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White Paper

The Role of BioPhorum Extractables Data in the Effective Adoption of Single-Use Systems

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The landscape for single-use systems

Single-use systems are increasingly popular in biomanufacturing. Their adoption offers numerous advantages for greater efficiency and productivity in applications such as final filtration, mixing and aseptic connections. Single-use systems can reduce capital investment in facilities and equipment, eliminate the need for cleaning procedures and their required validation, reduce startup times and decrease the risk of cross-contamination. Further benefits include more dependable lead times and faster delivery of higher-quality, lower-cost products.

Despite these advantages, single-use systems can also contain risk. Polymeric materials in single-use systems can introduce a range of unwanted chemicals into the manufacturing process fluid. Substances such as styrene monomers, stabilizers, lubricants and slip agents, pigments and antioxidants such as BHT may either leach out of the system or be extracted by solvents used in the manufacturing process. For patient safety, biomanufacturers must systematically assess and mitigate the risks posed by any extractables and leachables (E&L) in these systems. Unfortunately, the burden of obtaining E&L data and assessing and mitigating any risk found is the top reason some researchers restrict their use of single-use systems.

Guidance for evaluating SUS for extractables and leachables

Although formal guidelines for E&L assessments have not yet been enacted, there is nonetheless a regulatory expectation that researchers will test for these potentially harmful contaminants. Agencies such as the U.S. Food and Drug Administration (FDA) Center for Biologics Evaluation and Research (CBER) recommend a risk-based approach to evaluation.

In such an approach, indication, safety, product characteristics, dosage, formulation and stability are all factors.

If there appears to be lower risk with the materials in question, the sponsor can submit supplier data, a detailed justification for applying this data and an explanation of why no more testing is required. Guideline on process validation for the manufacture of biotechnology-derived active substances and data to be provided in the regulatory submission, EMA/CHMP/BWP/187338/2014 If there is relevant risk, the sponsor may have to determine toxicity based on maximum dosage of potential leachables derived from extractables data. If the risk of maximum dosage of potential leachables remains, leachable evaluation and testing may be necessary. Furthermore, if product quality could be affected by a potential leachable, studies may need to assess the effect on product quality, including efficacy. These evaluations are possible when the supplier provides extractables data, which can supplement final product quality assessments.

Beyond the regulatory sphere, numerous industry organizations have created best-practice strategies for implementing extractables studies. Prominent among these coalitions is BioPhorum formerly known as BioPhorum Operations Group (BPOG), a global consortium of large biopharmaceutical manufacturers. Many manufacturers are fulfilling regulatory expectations following BioPhorum extractables test methods and using compendial methods from U.S. Pharmacopeia. USP <665> which is effective in May 2026 is the first guidance specific to extractables evaluation of single-use components used in manufacturing. An overview of these standard extractables testing protocols is shown in Table 1.

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Table 1. Comparison of BioPhorum and USP protocols for extractables testing

	BioPhorum Requirements	USP <665> (November 2021)
Scope	Single-use components in contact with fluid path (for biopharmaceutical manufacturing)	Single-use and multi-use components and devices with fluid path contact (for pharmaceutical and biopharmaceutical manufacturing)
Solvents	1. 50% Ethanol 2. 0.5 N NaOH 3. 0.1 M Phosphoric Acid 4. WFI	1. 50% Ethanol 2. 0.2 M KCl, pH 3 3. 0.1 M Phosphate buffer, pH 10
Analytical methods	HPLC-DAD/MS (APCI, ESI, +/–) NVR ICP/MS TOC DI-GC/MS, HS-GC/MS pH	Described in USP <1663>. Broader scope in methods selection.
Time points	1–3, dependent on component	1, dependent on component
Pre-treatment	If an item is pre-treated prior to actual use, the item should be pre-treated exactly the same way before being tested for extractables.	Polymeric components are most appropriately tested when they have been conditioned or processed in a manner consistent with their intended use and as specified in the manufacturer's instructions for use.
Timing	Revised BioPhorum Protocol published in Apr 2020	Effective May 2022

Note that while these protocols are not identical, the general idea is similar and at least one or the other can apply to most drug products and substances. Testing is performed at various time points and temperatures. Resulting extraction solutions are subjected to robust and extensive analyses to determine what levels of volatile, semi-volatile and nonvolatile organic compounds, and metals, have been extracted. Either approach will produce an array of data that must then be evaluated in the context of the proposed process.

