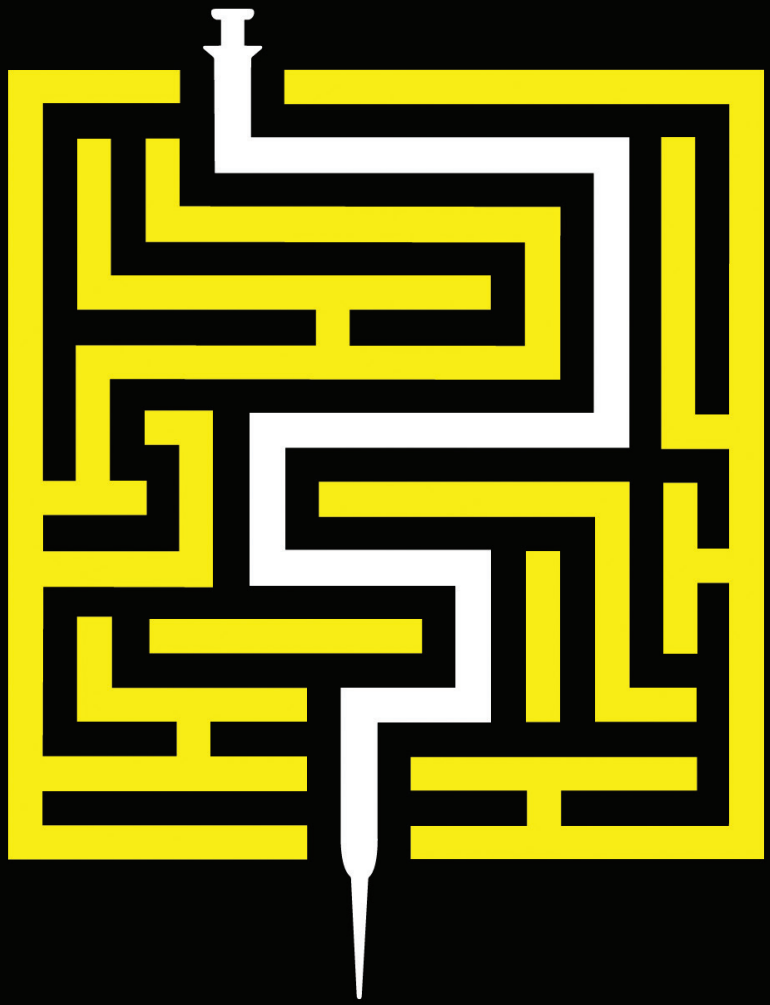


Simplifying the Route to GMP with a Risk-Based Approach to Single-Use Implementation

BioProcess International
SPECIAL REPORT

SARTORIUS

Simplifying the Route to GMP with a Risk-Based Approach to Single-Use Implementation

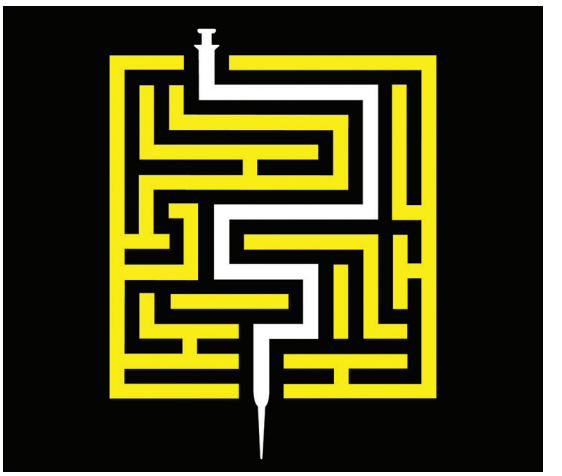
Lorraine Borland, Samuel Dorey, Armin Hauk, and Cecilia Annerén

Adopting single-use systems (SUS) is an attractive solution for current challenges within the biopharmaceutical industry. Such tools help manage risk by allowing developers to postpone capital investment until there is greater certainty of their drugs' market approval. Single-use solutions offer the flexibility to design a purpose-built facility that can be redesigned rapidly in response to increasing product diversity. Selecting SUS with demonstrated scalability facilitates technology transfer and supports rapid adoption of new processes.

Single-use solutions also lower cost of goods (CoG) while helping to improve sustainability. They use 70–80% less water compared with stainless-steel systems, which rely on steam-in-place (SIP) and clean-in-place (CIP) activities, and they support intensified processing, minimizing facility footprint and downsizing heating, ventilation, and air conditioning (HVAC) requirements. SUS also ease validation burdens, simplifying and accelerating a drug's journey to the clinic and removing internal costs at the site of manufacture.

However, before implementing single-use solutions, biomanufacturers must ensure that the required SUS product quality attributes are met. The BioPlan Associates *19th Annual Report and Survey of Biopharmaceutical Manufacturing Capacity and Production* found that four of the top five ranked attributes to consider when selecting SUS were quality-based, including general assurance of SUS-product quality, extractables and leachables (E&L), and integrity and robustness (**1**). Providing sterility assurance is paramount, and the industry is exploring adoption of X-ray sterilization to meet that demand.

Herein, we provide insights into regulatory guidelines for SUS implementation in biopharmaceutical manufacturing. Our discussion encompasses four pillars: forming a regulatory strategy, addressing flexible radiation-sterilization processes, assessing E&L, and assuring integrity and robustness. In the next chapter, we present a customer case study demonstrating how Sartorius Confidence validation services used a risk-based approach to carry out a safety assessment of leachables risk for a client's SUS-based manufacturing process. The guidance given in this report is meant to help enable a data-driven, risk-based approach to implementing SUS for protection of drug quality and patient safety.



