitability and ensuring that they do not pose a risk to product quality or patient safety. Suppliers are expected to provide characterization data aligned with regulatory and compendial requirements, according to the standard extraction protocol, outlined in USP <665>.

Establishing risk level for generating component data

SUCs are used in a wide range of biopharmaceutical manufacturing applications, posing different risks to the drug manufacturing process and final drug products. The USP <665> risk assessment requires in-scope materials to be classified as low, moderate or high risk. Two generally used risk assessments can be adapted by end-users: the BioPhorum Leachables Risk Assessment and the risk assessment approach described in the USP <1665> informational chapter (see Appendix). Ultimately, responsibility for defining risk levels and risk assessment processes rests with the end-user. However, if a supplier is generating extractables data for components, they typically evaluate representative use conditions to define appropriate testing strategies, based on material characteristics and aligned with the component's marketed applications. Because end-users perform risk assessments according to their specific formulations and conditions of use, risk classifications for the same component may differ between applications. As suppliers cannot anticipate all potential uses of their products, they should ensure comprehensive extractables data is sufficiently comprehensive to address a broad range of risk levels and contact solutions relevant to common biomanufacturing scenarios.

In general, the most critical single-use bioprocess applications tend to focus on final clearance/purification steps to downstream final filling. Components related to upstream processing steps (pre-UF/DF) would generally be of a lower risk level. Risk associated with a specific production step is addressed through the mitigation factors described in USP <1665> and, more directly, through the BioPhorum risk assessment factor of distance along the production stream (DAS). Beyond patient safety concerns, SUCs used in certain processing steps, such as bioreactors, may warrant a high-risk classification due to their potential impact on biologics production, the significant costs associated with this stage of manufacturing and the consequential risk of supply disruption or drug shortages (Figure 1).

Figure 1: Typical single-use bioprocess applications



(Image courtesy of Cytiva)

Potential risk rankings for risk dimensions associated with the location of the SUS in the process are shown here for visualization purposes. Risk ranking assignment for this specific risk dimension takes into consideration that leachables generated early in a manufacturing process have more opportunity to be separated from the product through downstream purification or clearance steps (lower risk) compared to those generated at the end of the process (higher risk), where there is limited or no further processing. This risk dimension, and its associated risk rankings, is one of the various factors incorporated into an overarching leachables risk assessment.

These higher risk applications have been prioritized by SUS suppliers for component extractables availability and USP <665> reporting. It is expected that most or all components used in higher-risk applications are fully supported by USP <665> data by May 2026. It is also expected that, in the longer term, more components will be supported by USP <665> datasets. However, by the May 2026 date, there may be some components used in upstream or early downstream processing, that are not yet fully supported by the USP <665> approach, as the breadth of components used in these lower risk applications can be quite broad. In these cases, it is the

opinion of the BioPhorum community that existing risk assessment strategies should remain a scientifically valid approach for risk assessment. Moreover, currently there are no established acceptance criteria for USP <665> low-risk data.

Expectations for low-risk characterization data under USP <665> (and BioPhorum protocol)

USP <665> requires organic extraction with 50% ethanol followed by non-volatile residue (NVR) and UV absorbance testing of the extracts for low-risk components. Note: If testing is performed at moderate- or high-risk level characterization, then low-risk testing is not required, per the chapter, "the test results are reviewed in the context of the validity of the risk classification." The chapter does not establish clear limits or requirements for assessment of such data and leaves the validity of the risk classification up to the discretion and/or interpretation of the user. Under the BioPhorum extraction protocol, analytical techniques such as total organic carbon (TOC), pH and NVR are optional for use, if deemed appropriate by the component manufacturer.

Components are typically classified as low risk due to the inherent nature of the components themselves or the conditions of their use; these usually have a small surface area to volume ratio, short contact times, are further upstream in the process and have less overall potential to impact product quality. In the context of a holistic E&L control strategy, these items may be further supported by selection of well-characterized and established polymeric materials, and by significant component/product/process history. Available information on inherent chemical compatibility with a range of chemicals and/or products, other relevant compendial compliances (such as previously leveraged USP <661>, USP <383>, EP 3. X chapters, food grade, etc.) and historical biological reactivity testing as a generic baseline material assessment (per USP <88>, <87> and/or ISO 10993-5) can be leveraged to support safety of the materials. This approach is aligned with guidance on qualification requirements for low-risk applications outlined in the BioPhorum best practices guide for evaluating leachables risk[15].

Expectations for moderate- and high-risk characterization data for USP <665> (and BioPhorum protocol)

High-risk characterization involves organic extractables profiling with multiple solutions, reflecting the effects of pH and polarity on extraction*. In general, the USP <665> extraction protocol is considered a subset of that described in the BioPhorum protocol[12]; this includes contact durations, solvents and test conditions. Fifty per cent ethanol is the organic solvent for both and is the only solvent for low- and moderate-risk characterization testing. Water is not included in the required USP extraction solutions as the extractables profile obtained in water will be intermediate to the combined extractables profiles obtained from the pH extreme aqueous solvents based on the guidance in USP <1665>. Because it lacks buffer and salts, water can demonstrate greater extraction potential compared with buffered solvents and therefore provides significant value to end-users as an expedient and broadly representative solution for assessing aqueous process streams. Water-based extracts more closely reflect the extraction behavior of neutral or near-neutral product formulations, offering regulators a more representative assessment of extractables. Accordingly, BioPhorum advocates use of water as an extraction solvent and recommends that suppliers continue to provide water-based extractables data. Where water extractables data is not available, combined acid and base extraction may be used as a conservative surrogate for pH-neutral products. However, this approach is likely to represent an overly conservative worst-case scenario. In such cases, additional work may be required, either to conduct a more representative extraction study or to fully characterize highly acidic or basic extractables (including structural identification and Chemical Abstracts Service (CAS) number assignment) and assess their relevance for downstream evaluation, including patient safety assessment.

USP <665> uses a less aggressive acidic solvent (potassium chloride (KCl) pH 3) as compared to the BioPhorum 0.1M phosphoric acid (H_3PO_4) solvent. Likewise, the basic solvent, phosphate buffer (Na_2HPO_4, pH 10), is significantly less aggressive when compared to the BioPhorum 0.5N sodium hydroxide (NaOH). With the changes to the acid and base solvents in USP <665>, most respondents to a 2024 internal BioPhorum member survey felt that there would be acceptance of the BioPhorum model solvents. For the acid solvent stream, 0.1M phosphoric acid is specifically referenced in USP <1665> as being interchangeable with the model solvent advocated by the BioPhorum extractable protocol. Conversely, for the basic solvent, 0.5N NaOH is not considered comparable to the phosphate buffer pH 10 based on USP guidance , as it is "too worst case" for standard processes. Nevertheless, use of a more alkaline solvent can be justified in worst-case situations if there are no chemical

* Note: That extraction is a complex interplay between material, solvent and the target analyte; the solvent may be optimized for certain target analysis. The standardized solvents proposed by BioPhorum and USP <665> will cover most applications and allow for comparison of extractables data.

compatibility issues. With the late addition of phosphate buffer pH 10 to the chapter, several existing extractables datasets do not include this USP-designated solvent and therefore technically deviate from the specified high-risk testing conditions. If a given component has been demonstrated to be chemically compatible with 0.5N NaOH, then, in combination with available water extractables data, the criteria for USP <665> evaluation of high-risk components and subsequent patient safety evaluation may be considered satisfied. From a risk-based perspective, evaluation using both solvents effectively brackets the pH 10 condition and addresses worst-case conditions. Where this evaluation demonstrates that patient safety is acceptable, the material may be considered suitable for its intended use.

For time points, BioPhorum indicates 24 hours, 7 days, 21 days and 70 days based on use of the component and, for some components, like film, up to three time points may be recommended. The time points in USP <665> are some of those recommended by BioPhorum. Use of multiple time points can provide valuable insight into changes over time and may benefit manufacturers by increasing the likelihood that at least one time point approximates, while remaining conservatively worst-case, their typical processing conditions. Previous studies and supplier data have shown that extractables generally increase with time, but the change is smaller as the extraction time increases. The mode of extraction like agitation (orbital shaking) can accelerate this equilibrium process. In certain cases, however, volatile compounds may be lost or degraded during extended extraction, potentially resulting in minor variations in extractables profiles.