Supplier-provided single-use system extractables data saves time and resources

The task of gathering all the product information required to assess risk and optimize E&L test strategies for a process can be daunting. Consider the filtration setup shown in Figure 1. This one assembly exposes the contact fluid to multiple components as indicated.

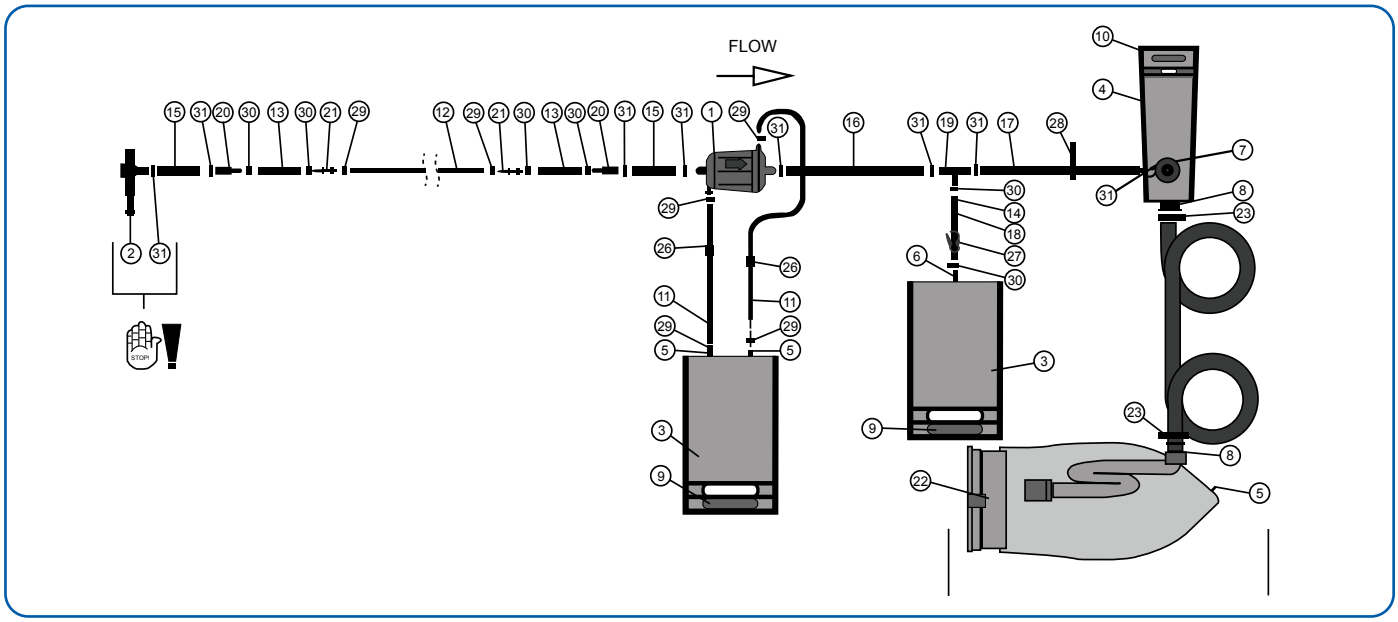


Figure 1. Schematic of a 32-component Mobius® filtration assembly containing various types of single-use filters and bags

A proper E&L assessment must account for the risk imposed by each component in the fluid path. Once each component's materials of construction (MOC), resin identity and manufacturing process have been documented, similar components may be grouped together for analysis. Two to three lots of worst-case representative samples are extracted as per the chosen testing protocol, under worst-case conditions. Finally, sample analysis yields an extractables profile quantifying all volatile, semi-volatile and nonvolatile organic compounds found, as well as elemental impurities.

This complex process is much simpler when the supplier provides comprehensive, organized datasets for its single-use components, such as filters and bags.

The overall strategy for E&L validation

Determining risk and interpreting extractables data to construct a reasonable validation strategy is a complex process; expert assistance can be valuable. The general steps in evaluating each single-use component are as outlined in Figure 2.