WWW.SARTORIUS.COM

Forming a Regulatory Strategy

A regulatory strategy — including a risk-mitigation strategy for all materials and components entering into a manufacturing process — should be formed as early as possible in the drug-development life cycle. The regulatory landscape for SUS implementation and associated test methods is complex and continues to evolve, particularly for new modalities such as cell therapy and RNA-based therapeutics. However, resources for guidance include regulatory agencies, published methods and guidelines, and consortia and industry groups.

The starting point for forming a regulatory strategy is to consult good manufacturing practice

(GMP) guidance from the appropriate agency. The US Food and Drug Administration (FDA) and the European Commission provide globally accepted guidances that should be incorporated into your quality management system (QMS).

US Code of Federal Regulations (CFR) Title 21 Part 211.65, which addresses equipment construction within current good manufacturing practice (CGMP) for finished pharmaceuticals, places SUS in line with other product-contacting materials applied in a biomanufacturing process:

> Equipment shall be constructed so that surfaces that contact components, in-process materials, or drug products shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements. (**2**)

In the European Union, the August 2022 update to *Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use — Annex 1: Manufacture of Sterile Medicinal Products* goes further than does the CFR to provide detailed guidance in a dedicated SUS section (8.131–8.139) (**3**). For our purposes, we focus on sterility assurance, E&L, and robustness and integrity requirements for SUS implementation in accordance with Annex 1 guidance.

Regarding sterility assurance, the guidance states:

> 8.133. Sterilisation processes for SUS should be validated and shown to have no adverse impact on system performance.
>
> 8.134. Assessment of suppliers of disposable systems including sterilisation is critical to the selection and use of these systems. For sterile SUS, verification of sterility assurance should be performed as part of the supplier qualification and evidence of sterilisation of each unit should be checked on receipt. (**3**)

Specific requirements for E&L safety assessment are detailed as follows:

> 8.136. The extractable and leachable profiles of the SUS and any impact on the quality of the product, especially where the system is made from polymer-based materials, should be evaluated. An assessment should be carried out for each component to evaluate the applicability of the extractable profile data. For components considered to be at high risk from leachables, including those that may absorb processed materials or those with extended material contact times, an assessment of leachable profile studies, including safety concerns, should be taken into consideration. If applying simulated processing conditions, these should accurately reflect the actual processing conditions and be based on a scientific rationale. (**3**)

It is important to form your regulatory strategy AS EARLY AS POSSIBLE in the drug-development life cycle, including your risk-mitigation strategy for all materials and components entering your manufacturing process.

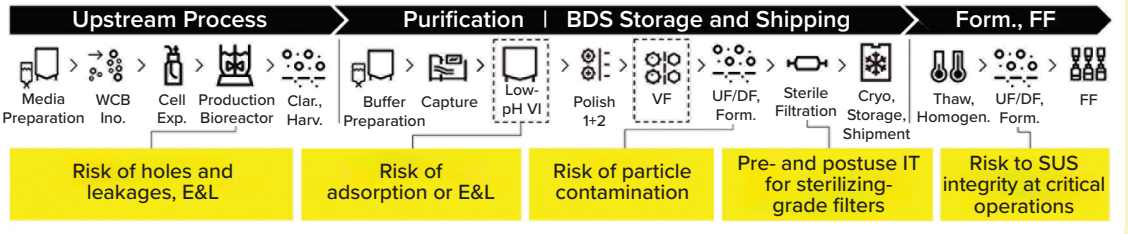
—Lorraine Borland, global manager for SU process technologies at Sartorius

SUS integrity relates directly to a given manufacturing process and operational conditions:

> 8.137. SUS should be designed to maintain integrity throughout processing under the intended operational conditions. Attention to the structural integrity of the single use components is necessary where these may be exposed to more extreme conditions (e.g., freezing and thawing processes) either during routine processing or transportation. This should include verification that intrinsic sterile connection devices (both heat sealed and mechanically sealed) remain integral under these conditions. (**3**)

Once a robust regulatory knowledge base has been established, SUS must be implemented using a quality risk management (QRM) approach that proactively identifies, scientifically evaluates, and satisfactorily controls potential safety risks. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q9 (R1) guidance provides principles and examples of tools for QRM that can be applied to different aspects of pharmaceutical quality (**4**).

Figure 1: A quality risk management (QRM) approach is essential for proactive identification, scientific evaluation, and satisfactory control of safety risks for single-use systems (SUS) throughout a biomanufacturing process.



BDS = bulk drug substance **Cell Exp.** = cell expansion **Clar., Harv.** = clarification and harvest **Cryo** = cryopreservation
E&L = extractables and leachables **FF** = fill–finish **Form.** = formulation **Homogen.** = homogenization **IT** = integrity testing
UF/DF = ultrafiltration/diafiltration **VI** = virus inactivation **VF** = virus filtration **WCB Ino.** = inoculation of a working cell bank

The two primary principles of QRM are that

- evaluation of risk to quality should be based on scientific knowledge and ultimately should be linked to the protection of patients
- the level of effort, formality, and documentation in a QRM process should be commensurate with the level of risk.

Those principles can be applied to assess SUS as part of overall processes for materials management and manufacturing risk assessment. For a streamlined approach, start with a simple process map. Then, identify your manufacturing-process steps and create flow diagrams detailing all SUS and how they will be used during each step (Figure 1). For each process step, ask these fundamental questions:

- What could go wrong?
- What is the likelihood (or probability) that it will go wrong?
- If it does go wrong, what consequences will result, and how severe will they be?

Figure 1 shows some considerations specific to SUS. A risk-assessment process should include consideration of all data and prior knowledge available to help with the analysis of risk.

SUS safety assessments should also consider the criticality of a given process step. Two key areas of concern are business continuity and assurance of drug-product quality and patient safety. For upstream and downstream process steps, the critical factors are those that would affect a company's ability to manufacture. For example, a company should ensure that SUS introduce no cell-growth inhibitors into a culture system, confirm chemical compatibility between a process and SUS, protect SUS integrity, and elucidate the effects of E&L at each process stage.