Availability of both BioPhorum data and USP <665> high-risk datasets for high surface area components used in drug product and final fill applications, such as filters, film and tubing, can provide useful additional information to support a robust assessment of patient safety. If 0.5N NaOH is not compatible or appropriate for the component, its use may be omitted, as the resulting extractables would not be representative of realistic processing conditions. Therefore, for high-risk components, characterization data following the combined USP <665> and BioPhorum solvent systems and time points provides a comprehensive evaluation for critical applications; 5 solvents of water, 0.1M phosphoric acid, phosphate buffer pH 10, 50% ethanol and 0.5N NaOH (where compatible) are recommended.

Additional single-use extractables data considerations

Single-use versus multi-use components. Some polymeric components intended for single-use applications, when properly validated, can be used more than once during processing of additional bioprocessing batches or in prolonged use during process intensification. In these cases, the industry-aligned USP <665> or BioPhorum approaches may well support the initial use. In general, for multi-use components, the number of extractables would be greatest during first use and progressively less during subsequent uses. Therefore, evaluation of extractables when a component is used for the second time, etc. is typically covered by the first use as this is the worst case and does not need to be reassessed.

Untreated, irradiated or heat-sterilized components. Ionizing radiation and heat sterilization each stress polymeric materials in different ways. Ionizing radiation typically generates a more complex extractables profile compared to heat sterilization[16]. In general, it is accepted that different sterilization modalities can result in different extractable profiles; hence, separate datasets should be generated for SUCs that are compatible and recommended for both sterilization methods, and these are generally considered worst case compared to the untreated material. The sterilization treatment parameters should be representative of the typical worst-case conditions. For irradiation, the higher end of the validated or intended dosage range is recommended. Typically, the upper end of this range should be used for testing, provided it remains within the material's qualification limits. If a dataset contains both irradiated and steam-sterilized pretreatment on the same test article before extraction, this would likely not be representative of irradiated alone, due to the potential removal of volatile compounds, during the steam process. Extractables profiles obtained where sterilization technologies or conditions different from the actual process conditions may require additional justification. For example, qualification of x-ray sterilization as equivalent to gamma, though significant industry progress has been made in this area[16, 17].

Lifecycle data management. During the lifecycle of components, many changes may occur, including manufacturing site, process, resin and additive changes. It is important to understand how any change can impact the product and its extractables dataset.

Depending on the type of change evaluation may be based on known information in a standalone assessment or may require comparative extractables testing against the original. For example, if a resin change occurs in the filter membrane, testing may be limited to the membrane component itself. Because comparison testing focuses specifically on the modification, it is typically performed using 50% ethanol as the worst-case model solvent. This solvent generally produces a large number of extractables and is regarded as providing the most inclusive profile of the solvents. Depending on the change, other solvents (e.g. aqueous) may also be evaluated. Limited-scope verification extractables testing conducted for change management purposes aims to determine whether the existing dataset remains applicable. If the material is demonstrated to be comparable with no impact on the original data (e.g. without novel compounds or significant changes in levels of compounds), original extractables dataset may be retained. This approach ensures that risk assessments performed for each drug product do not have to be repeated or changed.

Supplier data expectations. Depending on when component data was generated, testing may vary based on changes to extraction protocols over time. In general, extractables data previously generated following the BioPhorum extraction protocol will cover the intent of the extraction protocol in <665>. However, suppliers should evaluate their portfolios and existing extractables data to identify testing gaps and update their approaches in line with current expectations.

End-users own the qualification of components and systems for their intended use, establishing risk levels for their specific processes, determining suitability and ensuring these do not pose a risk to quality and/or patient safety. Suppliers cannot claim compliance with USP <665> but can align with the testing strategy. Supplier claims and reporting in relation to USP <665> are limited to descriptions of characterization data generated according to the standard extraction protocol; these claims/statements should clearly, and transparently, identify what solvent data is available for end-users to leverage in their qualification activities (Sections 4 and 5).

As USP <665> only details extraction conditions, the BioPhorum protocol may be leveraged to guide analytical methods and data reporting requirements. Depending on when component data was generated, testing may vary based on changes to protocols over time. For component-family testing approaches, extractables data reports should include detailed documentation of extraction conditions, identification of the selected worst-case representative component and justification for the family designation. A component family may be defined, for example, as components manufactured in different sizes from the same materials using the same manufacturing process. All components meeting the defined family criteria should be listed within the supplier's extractable data report. Component-family information may be prepared and shared using the BioPhorum extractables component family template. When providing extractables datasets under these assumptions, the information provided in Table 2 should be included.

Table 2: Test information

Extractables test information	
Test identifier	Report number or tested component
Test condition	Time and temperature of extraction
Sterilization type/conditions	Irradiation, autoclave sterilization, etc.
Pretreatment	None, flushing, sanitization
Reporting limit: limit of detection (LOD)	Unit µg/cm^2 or mg/device
Product family coverage	See component family information table

Table 3: Comparison of USP <665> and BioPhorum extraction solvents

		Extraction solvents							
		Organic		Acidic		Basic		Neutral	
		USP	BioPhorum	USP	BioPhorum	USP	BioPhorum	USP	BioPhorum
		50% ethanol		0.05M KCl pH 3	0.1M H_3PO_4	0.1M Na_2HPO_4 pH 10	0.5N NaOH	N/A	WFI
Characterization testing level	USP <665> low risk	✓*		–	–	–	–	–	–
	USP <665> moderate risk	✓		–	–	–	–	–	–
	USP <665> high risk	✓		✓	–	✓	–	–	–
	BioPhorum protocol	✓		–	✓	–	✓	–	✓
	BioPhorum + USP <665>	✓		✓**		✓	✓	–	✓

* USP extraction is limited to NVR and ultraviolet (UV) characterization. BioPhorum minimum extraction level (40˚C for 24hrs) includes extractables profiling (identity and amount).

** Acidic extraction solvents 0.05M KCl (pH 3) and 0.1M H_3PO_4 are considered interchangeable, therefore extraction with either is acceptable.

Table 4: Component family template example of completed component template

Component family information			
Extractables study ID	Study 1		
Component name	Component part number	Material(s) of construction	Fluid contact surface area (cm^2) or component mass (kg)
Component included in extractables study			
Connector L – small	123LS	Polypropylene (PP), silicone	10
Components included in the family			
Connector L – medium	123LM	Polypropylene (PP), silicone	20
Connector L – large	123LL	Polypropylene (PP), silicone	50
Connector T – small	123TS	Polypropylene (PP), silicone	15
Connector T – medium	123TM	Polypropylene (PP), silicone	25
Connector T – large	123TL	Polypropylene (PP), silicone	55

Source: **BioPhorum extractables assembly family template**

Call to action #1! Utilize the BioPhorum extractables component family template to clearly summarize critical supplier information linking the extractables study to the specific part numbers covered by the assessment.

3.0

Applying standardized component data to single-use assemblies

While USP <665> and BioPhorum extractables protocols focus on testing at component level, it is the integrated assembly of components (i.e. single-use systems) or groups of assemblies, used together as part of a larger biopharmaceutical manufacturing campaign that ultimately require assessment.

This may include component assemblies for specific operations such as intermediate storage, fluid transfer, mixing, final filling, continuous processes as examples. Hence, a key risk in evaluating components individually, rather than the entire assembly, is failing to address the potential cumulative effects (see Section 1, footnote).

Component-level testing offers important benefits, including improved traceability of compound origin, which can support the evaluation of unknowns. In addition, as SUS can change, instead of a need for a new assembly test, only the new component(s) would need to be evaluated, or if a length of tubing changed, then only the calculations would be updated. Historically, assemblies were often evaluated through extractables testing of a worst-case SUS configuration incorporating all possible components. However, this approach limited flexibility in accommodating future design changes, scaling component-level data and independently assessing individual components.

For large assemblies, it also introduced analytical challenges due to complex extraction profiles. In some circumstances, sub-assemblies or smaller (pre-assembled) configurations may be used, providing sufficient surface area-to-volume ratios are maintained, and appropriate scientific justification is documented.