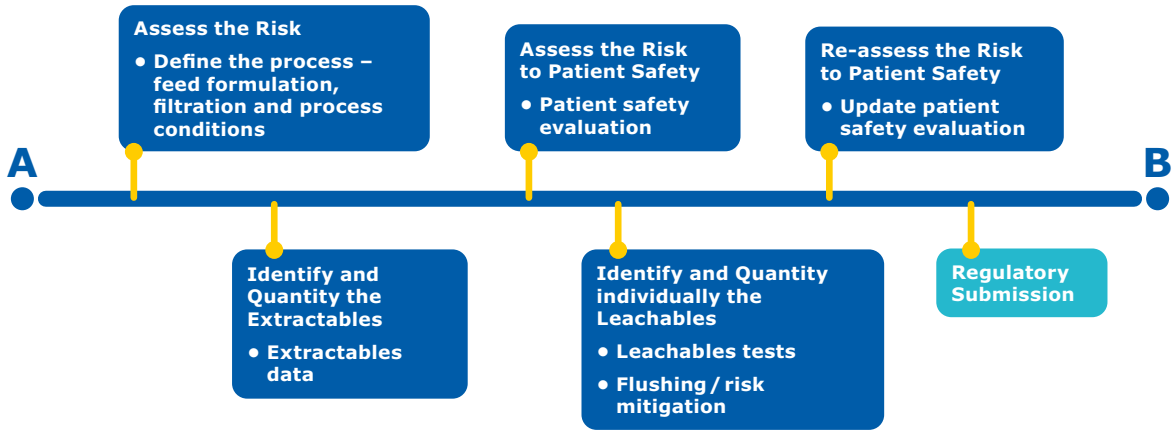


Figure 2. Risk-based approach to E&L assessment

In general, drug manufacturers must evaluate the single-use components that are in product contact under the given process conditions such as duration, temperature, solvents, material characteristics, etc. Any risk-mitigating steps occurring later in the process can be taken into consideration. At this stage, an extractables profile, if available, would facilitate product, process and dosage-specific assessment. If, thus far, the patient safety evaluation indicates no risk, the findings may simply be reported and monitored for future changes. On the other hand, if there is a risk to the patient, a leachables study may be conducted under normal product application or storage conditions. Risk may also be mitigated through a process-step modification, such as including an additional flush. A final option could be to change the material of the component.

The following case study demonstrates this approach to risk assessment.

Case study: A risk-based approach to E&L assessment of a final sterilization, single-use assembly

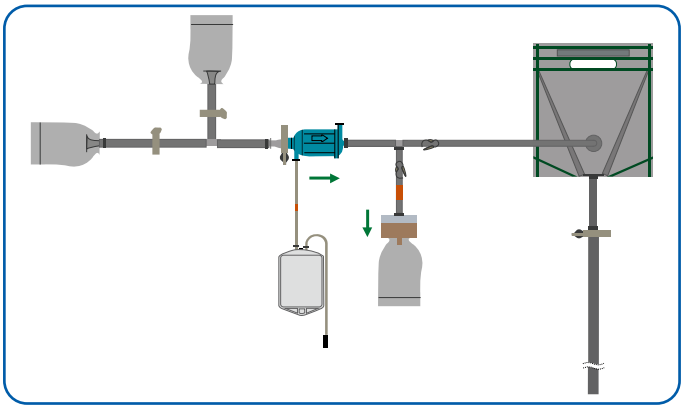


Figure 3. Monoclonal antibody final sterilization system including a single-use Millipore Express® SHC filter and a PureFlex™ Plus Mobius® single-use bag

Figure 3 illustrates a representative, single-use system for which E&L assessment might be required: a single-use final sterilization assembly including a capsule filter and a product collection bag. This assembly serves a commercial monoclonal antibody (mAb) application with typical excipients. The aqueous drug product solution includes 2% API, 0.02% PS80 surfactant and 2% dextrose, buffered to a pH of 5. Filter contact time is eight hours with a filtration temperature of 25 °C.

Extractables test data prepared by the single-use system component manufacturer can be used to analyze this system. The availability of such data in an easy-access format saves significant time and effort. If these components were evaluated in accordance with BPOG guidelines, they were tested under worst-case conditions, as shown in Table 2.