As a process moves toward more SUS-critical applications (e.g., storage and shipping of bulk drug substance), the level of risk increases. It is important, for instance, to consider the effects of E&L on a product and what could happen to SUS integrity during storage and shipping to another site for final filling.

Formulation and filling are critical steps, requiring the highest burden of proof. Therefore, all data packages must be validated to assure SUS chemical and biocompatibility, integrity, purity, and cleanliness.

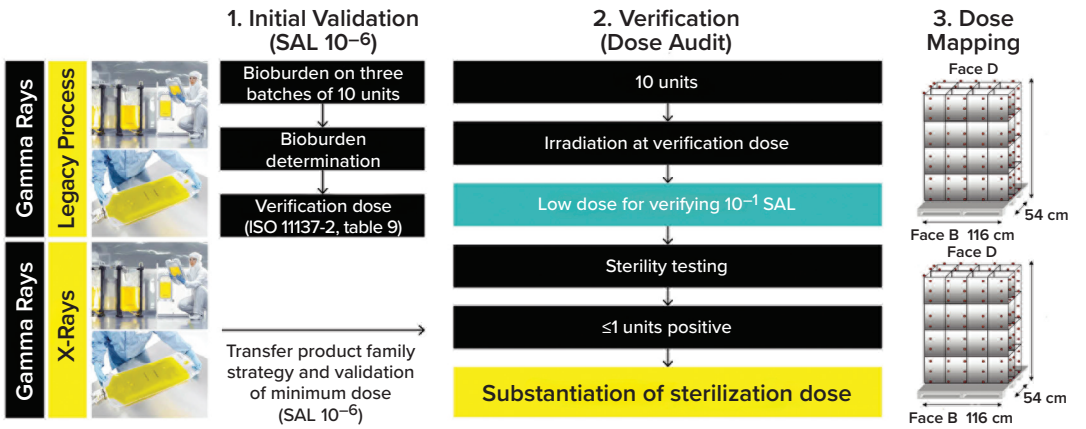
Addressing Flexible Radiation-Sterilization Processes

Sterility assurance is foundational for SUS implementation and must be validated with robust quality data to ensure sufficient performance throughout a product's shelf life. Part 1 of the International Organization for Standards (ISO) 11137-1:2006 document on *Sterilization of Healthcare Products Radiation* provides guidance on requirements for radiation-sterilization processes (**5**). It is critical that SUS meet those requirements.

Gamma irradiation has been the SUS-industry standard for meeting finished-product requirements. Interestingly, the standards outlined in ISO 11137 are agnostic to the type of irradiation technology applied. As SUS implementation becomes increasingly prevalent in biomanufacturing processes, flexible radiation-sterilization processes will become essential. Achieving sterilization with flexible and interchangeable technologies such as gamma irradiation, X-rays, or electron beams addresses potential disruptions to SUS supply and helps to ensure business continuity.

The purpose of all irradiation technologies is the same: to achieve sterility by disrupting biological processes within all microorganisms present, ultimately leading to their death. Radiation sterilization is the physical process of delivering a dose of radiation to SUS products; sterility is

Figure 2: Validating sterility according to the ISO 11137 standard and maintaining processes (SAL = sterility assurance level)



validated irrespective of the irradiation technology used as long as the dose is sufficient.

Validating sterility per the ISO 11137 standard is mandatory. Sterility also should be maintained throughout a process (Figure 2). For gamma irradiation, validation first requires determination of the nature and level of bioburden. Next, the dose is substantiated, and dose mapping is performed in all irradiation centers. Dose mapping ensures that a 25-kGy dose is received at all points of a pallet and product and that products do not receive more than the maximum dose defined during product-development qualification. When implementing X-ray technology as an alternative to gamma processing, sterility validation follows the same pathway, taking into account legacy results obtained during validation with gamma irradiation.

It is important to acknowledge that radiation sterilization can affect plastic materials. Exposure to radiation causes polymer materials to undergo changes that are influenced by factors such as the radiation dose, the chemical composition of the polymers, the presence of additives, and the presence of oxygen in the environment during irradiation. Changes to polymers must be mitigated when applying different sterilization technologies. The impacts of gamma and X-ray irradiation on SUS properties have been compared by leveraging plastics and product knowledge and using statistical evaluation. Products are eligible for both gamma and X-ray sterilization when the impacts are equivalent and when sterility is validated. Through this process, the experimental dose is substantiated, and a sterility assurance level (SAL) of 10^{-6} can be reached.

Sterilization must be considered as part of the development and manufacturing processes for single-use systems. Sterility is validated AGNOSTICALLY to the irradiation technologies applied, and products sterilized by gamma- and X-ray technologies have shown EQUIVALENT performance.

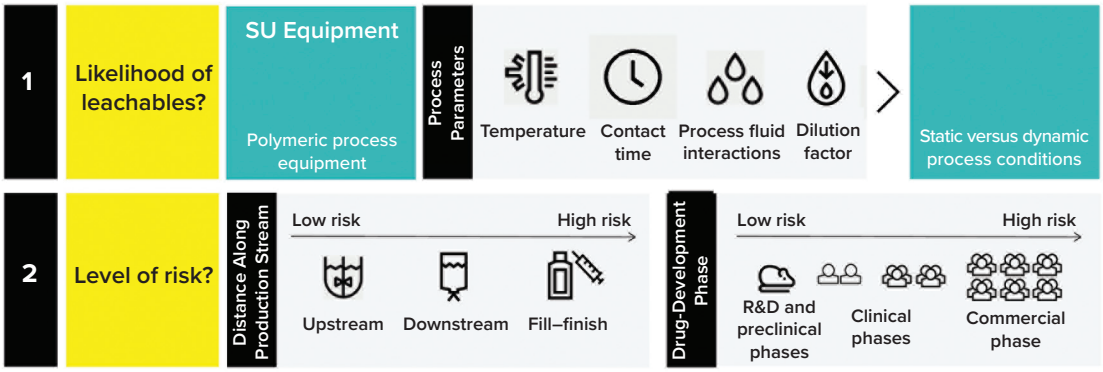
—Samuel Dorey, principal scientist in materials and irradiations at Sartorius

Assessing Extractables and Leachables

Typically, all parts of a production process are exposed to intermediates, and drug-product solutions are potential sources of impurities. Consequently, single-use equipment and components that come in contact with product fluids should be checked for compounds that are released into the process stream.