The most difficult aspect of assessing an assembly is gathering all relevant extractables data packages and necessary details of the sub-components used, such as contact surface area information. Assemblies often incorporate parts from various suppliers, complicating the task for integrators and end-users, when for example, the original part number and supplier name may be considered confidential by the assembly's supplier. Additionally, grouping components into specific product families for collective assessment is challenging as detailed information on raw material types and manufacturing parameters – needed to justify such grouping – is typically not provided as standard practice.

The BioPhorum assembly family template (Table 5)[17] provides a standard approach to communicating the core requisite information needed for the comprehensive risk assessment, including: component name, integrator part number, component manufacturer and manufacturer part number, extractables study identifier, and fluid contact surface area (or relevant scaling parameter).

Table 5: Bill of materials explosion of three assemblies (Bag A1–Bag A3) following the BioPhorum assembly family template

Assembly family information								
						Assembly name	Assembly name	Assembly name
						Bag A1	Bag A2	Bag A3
						Assembly part no	Assembly part no	Assembly part no
						123B1	123B2	123B3
Component name	Component part number	Component manufacturer part number	Component manufacturer	Material(s) of construction	Extractables study ID	Surface area (cm^2)	Surface area (cm^2)	Surface area (cm^2)
Film	123film			PE, EVOH, XXX	Study 36	1,000	2,000	3,000
Filter A	152filter			PES, PP	Study 15	10,000	10,000	10,000
Tubing A	147tubing			Silicone	Study 25	100	100	100
Tubing B	136tubing			TPE	Study 26	100	100	100
Connector L – medium	123LM			Polypropylene (PP)	Study 1	20	60	80
Connector T – medium	123TM			Polypropylene (PP)	Study 1	25	50	75
Connector B								
Port A								

Alternative or interchangeable part numbers and applicable reports may also be indicated here. Source: **BioPhorum extractables assembly family template**.

Call to action #2! Utilize the BioPhorum extractables assembly family template to clearly summarize critical supplier information required for the risk assessment.

The overall data compilation process, starting with the assembly family template and working to obtain the current version of each appropriate study report, can be time-consuming and challenging. Adopting industry best practices, such as allowing integrators or end-users to freely access and utilize supplier component data, without restrictions, paywalls or extensive confidentiality agreements, will streamline the process. Moreover, such practices may accelerate evolution and adoption of digital technologies (e.g. electronic databases) that could automatically access and seamlessly scale the requisite data described in the example four-stage process below.

Negligible or minimally impacting components. It is expected that not all SUCs will have a complete USP <665> data package when the standard becomes official. Primarily, this will affect components used in upstream or very early downstream applications, or generally small parts, as defined in BioPhorum extractables best practice[12]. In such cases, alternative risk mitigation measures may be considered, such as assessing these components against the recommended BioPhorum low-risk application qualification requirements, for example biological reactivity testing. Alternatively, end-user internal risk assessment practices, using basic material qualification data, may be used. For example, diaphragms and gaskets subjected to cleaning or steam pretreatment prior to use are typically considered low risk, as are prefilters, which are thoroughly flushed before being used. Small parts made from polymer materials already present in the assembly are unlikely to introduce significant new extractables, a consideration especially for silicone components. As a consensus, components manufactured from well-controlled materials*,[18] meeting baseline material requirements (see above) with relatively small surface areas (e.g. surface area ratio of less than 1% of the overall assembly contact surface area) do not contribute significantly to overall E&L profiles** (see Appendix). Similarly, the concept of surface area-to-process-volume ratio (SA/V), identified as a risk mediator[15], can be used to support a rationale that small contact areas or large process volumes lead to a negligible impact for materials where the SA/V <<0.1cm^2/mL. In either of these approaches the number of small components should be considered.

* A well-characterized material is made of raw materials from a controlled supply chain. That control is verified by the qualification of suppliers and sub-suppliers. The material shows consistent and reproducible properties and it complies to applicable compendial standards.

** Based on the experience of BioPhorum members, SUCs representing relatively small fractions of the fluid-contact surface area (e.g. 1%), can reasonably be expected to exhibit minimal contributions to the profile below reporting limits.

4.0

Comprehensive end-user SUS E&L assessment

End-users are expected to qualify components and systems for their intended use, determining suitability and ensuring that they do not pose a risk to quality and/or patient safety. USP <665> clearly states that testing and initial evaluation should be performed at component level. For example, in the case of a component like a filter or a sterile connector containing multiple materials of construction (MOCs) manufactured together, the component itself should be tested.

Once the component data is gathered, the data should be evaluated at the assembly level for the fluid path or process contact pathway that could lead to final product leachables, as only these contact areas remain relevant for the calculation. Understanding that the same extractables compounds could come from multiple MOC. The evaluation is the responsibility of the end-user.

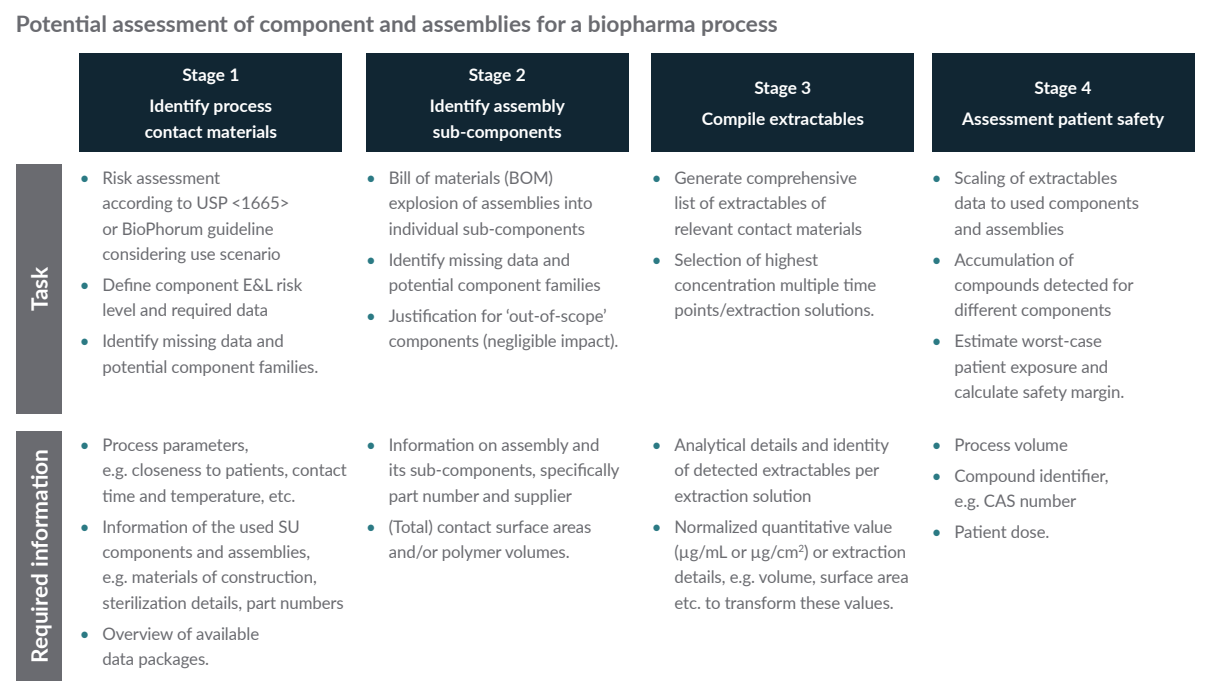
An example of a step-by-step E&L assessment workflow for a biopharmaceutical process using multiple SUCs and assemblies is presented in Figure 2. The assessment begins in stage 1 with identification and compilation of a list of all SUCs and/or assemblies used in each specific pharmaceutical process unit. This is followed by a risk assessment, for example based on either the BioPhorum or USP <1665> guidelines, to identify those with potential impact to the final product, determine the individual risk levels (risk rankings), appropriate qualification approach and test data requirements commensurate with the level of risk. As SUCs are used in a wide range of biopharmaceutical manufacturing applications, end-users are encouraged to evaluate the relevance and suitability of any published risk model to their unique operations to ensure they provide meaningful insights and support effective decision-making. Regardless of the risk model used to establish the different risk levels, these risk assessments must consider the different dimensions of E&L risk, including factors such as the nature of the plastic components (MOCs), contact surface area, the nature of the process stream, contact conditions (e.g. temperature, duration), pretreatment of components (e.g. sterilization), purification processes and the risk profile of the drug product itself.

In stage 2, unit SUS and/or assemblies are identified. These are assessed as individual sub-components and are grouped into MOCs/polymer families for further evaluation against the expected process contact fluid(s).

In stage 3, extractables data from vendor or end-user internal E&L reports is compiled and reviewed for applicability to the manufacturing process conditions. The most applicable extractables data is generated under worst-case conditions yet still representative of the actual manufacturing process.

In stage 4, identical compounds (potential leachables) are grouped together, and summed or scaled to reflect the actual assembly configuration. Compound (or elemental impurity) levels for assemblies are evaluated relative to the process volume and clearance steps to determine the total level or relevant compounds per dose for the specific end-user product presentation. Summing identical extractables from various materials or process steps supports a cumulative assessment of potential leachables per dose and is performed to avoid underestimating safety concerns. Finally, these calculated levels are compared to appropriate safety thresholds, such as the analytical evaluation threshold (AET)[10], thresholds of toxicological concern or a compound specific permitted daily exposure, to determine the margin of safety. As Jenke aptly summarizes: "...experience, introspection, and experimentation has established that the chances of PERLs accumulating in finished drug products are negligibly low, especially for PERLs derived from the downstream manufacturing components"[19].

Figure 2: Example workflow for a process risk assessment including assessment of a unit SUS and/or assembly sub-components



For low-risk SUS, no further action is required if the compendial requirements and other regulatory requirements are met per the end-user's application.

SUS with medium-risk scores require further evaluation of supplier extractables data and/or any existing leverageable leachables data. If each of the evaluated extractables (potential leachables) does not exceed the safety concern threshold (SCT) or AET and/or does not belong to the group of chemicals with structural alerts, then the SUS is qualified for intended use.

High-risk SUS or assembly units may require extractables and/or leachables studies while in contact with process fluid matrices (e.g. buffer, drug substance, drug product) or a representative solvent matrix. Existing information or the comparator approach described in USP <665> can be leveraged on a case-by-case basis with appropriate scientific justification in-line with end-user needs and considering the specific regulatory requirements. A case example of how data is compiled and assessed by a biomanufacturer is described in the Appendix. These tasks often involve complex, manual calculations, typically carried out in spreadsheets and prone to risk of human error. To streamline assessments and reduce risk, use of digital tools and appropriate software solutions can be advantageous and simplify the process. While suppliers generally provide officially signed reports in pdf format to meet quality standards, spreadsheet data summaries following the BioPhorum extractables data requirements are also expected to simplify the process. Generative artificial intelligence (GenAI) may be used to simplify the process but information must be double-checked before a quality document is generated from it.

4.1 End-user workflow considerations

In practice, implementation of the four-stage workflow by any end-user organization may be influenced by:

Risk assessment model. It is essential for end-users to recognize the thoughtful placement of the USP example risk model within chapter USP <1665>. An example of a risk evaluation matrix can be found in the Appendix. As the purpose of this example is education and illustration, it is not required that the matrix in the Appendix be adopted for a sponsor to claim that their assessment complies with <665>. This distinction highlights the

importance of aligning risk models with the specific context and needs of each manufacturing process. Comparative assessments of risk models, such as those from the USP, BioPhorum and ELSIE, demonstrate that although these models often yield similar outputs, they may vary in sensitivity and robustness depending on the modality, whether small molecule manufacturing, biologics, cell and gene therapy, or m based processes. A well-calibrated risk model enhances process understanding by offering nuanced assessments across each step. If a model lacks the resolution to differentiate risk levels, its utility diminishes. Therefore, tailoring the model to reflect the specific risk landscape of a process is key to unlocking its full value. Given the diversity of manufacturing approaches across companies, from single-product lines to complex portfolios, there is no one-size-fits-all risk model. Experienced auditors appreciate this and focus on identifying key risk factors, as outlined in USP <1665>, in relation to the end-user's specific manufacturing context. As science and manufacturing technologies evolve, so too must our risk models. Treating risk assessment as a dynamic, ongoing process ensures continued relevance and effectiveness. Periodic evaluation and updates are vital components of a robust risk model lifecycle, enabling organizations to stay aligned with emerging knowledge and innovations in material risk and manufacturing practices.

Impact of clearance steps. Components and assemblies upstream of final clearance steps, such as diafiltration or bind-elute chromatography, are generally associated with lower risk of PERLs persisting into the final product. Several publications have now demonstrated the log reduction capability of representative PERLs to be cleared or reduced by clearance technologies such as diafiltration[20, 21, 22], supporting an approach where SUS and assembly components upstream of the final clearance step may be expected to yield negligible impact on the final assessment. Hence, quantitative assessments of volume dilution or clearance may also be used as tools in evaluating risks associated with components upstream of final clearance steps.

Additional studies required to complete the SUS E&L assessment. In some cases, the end-user risk assessment based on standardized supplier data may not be sufficient to complete the full SUS assessment, and additional studies may be warranted. In these cases, process-specific studies with analytically expedient solvents that closely simulate the actual drug manufacturing process fluids and conditions may be conducted as a next step. Examples of such cases may include:

- Where there is a lack of extractables data or if available data is inadequate
- Where extractables and/or leachables data is not sufficiently representative of the process fluid or conditions (time, temperature, solvent characteristics).

Regardless of whether additional studies are warranted, the safety assessment of individual compounds and levels are conducted as described in stage 4 of the example four-stage model.

Comparator approach. Where SUS or assembly components have been used in an approved drug manufacturing process; this may fit the concept of a comparator component as described in USP <1665>. Historical supporting data and prior knowledge may serve as risk mitigation measures taking into account the nature of the materials or components, the characteristics of the contact solution, the conditions of use in the manufacturing process and the level of patient safety risk associated with the dosage form and route of administration. Use of similar materials/components in similar bioprocessing environments may be leveraged by end-users to establish matrixed/product-family approaches for assessment and qualification, to justify suitability of materials and components, and to support change management throughout the product lifecycle.

5.0

Navigating risk assessment approaches, improvement opportunities and regulatory expectations

With increased attention on single-use PERLs, an official USP <1665> guidance chapter (May 2022), a soon-to-be official USP <665> chapter (May 2026) and existing industry datasets and best practices, some opportunities to clarify and improve best practice alignment remain. In each of the specific areas described below, flexible approaches are required to address product risk, minimize redundant testing and align with industry best practices.

USP <1665> material risk scoring. The example risk assessment model included in the appendix of USP <1665>, includes as one of its four dimensions of risk, a score for the percentage-by-weight concentration of additives in the component. As most plastics formulations are regarded as confidential and proprietary by resin manufacturers, this information is often difficult to obtain and share, especially given the range of plastics frequently used in many components. Furthermore, the multiple processing steps involved in processing multiple raw materials into a finished component render such values impractical or impossible to determine in a meaningful way. This lack of information arbitrarily leads to a higher risk score. In addition, many low-risk additives, such as titanium dioxide whitening pigments which help improve readability of machine text or barcodes on external surfaces, are added at concentrations that yield the worst-case risk score. As such, flexible alternative approaches focusing on compatibility and process fluid characteristics may be more practical. However, the authors note that the USP <1665> example risk model is included as an example and is not intended as a prescriptive approach.

USP <665> low-risk data expectations and acceptance criteria. Given the range of plastics used in single-use bioprocessing, the different combinations by which they can be manufactured into components and the cost of testing, not all low-risk components will have USP <665> data by May 2026, when USP <665> becomes official. As historical and highly successful best practices for low-risk components frequently relied on compendial biological reactivity pass/fail compliance (e.g. USP <87> and <88>), it is anticipated that a new expectation for novel low-risk extractables testing may not add meaningful value, especially for low-risk components used at early stages in bioprocessing distant to the final product. Additionally, where existing low-risk biological reactivity testing has pass/fail acceptance criteria, there can be ambiguity or lack of industry alignment in how to interpret <665> low-risk test data. Hence, flexibility that relies on biological reactivity or other compendial data is needed in assessment strategies for low-risk components.