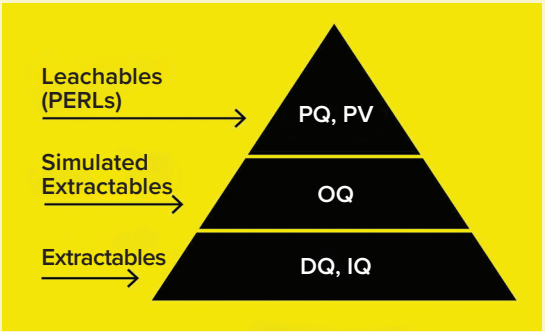
Extractables are substances that can migrate from an SUS into a contacting liquid. *Process-equipment–related leachables* (PERLs) are a subset of SUS extractables that can occur in an up- or downstream manufacturing environment. *Leachables* are PERLs that can survive downstream processing and become drug impurities. Such

Figure 3: End users must consider the likelihood of leachables and the level of potential risk with single-use (SU) equipment. (R&D = research and development)



ICH Q9: "Quality risk assessments begin with a well-defined problem description or risk question."

Figure 4: Qualifications triangle for extractables and leachables (E&L) (**6**)



PERLs = process-equipment–related leachables
PQ = process qualification **PV** = process validation
OQ = operational qualification **DQ** = design qualification
IQ = installation qualification

compounds can compromise process performance, drug-product quality, and patient safety. Thus, they are of significant concern. Consequently, drug makers should consider assessment of extractables from SUS and appropriate investigation of PERLs and leachables.

The methodology for extractables testing is listed in several guidance documents, including *United States Pharmacopeia* section ‹665› on "Plastic Components and Systems Used To Manufacture Pharmaceutical Drug Products and Biopharmaceutical Drug Substances and Products" (**7**), the BioPhorum Operations Group (BPOG) *Best Practice Guide on Extractables Testing of Polymeric Single-Use Components Used in Biopharmaceutical Manufacturing* (**8**), and the ICH Q3E guideline on E&L (for which a concept paper was proposed on 30 June 2020 and full publication is scheduled for some time in 2024) (**9**).

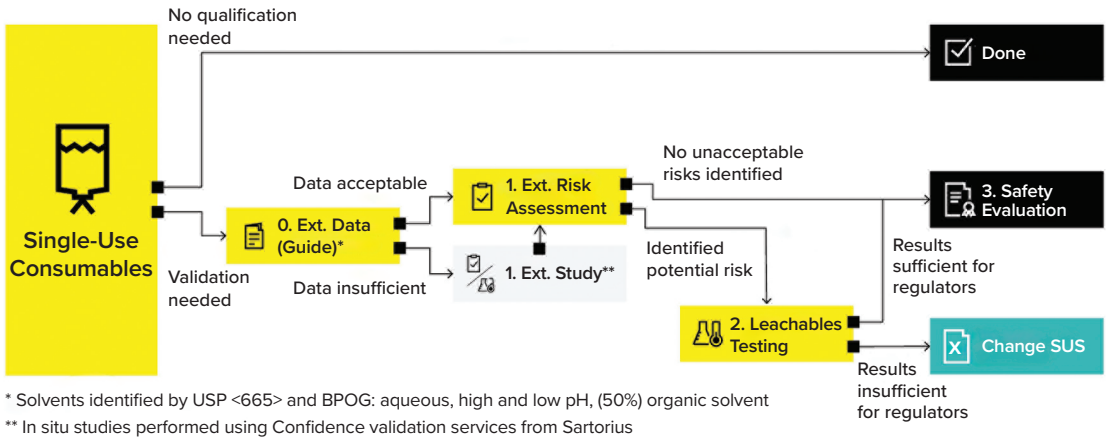
SUS extractables testing is performed under worst-case conditions to create an extractables profile. Extensive testing programs are required to achieve fully elucidated extractables profiles. However, comprehensive extractables data are available for the industry's most widely used SUS. Suppliers such as Sartorius can provide complete extractables profiles for their products. Such information is essential because only fully elucidated extractables profiles allow for conclusive safety assessments.

As part of such assessments, drug manufacturers must consider the likelihood of SUS-derived PERLs and leachables and their potential to harm process performance, product quality, and patient safety (Figure 3). High operating temperatures, long contact times, potential process-fluid interactions, low dilution factors, and dynamic process conditions can increase or decrease levels of compounds leached from plastics.

The level of risk relates directly to the position of the single-use device in the production stream and to the drug-development phase (Figure 3). Leachables may pose low risks for product quality and patient safety during initial upstream operations but then become high risks as a product moves closer to fill and finish. Similarly, risk levels increase as a product advances from early research and development or preclinical studies through to late clinical phases and commercial manufacture.

SUS manufacturers' extractables guides and validation packages are references of quality data that can be used as decision-making tools when implementing SUS during process development. Extractables guides should be used for initial design qualification (DQ) and installation qualification (IQ) to assess the material safety of the respective SUS;

Figure 5: An extractables (Ext.) and leachables (Leach.) testing approach for single-use systems (SUS); BPOG = BioPhorum Operations Group, USP = *United States Pharmacopeia*



* Solvents identified by USP <665> and BPOG: aqueous, high and low pH, (50%) organic solvent

** In situ studies performed using Confidence validation services from Sartorius

such data also should be consulted for further process qualification (PQ), including the design of a subsequent leachables study (Figure 4).