Justification of 0.5N NaOH in lieu of USP pH 10 (C3) extraction solvent. USP <665> section 4.3.2 allows substitution of higher-pH solvents only in specific circumstances where the process stream exceeds pH 10. The USP provides strong arguments for why pH 10 may make sense for a compendial standard (i.e. most applications are <=pH 10, plastics that are poorly compatible above pH 10 should not be disadvantaged, pH 10 exceeds the pKa of most additives), end-users who already have 0.5N NaOH data, or have some processes which exceed pH 10, should be able to leverage the 0.5N NaOH data without redundant testing of a different alkaline solvent.

Neutral process fluids and expectations for low and high pH data. In many high-risk applications, the process fluid may be relatively pH neutral, and significantly different from the limiting extraction solvents of pH 3 (USP C2) and pH 10 (USP C3). In such cases, low and high pH data may provide limited additional value compared with more representative semi-polar (50% EtOH/water) or aqueous solvents. Similar considerations may apply to components intended for use in low-pH virus inactivation steps, where high-pH solvents may not enhance the risk assessment.

Superior process-specific or simulation data and expectations for/in lieu of USP <665> component data. In some cases, process-specific or simulation data may have been generated using analytically expedient solvents designed to bracket specific drug-manufacturing process conditions. In such cases, additional testing aligned strictly to USP <665> or BioPhorum component protocols may not provide incremental scientific value. While standards can support overall industry alignment, these scenarios warrant flexibility to ensure that process risks are appropriately assessed using relevant worst-case simulation data, without imposing redundant or non-meaningful testing requirements.

Elastomers. USP <665> is intended for plastic components, which frequently contain small elastomeric sub-component materials or seals. However, USP <381> and now <383>, or EP 3.1.9 are often recommended by compendia specifically for elastomers. In addition, biological reactivity testing (e.g. USP <87>, ISO 10993-5), has historically served as a baseline assessment of suitability for all materials, including elastomers, used in single-use bioprocessing. BioPhorum testing recommendations, while largely aligned with <665>, do not differentiate between plastics and elastomers. Flexibility to use available data to support science-based risk assessments will continue to be essential in practical, risk assessment strategies.

5.1 Variation in BioPhorum and USP <1665> risk level determinations

Both BioPhorum risk assessment best practice and the USP <1665> example risk assessment employ multiple risk dimensions to establish one of three levels of risk. Both models generally align in how some dimensions lead to increased risk. However, as risk dimensions and mitigation factors are defined differently, some different risk levels are calculated depending on the model, which could lead to increased or decreased expectations for supporting data.

BioPhorum risk assessment ratings are based on risk factors including the location of the SUS or distance along production stream (DAS), exposure temperature (ET), exposure duration (ED), process fluid interaction (PFI) or solvation power and contact surface area to process liquid volume ratio (SA/Vol). Each of these factors has ratings ranging from 1 to 9 and has corresponding weightings (%). The weighted sum of the risk ratings yields a SUS-specific risk score, categorized as either low (1.0 to 3.6), medium (3.7 to 6.2) or high (6.3 to 9.0). See Appendix for examples illustrating this scoring approach.

The USP <1665> example risk assessment ratings are based on risk factors including duration of contact, temperature of contact, chemical composition of the process stream and nature of the component's MOCs. Each of these factors has a rating from 1 to 3; a numerical risk sequence is then assigned based on the scoring of each factor which is used to categorize the risk level as low, moderate or high. Mitigation factors can be used to adjust the final risk level.

The Appendix provide risk scoring examples for several different component applications according to BioPhorum and USP <1665>. In general, the resultant scores are the same or differ by a single level. For scores that differ, no consistent trend indicates that one model is systematically more conservative than the other; in some cases, each model may yield higher or lower outcomes. Given the substantial experience and established track records that both industry and individual companies have with their existing models, often tailored to their specific manufacturing portfolios, flexibility to apply scientifically justified risk models remains an essential element of an effective and holistic E&L risk assessment ecosystem.

5.2 Health authority expectations and queries

With the advent of the USP <665> and <1665> chapters, as well as more attention to single-use bioprocessing, communication and education will remain paramount. In a recent survey of ELSIE member companies reviewing health authority feedback related to <665>/<1665> regulatory submissions, two representative (paraphrased) comments were identified.

- *"With respect to leachables/extractables of the filter: carry out a risk assessment according to USP 1665"*
- *"Please provide results from risk assessment of the new bag and the risk evaluation matrix used for the risk assessment. Risk assessment is accomplished via application of a risk assessment matrix detailed in United States Pharmacopeia (USP) 1665. Please provide full E&L profiling; please refer to the guidance provided in USP 665 and 1665."*

Although the comments of a single regulator may not reflect consensus thinking of an agency, they do highlight how the USP chapters may be interpreted, and where there may be opportunities to improve communication and alignment. In the context of the comments above, most end-users will already have completed risk assessment and testing of materials through their existing processes prior to submission. In such cases, the applicant may include some of the points below in their response:

- The example risk assessment approach described in the appendix of the USP <1665> guidance chapter is helpful in sharing the thinking of the USP and providing a starting point for companies that may not already have a risk assessment approach. However, other science-based, risk assessment approaches (BioPhorum, ELSIE or existing company-specific approaches) are considered acceptable.
- The USP <1665> guidance chapter, section 4.2.1, states the core requirement of the risk assessment approach is that the outcome must align with the three levels required in USP <665>, and that at least one of the sponsor's potential risk levels must be aligned with the USP <665> high risk level. The risk assessment approaches described above meet these requirements.
- USP <1665> section 6.3 also considers that alternative approaches to qualification may also be appropriate in justified circumstances, subject to agreement with regulatory authorities.

In the health authority query examples shared above, the sponsor's response indicating their alternative risk assessment model, and appropriate level of supporting data, was well-received without the need to repeat additional extractions per <665>.

6.0

Application of standardized risk assessments and data to evolving modalities

USP <665> and USP <1665> were developed for qualification of SUS used in classic biopharmaceutical processes for the manufacturing of, for example, monoclonal antibodies (mAbs), vaccines or bioconjugates. New technologies/modalities like cell and gene therapies (CGTs), intensified and continuous bioprocessing (ICB) and/or antibody drug conjugate (ADC) production have not been the primary focus[23]. Whether the standard can be applied to these emerging technologies requires a comparison of their characteristics with those of classical bioprocessing.

The most relevant parameters to consider are provided in Table 2. Many of the SUCs used for these new modalities are the same as for those used in classic biomanufacturing and are mentioned as components in USP <665> or in assemblies thereof. However, new designs might be specifically developed for these modalities, with some examples given in Table 6.

Table 6: Parameters of CGT, ICB and ADC production[24] that may differ from classical bioprocessing influencing an extractables testing and the safety assessment strategy

Modality / Factor considered	Traditional biomanufacturing (mAbs)	CGT	ICB	ADC
SUS types to be considered, but not mentioned explicitly in USP <665>	Bioreactors, various filters, bags, tubes, transfer sets, connectors, chromatographic systems, tangential flow filtration (TFF) and fill and finish (F&F) systems	Isolators, microcarriers (MCs), perfusion bioreactors, aliquoting systems, bags and bottles for low-temperature storage	Perfusion bioreactors, surge tanks, CVI* and continuous chromatographic system assemblies	Reaction and liquid exchange systems, e.g. combined as a complex TFF assembly
Process temperature	≤37°C	≤37°C	Typically ≤40°C	Typically ≤40°C
SUS size	Large	Small	Medium–large	Medium–large
Process liquid	Aqueous to aqueous-organic	Aqueous to aqueous-organic	Aqueous to aqueous-organic	Aqueous and highly organic
Process time	Hours–weeks	Hours–several weeks	Hours–months	Hours–days
Downstream purification efficacy (ability to remove leachables)	Very high	Moderate/High	High, decreasing along the DS process	High, as organic solvent needs to be removed
Patient proximity**	Low	High	Low	Low

* CVI – continuous virus inactivation

** Fill-finish operations are always considered high risk

6.1 Consideration on risk assessment according to USP <1665>

The risk assessment of SU components used, as outlined in USP <1665>, will probably lead to a moderate- and high-risk rating. For example, in cell therapy, there is a probability that PERLs remain in the drug product – the therapeutic cells – due to an absence of comprehensive downstream purification steps (besides a few final washing and elution steps), and high proximity to the patient[25, 26, 27]. The organic solutions used as CGT cryo-preservatives are typically low risk when frozen but need to be considered during freezing and thawing before filling. In ICB processing, time can be prolonged. And with ADC applications, organic solutions with a higher extraction 'propensity' may be encountered, particularly during the conjugation step. Therefore, the safety assessment may consider the applicability of readily available standard extractables data in relation to the pharmaceutical manufacturing process, including downstream processing and clearance steps (e.g. UF/DF).

6.2 Applicability of 50% ethanol/ water mixtures for non-traditional biomanufacturing

CGT, ICB and the antibody-related product-stream in ADC require physiological process conditions with temperatures slightly below 40°C with aqueous serum-containing (CGT) and/or serum-free media buffered at pH values close to neutral. USP <665> as well as BioPhorum recommendations meet these conditions, requiring testing at 40°C with a 50% EtOH extraction solution for aqueous-organic process fluids. The low- and high-pH extraction solutions exaggerate typical process conditions encountered in all three new modalities. In CGT, product storage often requires the application of dimethyl sulfoxide (DMSO) as cryo-preservative in concentrations up to 10%. In ADC production, DMSO, dimethyl formamide (DMF), isopropyl alcohol (IPA) and other water-miscible solvents are used to solubilize the active small molecule drug and linker, before they are added to the aqueous antibody-containing product stream. And whereas these process solvents are excellent solvents for organic compounds in their pure form, their extraction 'propensity' becomes significantly reduced when diluted as aqueous solutions, making an extraction solution like 50% EtOH an appropriate surrogate[28, 35]. Detailed discussions and data reviews within BioPhorum, including laboratory data and assessments of extraction propensity, support the use of 50% EtOH/water data to serve as surrogates for CGT and the diluted ADC solvents and are further detailed in the Appendix.

6.3 Scaling considerations

CGT applications typically employ relatively small form factor versions of components or devices typically used in larger, classical bioprocessing. The test protocol in USP <665> and BioPhorum prescribe high surface area to extraction solution volume ratios (S/Vs) of 6cm^2/mL for most components. These S/Vs are still higher than those for the majority of the small form factor devices in a CGT use scenario. Therefore, data obtained from USP <665> is suited to the risk assessment. For ICB and ADC production, device sizes and process volumes are similar to those in classical bioprocessing.

6.4 Contact duration

Process times for filtration processes and liquid transfer in CGT and ADC production will be comparable to classic bioprocessing (not exceeding 24 hours) and therefore aligned with USP <665> testing conditions for filtration and fluid transfer devices. Manufacturing steps, such as cell differentiation and proliferation in CGT in SU bioreactors can run for several weeks, and media and product storage can last longer. Processing times for ICB can easily exceed the 21-day extraction time given in USP <665> (e.g. surge tanks, tubing, CVI). In this context, a 21-day extraction at 40°C for a bag system, for example, approaches an extractables concentration close to the equilibrium concentration – the highest possible concentration one can reach in a given, closed extraction system[29]. Longer extraction times will not result in higher extractables concentration, making the 21-day extractions data from USP <665> suitable. Furthermore, the transient contact time of a given volume of process fluid with different SU components in ICB is much lower compared to the total process time.

6.5 Applying standard data to evolving modalities

Extractables profiles obtained for SU components following USP <665> are well-suited for assessing most evolving modalities, including CGT. However, actual use of the data for the safety assessment may require more careful consideration. One example concerns the well-mixed, but thermodynamically closed nature of standard extraction conditions where new fluid does not continuously enter and exit the system, as may be the case in many bioprocessing applications. While highly practical for standard laboratory extractions, these thermodynamically closed conditions are only directly applicable to applications such as storage containers and stirred tank reactor (STR) bioreactors. By considering dynamic exposure considerations, such as rinsing, flow, migration kinetics, clearance or product discard, such calculations can greatly simplify safety evaluations even in situations where a reliable leachables analysis is not feasible, e.g. along a running ICB process or in a cell suspension in CGT[25, 28].

7.0

Streamlining the extractables ecosystem and assessment process

Standardizing practical and industry-accepted expectations for component extraction conditions, analytical methods and data reporting represent major milestones in simplifying and streamlining the SUS extractables ecosystem; improvements in these areas include the following:

- Solvents, time points, temperatures and S/V extraction conditions
- Analytical method guidance and reporting limits
- BioPhorum component family templates linking test data to the applicable component part numbers and scaling parameters
- BioPhorum assembly family template linking the final SU assembly to the individual component part numbers and relevant datasets
- BioPhorum guidance for structured spreadsheet summaries of organic compounds and elements extraction data.

In addition, industry publications ICH Q3C and Q3D[7] provide safety data for many commonly encountered extractables and relevant safety data, which can help build confidence and reduce uncertainty when conducting risk assessments. Industry consortia, such as ELSIE, offer ways to build shared libraries of compound safety data with its members. Success with published data and databases requires either peer review or proper curation to ensure utility and acceptance.

The goal of such industry efforts is to streamline and accelerate the often complex data-compilation process, enabling final PERL levels to be estimated easily or computationally with a high degree of certainty. Some early industry efforts explored the creation of third-party-hosted databases of supplier extractables data but these initiatives were ultimately unsuccessful due to a number of challenges including data ownership, access, confidentiality, the level of data required, data maintenance and agreement on how the data would be used. Proprietary databases are also being developed within individual companies[29, 30, 31], which incorporate extractables data, associated physical constants and toxicological parameters, which may help streamline assessments for their assemblies. As availability of standardized, robust characterization data increases, evolving AI tools may further facilitate data compilation, review of compound assignments and overall assessment of materials for their intended use.

8.0

Conclusion

Historical experience across biopharmaceutical manufacturing demonstrates that E&L risk from SUCs is generally low, particularly for applications upstream of final purification. In the absence of an official USP <665> requirement, BioPhorum aligned extractables data provides a scientifically robust foundation for patient safety assessments and regulatory submissions.

Looking forward, new polymer formulations introduced into SUS are expected to be supported by USP <665> data from the outset, while existing materials will continue to rely on established extractables data packages. Furthermore, the use of 50% ethanol as an extraction solvent effectively brackets the extraction potential of DMSO and similar solvents, eliminating the need for additional organic solvents to represent current biologics platforms.

By maintaining alignment with standardized protocols and leveraging existing best practices, the industry can ensure patient safety while avoiding unnecessary testing, supporting a streamlined and science-based approach to E&L risk management.

Appendix

Appendix A – Detailed assessment of the extraction propensity of ADC solvents compared to USP <665> and BioPhorum extraction solvents

Dimethyl sulfoxide (DMSO) is commonly employed as a solvent in the manufacturing processes of cell and gene therapies (CGTs) and antibody-drug conjugates (ADCs), typically at concentrations ranging from 5% to 10% for CGTs and 10% to 20% for ADCs. However, DMSO is not included as a standard extraction solvent in USP <665> or the BioPhorum extractables best practice guidance. There is ongoing discussion within the pharmaceutical industry and among material suppliers regarding the assessment of DMSO for CGT and ADC applications, and whether it may be needed as an additional solvent profile.

The primary aim of this section is to stimulate industry-wide discussion, facilitate comparison of research findings across the sector, and ultimately reach a consensus among end-users and suppliers on whether DMSO should be incorporated into the standard extraction solvent panel.

In evaluating the extraction power of various organic solvents, dielectric constants octanol to water partition coefficients are frequently used. Dielectric constants measure the relative permittivity of free space, or the ability to store electrical energy, and can be used to evaluate solvent polarity. Higher values tend to represent more water-like (80.1 at 25°C) behavior, whereas lower values, such as for hexane (1.88 at 25°C) tend to exhibit very low permittivity of free space. Compared to pure ethanol (24.5 at 25°C), DMSO exhibits a higher permittivity (46.7 at 25°C) indicating it may have less organic extraction power than ethanol.

Similar polarity index values are described in ISO 10993-18 for evaluating solvents used for extraction of polymeric medical devices, with water (10.2), a highly polar compound, exhibiting a much higher polarity index than hexane (0.1). Compared to pure ethanol (4.3), DMSO (7.2) exhibits more polar qualities, indicating a lower propensity for extraction of organic compounds than ethanol. The polarity index also allows for estimation of mixtures. Based on these empirical assessments, DMSO would be expected to exhibit a lower extraction power for organic compounds from plastics than ethanol, and hence the USP 50% ethanol/water mixture may be expected to bracket applications using 10–20% DMSO.