Following a standard E&L evaluation approach ensures compliance with regulatory expectations, such as those expressed in Annex 1. Figure 5 presents a simple decision tree to guide you through key points to address during E&L study.

For low-risk materials (e.g., a funnel used during upstream production), E&L validation might not be required. If validation is deemed to be necessary, start with a review of extractables guides to determine whether sufficient data are available for an extractables risk assessment (Figure 5). When available data are insufficient, that information can be supplemented with in situ and leachables-simulation studies performed by SUS-manufacturer validation programs, such as Confidence validation services from Sartorius.

Outputs from extractables risk assessment will determine whether specific leachables testing is required or whether you can proceed directly to safety assessment. If you find that a tested single-use solution presents an unacceptable leachables safety risk, then you might decide to change your SUS (Figure 5).

An elegant and highly sustainable way to meet safety-assessment criteria listed in Annex 1 is to apply in silico modeling. Based on physical principles and comprehensive extractables profiles available in curated databases, in silico tools are scientifically justified and can be used to predict extractables levels, which then can be extrapolated to PERLs and leachables (**10**). Extrapolating data for PERLs and leachables assessment is particularly necessary for complex single-use assemblies — e.g., in cases with components from different suppliers and when leachables simulation testing has been inadequate.

Curated databases such as those included with Sartorius ExSim software provide extractables profiles obtained by standardized test protocols, ensuring the availability of physicochemical properties for exposure calculations and modeling. Access to such information helps to simulate both equilibrium and dynamic process conditions. With a curated database, permittable daily exposure (PDE) and tolerable daily intake (TDI) values can be collected to supplement threshold of toxicological concern (TTC) values in safety assessments.

Coupled with databases containing prior knowledge, in silico tools enable predictions,

Today, E&L assessment can be SIMPLIFIED and ACCELERATED using both prior knowledge accumulated in state-of-the-art databases and a breadth of in silico methodologies and tools. The days of repetitive, empirical E&L studies are over.

—Armin Hauk, principal scientist in process-development science and testing at Sartorius

Figure 6: Testing of 45 typical process-equipment–related leachables (PERLs) with a modern, high-throughput cell-painting assay using a human cell line; experiments carried out at the Max Planck Institute for Molecular Physiology in Dortmund, Germany (**11**).

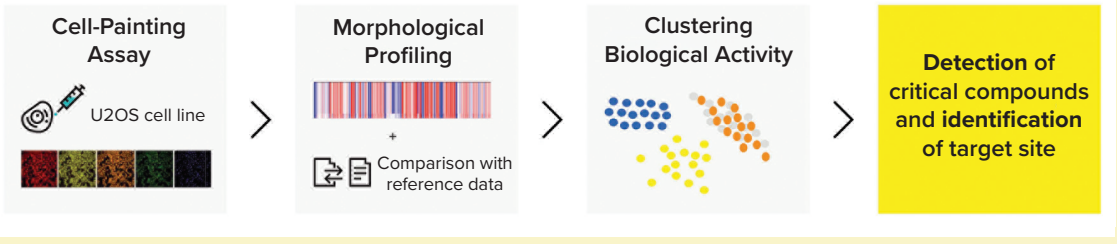


Photo 1: The Linkit AX aliquoting solution

assessments, and evaluations to be carried out without time-consuming and expensive physical testing (**12**). In summary, building a comprehensive understanding of SUS extractables and leachables profiles and behaviors can be achieved in partnership with SUS manufacturers and their expert knowledge.

Example Safety Assessments: The following case study describes how a state-of-the-art safety assessment might proceed and shows how it should be modified to serve advanced-therapy medicinal product (ATMP) applications.

The Linkit AX system is a multicomponent, fully closed single-use technology that directs fluid from a peristaltic pump into 10 Flexsafe single-use bags for simultaneous filling (Photo 1). The system can be used for aliquoting small quantities of fluid for applications such as small-batch media preparation and ATMP processing. The aim of this case study was twofold: to perform a risk assessment regarding patient safety for Linkit AX technologies used in classical biopharmaceutical production and to perform a safety assessment for Linkit AX use in ATMP applications.

When assessing a multicomponent product, it is important to consider all aspects of the equipment

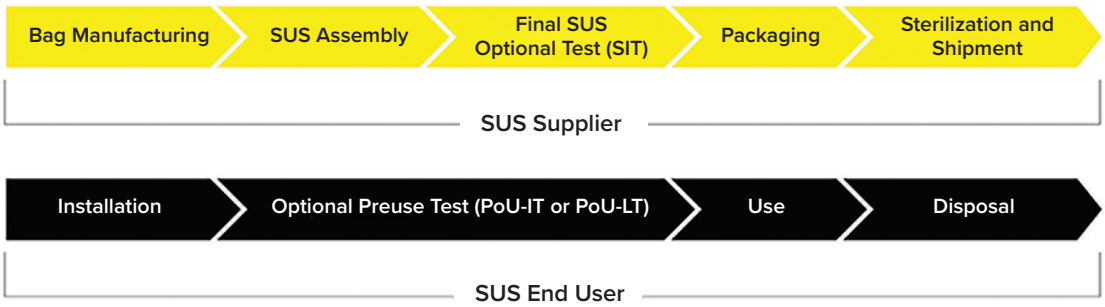
Our data demonstrated that the high-throughput cell-painting assay could be a POWERFUL TOOL for screening and investigating the effects of process-equipment–related leachables on human cells manipulated ex vivo.

for potential extractables. This case required evaluation of extractables data for the single-use manifold, the tubing, and the single-use bag film. The Sartorius ExSim system retrieved and combined the respective data. For the patient-safety assessment considering a classical biopharmaceutical application, safety thresholds and limits (PDE values) were taken from ExSim databases and compared with the expected exposure. ExSim software enabled us to perform the whole exercise within a few minutes, returning robust data and evaluations that were scientifically justified and fully traceable.