Published data comparing extractions in DMSO to 50% EtOH/water profiles are limited, but several key studies have provided valuable insight. One study[28] investigated the extractables profile of various materials, including polyethylene (PE) and ethylene-vinyl acetate (EVA) films, when exposed to a range of solvents. These solvents included 3M NaOH, 1M HCl, 20×DPBS, 20% ethanol, pure water, pure ethanol, 10% DMSO and several other chemical solutions. The study, which was conducted at 40°C over a 112-day period, concluded that 10% DMSO did not provide additional useful data for the extractables and leachables profile when compared to more commonly used solvents, such as pure water and pure ethanol. Another study[27] focused on the challenges of designing E&L studies for CGT therapies, notes the lack of extensive published extractables data using DMSO. A third study[32], which examined extractables from SUSs used in ADC manufacturing, found that exposure to 15% to 20% DMSO at 30°C for 24 hours resulted in a low E&L risk.

There is ongoing review regarding current data on DMSO extractability, based on controlled extraction studies (CESs) conducted on filters, tubing, bags and assemblies from various suppliers. The detailed data will be published elsewhere in a peer-reviewed journal. Critical findings are summarized here: Solvents with higher organic content, such as 50% ethanol or more, exhibited significantly higher extractability compared to 10% DMSO. One supplier's data indicates that higher concentrations of DMSO (11% to 44%) did not significantly impact the E&L profile. However, another supplier's study found that 20% DMSO extracted significantly higher concentrations of di (2-ethylhexyl) phthalate (DEHP) than 20% ethanol, which may be attributed to the lower ethanol concentration used in the study (below 50%) with respect to the recommended 50% ethanol extracting solvent.

Therefore, it may not be necessary to include 10% DMSO in the standard extraction solvent panel (which typically includes 50% ethanol) when designing extraction studies for SUS in CGTs. However, for ADC applications that involve higher DMSO concentrations (10% to 20%), more data is needed to determine whether solvents such as 50% ethanol can bracket 20% DMSO in extractability. Additional research is essential to further support this assessment and clarify the relative impact of these solvents on E&Ls in ADC production. Ultimately, continued collaboration and data comparison across the industry are crucial to reaching a consensus on whether DMSO should be included in the standard extraction solvent panel. Such efforts will help standardize the testing of SUS used in CGT and ADC manufacturing, ensuring that all materials are assessed for E&L risks in a consistent and reliable manner.

Appendix B – Examples of end-user risk assessment and compilation approaches

Example of cumulative assessment following a four-stage process

An example of how data is compiled by a biomanufacturer to support cumulative risk assessment is described below; the template is available to download. This specific process is intended to be illustrative, and not prescriptive, and may be adapted to fit individual company needs and workflows.

Figure 3: Stage 1 – E&L risk review

Molecule Name	Material Description	PCM Type	Material of construction (MOC)	Product Contact Material (PCM) Manufacturer	Product Contact Material (PCM) Manufacturer Part Number	Sterilization Method	Pre/Post final purification	Distance along the production stream (DAS)	Dilution ratio (DR)/(SA:V)	Exposure temperature (ET)	Exposure duration (ED)	Process-fluid interaction (PFI)	E&L Risk Total	E-L Risk Level (Low, Med, High)	Produt Contact Material Manufacturer E-L Data Available (Yes, No)	Is Additional Data Needed (Yes, No)
	125 Ml flask	Flask	PC		246	gamma	pre	1	1	3	1	3	1.6	low	yes	No
		Bag	LDPE		345	gamma	post	5	3	3	5	5	4.4	Medium	yes	No
		Filter	PVDF		1234	autoclaved	post	9	3	3	5		5.25	High	Yes	Yes
		Tubing											0			

Figure 4: Stage 2 – Sub-component data compilation

Product Contact Material (PCM)	Assembly / Sub-Component	Molecule Name	Product Contact Material (PCM) Type	SAP Name (Material Description)	Product Contact Material (PCM) Manufacturer	Product Contact Material (PCM) Manufacturer Part Number	Subcomponent Qty	Sub Component Manufacturer/ 3rd party PCM Manufacturer	Sterilization method (SM)	Distance along the production stream (DAS)	Material of construction (MOC)	SA (cm2)	Total Subcomponent Surface Area Per assembly (cm2)	PCM Manufacturer E-L Data Available (Yes, No)	Is Additional Data Needed (Yes, No)	E&L Profile ID	Reference to PCM Manufacturer Data
CM1234	Assembly		TUBE	TUBE,7FT,1X1.375,SIL,LB,TC					Gamma	DS After TFF/Final Purification	Silicone	3,364					
CM1234	Sub-			FITTING-CAP 1-1/4" TC			2					NPC		Not Required			
CM1234	Sub-			CLAMP 1-1/2"TC			2					NPC		Not Required			
CM1234	Sub-			BACKUP CUP - 1-1/2" TC			2					NPC		Not Required			
CM1234	Sub-			TRI CLAMP - MOLDED LIM 1-1/2"			2					NPC		Not Required			

Figure 5: Electronic data linked to compilation

Profile ID (specifc reference attached to Data pack	Product Contact Material (PCM) Manufacturer	Brand	Membrane MoC	Catalog #	Sterilization Method	Extract Solution	Identification of Compound	CAS #	Analytical Technique (Detection Method)	Max in units to convert (ug/mL)	Duration	Timepoint (30 min)	Timepoint (1 day)	Timepoint (7 day) ug/cm^2
4			Silicone	N/A	Gamma Irradiation (25-45 kGy)	50% Ethanol	Ethoxytrimethylsilane	1825-62-3	HS-GC-MS			N/A	<RL	N/A
4			Silicone	N/A	Gamma Irradiation (25-45 kGy)	50% Ethanol	Trimethylsilanol	1066-40-6	HS-GC-MS			N/A	<RL	N/A
4			Silicone	N/A	Gamma Irradiation (25-45 kGy)	50% Ethanol	Ethoxytrimethylsilane	1825-62-3	HS-GC-MS			N/A	0.6	N/A
4			Silicone	N/A	Gamma Irradiation (25-45 kGy)	50% Ethanol	Hexamethyldisiloxane	107-46-0	HS-GC-MS			N/A	0.5	N/A
4			Silicone	N/A	Gamma Irradiation (25-45 kGy)	50% Ethanol	1,1-Diethoxyethane	105-57-7	HS-GC-MS			N/A	0.1	N/A
4			Silicone	N/A	Gamma Irradiation (25-45 kGy)	50% Ethanol	Trimethylsilanol	1066-40-6	HS-GC-MS			N/A	0.1	N/A
4			Silicone	N/A	Gamma Irradiation (25-45 kGy)	50% Ethanol	Toluene (common lab contaminant)	108-88-3	HS-GC-MS			N/A	0.2	N/A
4			Silicone	N/A	Gamma Irradiation (25-45 kGy)	50% Ethanol	Unknown siloxane	N/A	HS-GC-MS			N/A	0.1	N/A

Figure 6: Stage 3 – Patient safety assessment

E/L Data Reference # (Path)	Component Category	Manufacturer	Brand	Extract Solution	Identification of Compound	CAS #	Analytical Technique (Detection Method)	Max. Reported Extractable concentration (ug/cm^2)	Potential Leachables (ug/mL)	Total Potential Leachables (ug/mL)	Potential Exposure	ADI	MOS
1	SUV			1% PS80	1,3-di-tert-butylbenzene	1014-60-4	DI GC-MS	0.205	0.155	0.16	16.24	180	11
5	Tubing			50% Ethanol	1,3-Ditert-butylbenzene	1014-60-4	GC-MS	0.04	0.000				
9	SUV			1% PS-80	Benzene,1,3-bis(1,1-dimethylethly)-	1014-60-4		0.03	0.007				
1	SUV			Ethanol:Water (1:1)	1,1-Diethoxyethane	105-57-7	HS GC-MS	0.163	0.123	0.22	22.47	TBD	
5	Tubing			50% Ethanol	1,1-Diethoxyethane**	105-57-7	HS-GC-MS	0.17	0.001				
6	Tubing			50% Ethanol	1,1-Diethoxyethane**	105-57-7	HS-GC-MS	0.29	0.048				
6	Tubing			50% Ethanol	1,1-Diethoxyethane**	105-57-7	HS-GC-MS	0.05	0.008				
7	SUV			50% Ethanol	1,1-Diethoxyethane	105-57-7	GC-FID/MS	0.03	0.002				
11	Tube			50% Ethanol	1,1-Diethoxyethane**	105-57-7	HS-GC-MS	0.42	0.042				

Example process review and risk assessment per BioPhorum leachables best practice

The BioPhorum final risk level determination is based on the weighted sum of risk ratings for each factor shown in Table 7. SUS-specific risk scores are classified as either low (1.0 to 3.6), medium (3.7 to 6.2) or high (6.3 to 9.0). The final risk level determination in the USP <1665> example risk assessment is based on the numerical risk sequence of the risk ratings of each factor shown in Table 8. SUS-specific scores are classified as low, moderate or high. Mitigation factors can be used to adjust the final risk level.

Table 9 shows a summary of three worked examples in a side-by-side comparison of BioPhorum and USP <1665> risk assessment templates.

An Excel-based risk assessment calculator is also provided as a supporting tool alongside this publication.

Table 7: BioPhorum leachables risk assessment matrix

Consideration	Ratings[a]		Weight[b]
Distance along the production stream (DAS)	1	Upstream: *e.g. working cell bank, vial thaw, inoculum, expansion, production, harvest, plasma and solution preparation*	0.40
	3	Purification: *e.g. filtration, chromatography, viral inactivation, viral filtration and UF/DF*	
	5	Bulk drug substance: *e.g. formulation, 0.22µm filtration, BDS storage*	
	9	Final formulation, fill/finish: *e.g. bulk drug product storage, potency adjustment, sterile filtration and filling*	
Exposure temperature (ET)	1	<0°C	0.15
	3	0 to 8°C	
	5	>8°C to 30°C	
	9	>30°C	
Exposure duration (ED)	1	Transient (≤60 minutes)	0.15
	3	Short (≤24 hours)	
	5	Medium (≤7 days)	
	9	Long (>1 week)	
Process fluid interaction (PFI)	1	Limited penetration into polymeric component (i.e. water)	0.15
	3	Low solvation power or low penetration of polymeric component *e.g. neutral pH without organics, surfactants, etc.*	
	5	Medium solvation power or medium penetration of polymeric component *e.g. surfactant, low-concentration organics, high/low pH solutions without organics/detergents*	
	9	High solvation power or high penetration of polymeric component	
Dilution ratio (DR)	1	$<1 \times 10^{-03}$ m²/L	0.15
	3	1×10^{-02} to 1×10^{-03} m²/L	
	5	1×10^{-01} to 1×10^{-02} m²/L	
	9	1×10^{-01} m²/L	

DAS = distance along production stream DR = dilution ratio ED = exposure duration ET = exposure temperature PFI = process fluid interaction.

[a] Parameter range definitions in this table represent examples only. Individual companies should develop their specific range definitions according to their internal policies/standard operating procedures.

[b] Weighting levels used in the table represent examples only. In this table, 0.40 is used for DAS rating, and 0.15 is used for all other considerations. Individual companies may use an equal weighting distribution or may assign weighting levels according to their internal policies.

(Source: **Guide for evaluating leachables risk from polymeric single-use systems**.)

Table 8: USP <1665> Risk assessment matrix

Risk dimension	Level	Description
Duration of contact	1	<24 hours
	2	1–7 days
	3	>7 days
Temperature of contact	1	Refrigerated (2–8°C)
	2	Ambient (15–25°C)
	3	Elevated (>30°C)
Chemical composition of the process stream	1	Aqueous (≤ 5% organic v/v; pH ≥3 and ≤9)
	2	Somewhat organic (>5% and ≤40% organic v/v)
	3	Highly organic (>40% organic v/v) or aqueous, extreme pH (<3 or >9)
Chemical composition of the component	1	Low risk (≤0.1% by weight total additives)
	2	Intermediate risk (>0.1% and ≤1% by weight total additives)
	3	High risk (>1% by weight total additives)

Table 9: Example component risk assessment scoring with BioPhorum and USP <1665>

Guidance	Material	Process stream (PFI)	Contact duration (ED)	Contact temperature (ET)	Location in the process (DAS)	SA/V ratio (DR)	Risk score	Risk level	Mitigating factors	Final risk level
Example A: Virus filtration *Contact time: 8 hours* *Contact temperature: 25°C*					*Sterilization method: None* *Process stream: Somewhat organic, pH 4* *SA/V: 1.53m²/100L = 0.0153m²/L*					
BioPhorum	N/A	5	3	5	3	5	3.9	Moderate	N/A	Moderate
USP <1665>	3	2	1	2	N/A	N/A	3221	Level B Moderate	UF/DF clearance step after	Low
Example B: Final filtration *Contact time: 8 hours* *Contact temperature: Room temperature*					*Sterilization method: Irradiation* *Process stream: Somewhat organic, pH 4* *SA/V: 0.1m²/20L = 0.005m²/L*					
BioPhorum	N/A	5	3	5	9	3	6.0	Moderate	N/A	Moderate
USP <1665>	3	2	1	2	N/A	N/A	3221	Level B Moderate	No clearance	Moderate
Example C: Biopharmaceutical cell-based production *Contact time: 72 days* *Contact temperature: 37°C*					*Sterilization method: Autoclave* *Process stream: 0.15M NaOH, aq. Solution later needed to adjust pH of product* *SA/V: <1 x 10⁻³m²/L*					
BioPhorum	N/A	5	9	5	1	1	3.4	Low	N/A	Low
USP <1665>	3	2	3	3	N/A	N/A	3332	Level C High	Clearance	Moderate

Appendix C – Justification for excluding components with a threshold surface area <1% (negligible or minimally impacting components)

There are various ways to justify excluding components from an assembly risk assessment using a threshold of 1% surface area for the entire assembly. Firstly, a safety-related justification, secondly an analytical consideration can be facilitated.

Safety-based justification using a TTC approach

A negligible surface area can be derived based on a conservative threshold of toxicological concern (TTC) for unknown compounds, an estimated patient dose volume, and an assumed maximum surface-related extractables release from the minimally impacting component.

For an example worst-case calculation, the following conservative parameters are applied and may be adjusted to the specific end-user scenario:

- Generic TTC for an unknown compound: 1.5µg/day
- Patient dose volume: 100mL (worst-case large-volume parenteral)
- Process volume: 100L
- Assumed surface-related extractables release: 1µg/cm²

Based on these assumptions, the maximum tolerable concentration in the drug product is 15µg/L (1.5µg per 0.1L). This corresponds to a total allowable extractables amount of 1,500µg within the entire process stream (15µg/L × 100L).

Assuming a conservative surface-related extractables release of 1µg/cm², a total surface area of 1,500cm² would be required to reach this TTC-based exposure limit. Typical single-use assemblies such as mixing bags or final filling assemblies exhibit total fluid contact surface areas well above 10,000cm².

A component contributing ≤1% of the total assembly surface area would therefore have a maximum surface area of ≤100cm², which is clearly insufficient to reach a toxicologically relevant exposure. This surface area range is comparable to or smaller than common small single-use components such as connectors (approximately 10–200cm²), bioreactor ports (approximately 10–100cm²), or gaskets (<1cm²). Consequently, such components are considered toxicologically non-relevant with respect to extractables contribution.

Analytical justification

Extractables testing of single-use assemblies is performed on the entire fluid contact surface area using standardized surface-to-volume (S/V) ratios (e.g. 1cm²/mL or 6cm²/mL). Assuming a representative surface-related extractables concentration of 1µg/cm², this corresponds to a theoretical extractables concentration of 1µg/mL (or 6µg/mL) in the assembly extract.

A component contributing ≤1% of the total surface area can therefore contribute no more than 0.0µg/mL (or 0.06µg/mL) of a component-specific extractable to the overall extractables profile. Such concentrations are below typical analytical reporting thresholds and screening detection limits used in extractables studies.

As extractables testing is performed on individual components using standardized surface-to-volume ratios, the resulting extractables data are combined to represent a theoretical worst-case extractables profile of the entire assembly. Under this surface-area-based extrapolation approach, the contribution of components representing ≤1% of the total fluid contact area remains analytically insignificant and below typical reporting thresholds.

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