The PDE approach is insufficient for assessing ex vivo manipulated and cultivated cells in ATMP applications because toxicological effects on isolated cells cannot be compared with commonly available toxicological information. To address that limitation, we completed a safety assessment for a potential ATMP application using a high-throughput cell-painting assay using a human cell line (Figure 6). The test, established at the Max Planck Institute of Molecular Physiology in Dortmund, Germany, can evaluate more than 700 cell features in a single run by analyzing cellular and subcellular morphological alterations (**11**).

Our data demonstrated that the high-throughput cell-painting assay could be a powerful tool for screening and investigating the effects of PERLs on human cells manipulated ex vivo. The results were

Figure 7: Responsibilities of single-use system (SUS) manufacturers and end users to ensure equipment robustness; PoU-IT = point-of-use integrity test, PoU-LT = point-of-use leak test, SIT = system-integration testing



encouraging: High-risk PERLs identified by the assay were not present in the Linkit AX system, and no Flexsafe bag film extractables were induced in the assay. Of note is that levels of the cytotoxic leachable bis(2,4-di-tert-butylphenyl)-phosphate (bDtBPP) were low enough that they would not compromise cells, meeting requirements of the test set forth by Deutsche Gesellschaft für chemisches Apparatewesen (DECHEMA) and ASTM International (**13**). Such findings demonstrate that the Linkit AX system poses a low risk to human cells manipulated ex vivo and, therefore, that it is well suited for ATMP applications.

Assuring Integrity and Robustness

Ensuring SUS integrity is an essential part of a contamination control strategy (CCS). SUS should be designed to maintain integrity throughout processing under the intended operational conditions. Special attention should be given to the structural integrity of single-use components during exposure to extreme conditions (e.g., freezing and thawing processes), during both routine processing and transportation.

It is important to conduct a holistic approach to risk analysis and mitigation by assessing product and process robustness, quality, and process control. All steps of the SUS life cycle should be evaluated to identify and address risks for integrity loss. SUS manufacturers and end users have distinct responsibilities to ensure robustness across the product life cycle (Figure 7).

For low- to medium-risk operations, ensuring that an SUS manufacturer has adequate validation and controls in its manufacturing process may be sufficient. Packaging and shipping validation are of particular importance. End users also should ensure proper operator training for handling SUS.

High-risk or critical process steps should follow that essential mitigation strategy but with additional considerations. Those could include implementing point-of-use leak (PoU-LT) and integrity testing (PoU-IT) into critical process steps and/or adopting complete supplier integrity testing on a per-SUS-batch lot release. Ultimately, an IT strategy should ensure that SUS are secure before and during normal use cases. However, because testing processes can damage SUS, the risks and benefits of performing integrity testing should be evaluated to define the most relevant strategy for each use case.

Depending on the APPLICATION and the CRITICALITY of an integrity breach, specific supplier or point-of-use integrity/leak testing may be needed. Quality risk assessment should consider the feasibility and the risks and benefits of such testing.

—Cecilia Annerén, head of product management, advanced applications, at Sartorius

Case Study: Microbial Ingress and Liquid Leakage for Flexsafe Products

SUS integrity testing should confirm the defined barrier properties of a single-use product at the maximum allowable leakage limit (MALL). Integrity testing validation data from an SUS manufacturer can be incorporated directly into SUS-integrity risk assessment. However, it is important to ensure that supplied validation data have been generated over

Figure 8: Validation data for single-use system (SUS) setup over multiple pressure conditions

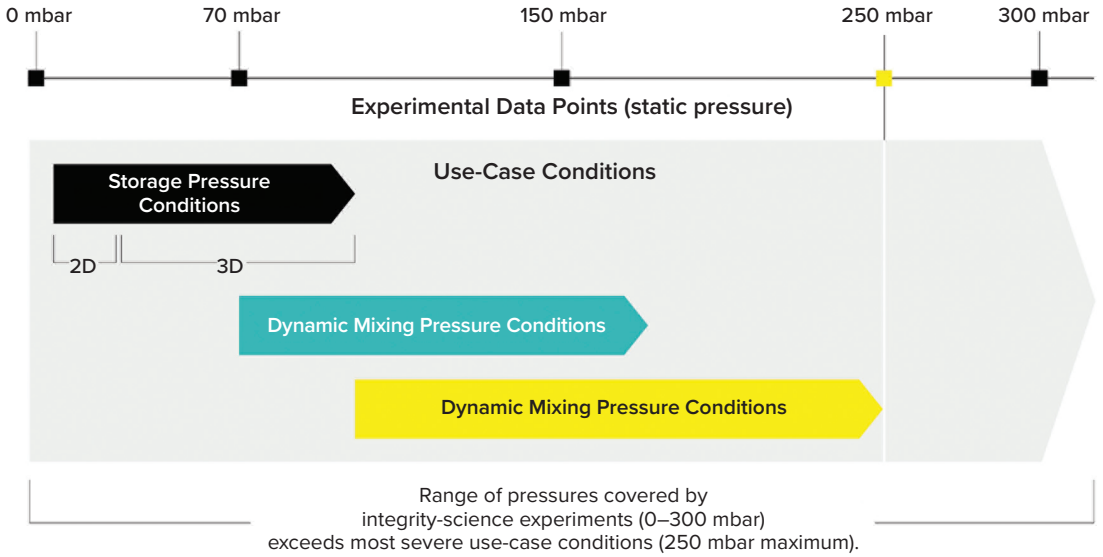
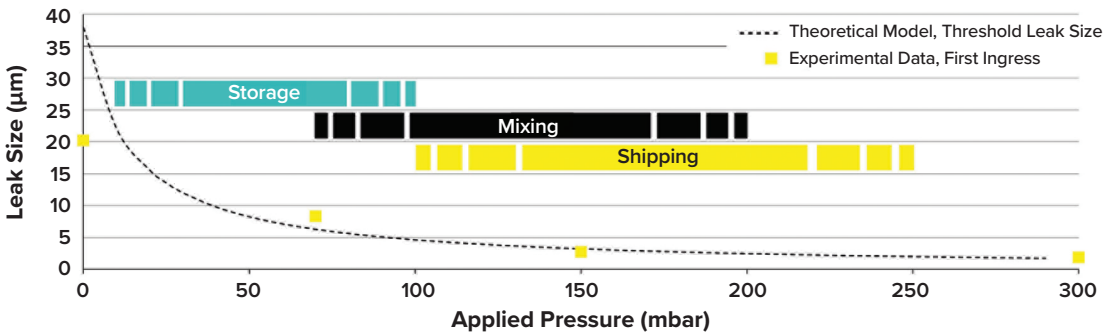


Figure 9: Example of a manufacturer's data package for single-use system (SUS) integrity testing



By leveraging SUS manufacturers' data packages, end users can determine the risk of integrity breach in their manufacturing processes and set mitigation strategies to PREVENT such events.

multiple use cases and under worst-case conditions. Figure 8 shows the experimental setup for scientific studies performed by Sartorius using a microbial-ingress test method to determine the MALL for Flexsafe SUS under different operational conditions (**14–16**). In such tests, storage pressure conditions for two- and three-dimensional bags represent the static pressure due to the liquid column height in the bag. Dynamic mixing pressure conditions represent dynamic pressure pulses from liquid motion during mixing. And dynamic shipping pressure conditions represent dynamic pressure pulses from acceleration and shocks during liquid transport (Figure 9). The most severe use-case condition was found to be 250 mbar. Sartorius carried out multiple integrity-science experiments over a range of pressures (0–300 mbar), exceeding even the most severe use-case condition (Figure 9). The results demonstrated that 2 µm is the MALL for liquid leaks and microbial ingress for all use-case conditions up to 250 mbar.

By leveraging SUS manufacturers' data packages, end users can determine the risk of integrity breach in their manufacturing processes and set mitigation strategies to prevent such events. Such strategies include ensuring selection of the most robust materials for the given process needs.

Simplify Your Route to GMP

Above, we presented a simplified framework for devising a robust and reliable SUS implementation strategy. The framework consists of four pillars: forming a regulatory strategy, addressing flexible radiation-sterilization processes, assessing E&L, and assuring integrity and robustness.

You can simplify your route to GMP with a robust QRM-based approach to SUS implementation. In the next chapter of this report, we provide a case study showcasing how Sartorius Confidence validation services support the speedy performance of robust E&L assessments.

References

1 *19th Annual Report and Survey of Biopharmaceutical Manufacturing Capacity and Production: A Study of Biotherapeutic Developers and Contract Manufacturing Organizations*. BioPlan Associates: Rockville, MD, 2022; https://www.bioplanassociates.com/biopharmaceutical-manufacturing-capacity-production.

2 21 CFR 211. *Current Good Manufacturing Practice for Finished Pharmaceuticals*. US Food and Drug Administration: Silver Spring, MD, 2023; https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=211.65.

3 *EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use: Annex 1 — Manufacture of Sterile Medicinal Products*. European Commission: Brussels, Belgium, 2022; https://health.ec.europa.eu/document/download/e05af55b-38e9-42bf-8495-194bbf0b9262_en?filename=20220825_gmp-an1_en_0.pdf.

4 ICH Q9 (R1). *Quality Risk Management*. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Geneva, Switzerland, 2023; https://database.ich.org/sites/default/files/ICH_Q9%28R1%29_Guideline_Step4_2022_1219.pdf.

5 ISO 11137-1:2006. *Sterilization of Health Care Products: Radiation — Part 1: Requirements for Development, Validation and Routine Control of a Sterilization Process for Medical Devices*. International Organization for Standards: Geneva, Switzerland, 2006; https://www.iso.org/standard/33952.html#.

6 Li K, et al. Creating a Holistic Extractables and Leachables (E&L) Program for Biotechnology Products. *PDA J. Pharm. Sci. Technol.* 69(5) 2015: 590–619; https://doi.org/10.5731/pdajpst.2015.01073.

7 USP ‹665›. Plastic Components and Systems Used To Manufacture Pharmaceutical Drug Products and Biopharmaceutical Drug Substances and Product. *USP–NF* February 2022; https://www.uspnf.com/notices-665-nitr-20220225.

8 Scott B, et al. *BioPhorum Best Practice Guide for Extractables Testing of Polymeric Single-Use Components Used in Biopharmaceutical Manufacturing*. BioPhorum Operations Group: Sheffield, United Kingdom, 2022; https://www.biophorum.com/download/extractables-testing-of-polymeric-single-use-components-used-in-biopharmaceutical-manufacturing.

9 ICH Q3E. *Final Concept Paper: Guideline for Extractables and Leachables (E&L)*. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Geneva, Switzerland, 2020; https://database.ich.org/sites/default/files/ICH_Q3E_ConceptPaper_2020_0710.pdf.

10 Hauk A, et al. Using Extractables Data from Single-Use Components for Extrapolation to Process Equipment-Related Leachables: The Toolbox and Justifications. *Eur. J. Pharm. Sci.* 163, 2021: 105841; https://doi.org/10.1016/J.EJPS.2021.105841.

11 Hauk A, et al. Extractables and Leachables in Gene and Cell Therapies: The Assessment of Extractables from Single-Use Systems. *Pharmind* 85(11) 2023: 1036–1045; https://www.ecv.de/beitrag/pharmind/Extractables_and_Leachables_in_Gene_and_Cell_Therapies.

12 Hauk A, et al. *Sartorius Extractables Simulator: Simplifying E&L Through In-Silico Modeling*. Sartorius: Göttingen, Germany, 2021; https://www.sartorius.com/en/whitepaper-nbsp-sartorius-extractables-simulator--754122.

13 ASTM E3231-19. *Standard Guide for Cell Culture Growth Assessment of Single-Use Material*. ASTM International: West Conshohocken, PA, 2019; https://www.astm.org/e3231-19.html.

14 Aliaskarisohi S, et al. Single-Use System Integrity I: Using a Microbial Ingress Test Method To Determine the Maximum Allowable Leakage Limit (MALL). *PDA J. Pharm. Sci. Technol.* 73(5) 2019: 459–469; https://doi.org/10.5731/pdajpst.2018.009688.

15 Aliaskarisohi S, et al. Single-Use System Integrity II: Characterization of Liquid Leakage Mechanisms. *PDA J. Pharm. Sci. Technol.* 75(3) 2021: 258–272; https://doi.org/10.5731/pdajpst.2020.012088.

16 Hogreve M. Single-Use System Integrity IV: A Holistic Approach Based on Compiled Scientific Study Data. *PDA J. Pharm. Sci. Technol.* 77(2) 2023: 133–144; https://doi.org/10.5731/pdajpst.2022.012794.

17 *EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use: Annex 15 — Qualification and Validation*. European Commission: Brussels, Belgium, 2015; https://health.ec.europa.eu/system/files/2016-11/2015-10_annex15_0.pdf.

18 TRS 937. *40th Report of the WHO Expert Committee on Specifications for Pharmaceutical Preparations*. World Health Organization: Geneva, Switzerland, 2019; https://www.who.int/publications/i/item/WHO_TRS_937.

Lorraine Borland *(lorraine.borland@sartorius.com) is global manager for SU process technologies,* ***Samuel Dorey*** *(samuel.dorey@sartorius.com) is principal scientist in materials and irradiations,* ***Armin Hauk*** *(armin.hauk@sartorius.com) is principal scientist in process development science and testing, and* ***Cecilia Annerén*** *(cecilia.anneren@sartorius.com) is head of product management for advanced applications, all from Sartorius.*

Support for Leachables Risk Evaluation

A Case Study in Confidence

Marine Cannuel and Nicole Liu

Following regulatory guidelines such as EU GMP Annex 15 on "Qualification and Validation," manufacturers should perform justified and documented risk assessments to provide evidence that their processes, operated within established parameters, can perform effectively and reproducibly to produce a medicinal product (**17**). The World Health Organization (WHO) Annex 4 supplementary guideline also recommends that manufacturers prove that critical aspects of their operations are controlled appropriately (**18**).

In this case study, the Confidence validation services team at Sartorius supported our client, mAbxience, with regulatory compliance and performance of a process risk analysis. The team provided global support through facilities in Europe and South America. The scope of the analysis was comprehensive, covering 24 product formulations used in 13 drug-substance manufacturing steps and investigating more than 240 single-use sustems (SUS) — including filters, containers, tubing, and bags — from different suppliers. In addition to the high complexity, the tight timeline required that the studies be carried out quickly.

To find the right extractables and leachables (E&L) strategy, the manufacturing steps were first classified based on their position in the given manufacturing process, with increasing risk classification given to process steps at the end of the process (in proximity to the final filling). To maximize efficiency, the validation experts suggested a grouping approach to sort SUS by manufacturer and contact material. The 24 product formulations were grouped into several types (surfactants and organic formulations, acid and alkaline solutions, buffers, culture media, and drug substances). To cover all potential risks, the team also considered a worst-case dilution rate for all tested SUS and evaluated contact time and temperature.

Using results from the grouping approach, the Confidence team classified the risk at each process step as low, medium, or high based on the BioPhorum *Best Practices Guide for Evaluating Leachables Risk from Polymeric SUS*, which covers the parameters fixed with a proposed scoring system to determine when a specific leachable evaluation is necessary (**8**). For low-risk process steps, references to biocompatibility and physicochemical tests can be used for justification. For medium-risk process steps, extractables data should be evaluated, and if needed, an extractables assessment report can be prepared based on specific process conditions. For process steps of high risk, leachables testing is recommended.

After the process risk analysis, six SUS for upstream steps presented as low risk, six SUS for downstream steps were of medium risk, and two SUS at the fill–finish step presented high risk. The testing recommendation was to use extractables guides to cover the low-risk equipment, performing an extractables assessment with ExSim software for the medium-risk SUS, and leachables testing for the high-risk SUS.

Thanks to its expertise in SUS and leachables risk evaluation, the Confidence validation services team at Sartorius performed the analysis (which aligned with regulatory expectations) more quickly and cost-effectively than if it had been carried on the customer's side. The team provided a report detailing the manufacturing process, the risk value associated with each parameter, and the leachables-risk ratings calculated. Leveraging more than 30 years of experience, the team also gave expert guidance on how to proceed based on scientific evidence, empowering mAbxience to perform the required testing and align with regulatory expectations.

Reflecting on the project, Roberto Fouces Martínez (manufacturing manager at mAbxience) notes,

> Our partnership with the Sartorius Confidence team was pivotal in streamlining our compliance with complex regulatory standards. Their expertise in process risk analysis and leachables risk evaluation not only enhanced our understanding of the risks associated with various manufacturing steps, but also guided us in implementing the most suitable materials and strategies. This collaboration has significantly bolstered our confidence in maintaining the highest standards of product safety and quality.

Marine Cannuel *is manager, and* ***Nicole Liu*** *is product manager, both in Sartorius Confidence validation services; marine.cannuel@sartorius.com.*