Table 2. BioPhorum conditions for extractables testing of the single-use components in the mAb system

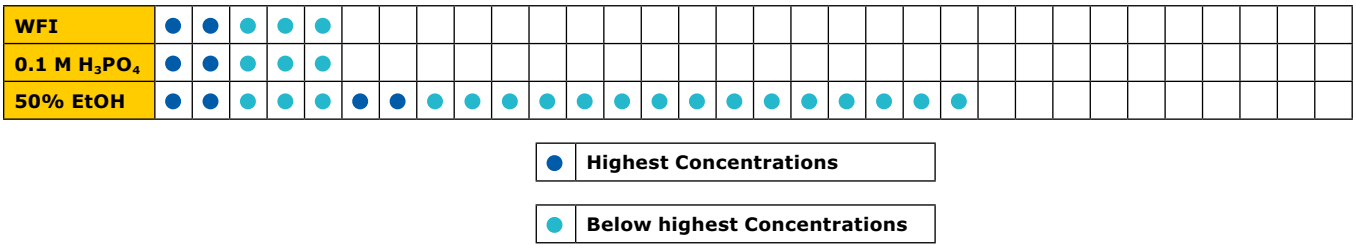
Gamma-compatible Millipore Express® SHC Filter	PureFlex™ Plus Mobius® Single-Use Bags
• Three different lots • Gamma-irradiated at 45–55 kGy • Two time points at 1 day/7 days • Temparature 40 °C • SA/V = 4:1	• Three different lots • Gamma-irradiated at 45–55 kGy • Three time points at 1 day/21 day/70 days • Temperature 40 °C • SA/V = 6:1

Summary results of this testing are shown in Figures 4 and 5. The data includes numerous compounds that were produced through extraction using the solvents listed. Darker dots indicate compounds found in the highest concentrations.

Component 1

Extractables Data for Gamma-Sterilized Millipore Express® SHC Filter

32 Individual Compounds Identified



Individual Quantitation

Compound Evaluation

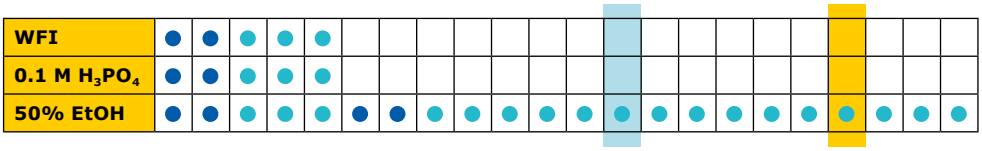
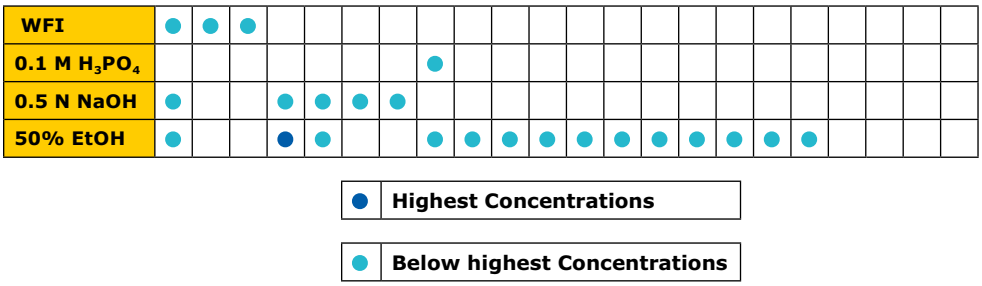


Figure 4. Summary of extractables test results for the single-use filter. The highlighted compounds are present in multiple components.

Component 2

Extractables Data for PureFlex™ Plus Film

22 Individual Compounds Identified



Individual Quantitation

Compound Evaluation

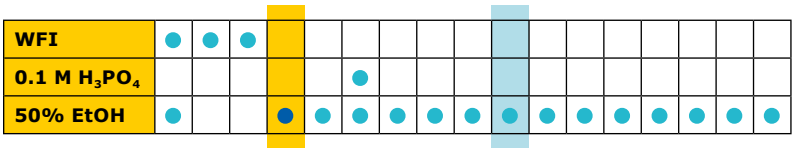


Figure 5. Summary of extractables test results for the film used to make the single-use bag. The highlighted compounds are present in multiple components.

Water (WFI), phosphoric acid (0.1 M H_2PO_4) and ethanol (50% EtOH) are most applicable to this process based on the pH and formulation of the drug product. Hence, the focus is on the compounds extracted by these solvents. One observation is that the two compounds highlighted – 2,4-di-tert-butylphenol and 1,3-di-tert-butylbenzene – are common degradants and appear in the extraction data for both the filter and the single-use bag. In such cases, once the single-use system contributions have been scaled to the actual system in question, it is the sum of these contributions that must be evaluated for toxicity.

To illustrate this calculation, here is the analysis of 2,4-di-tert-butylphenol. The first step is to scale the data for each component's contribution. Filter data is provided as 0.36 mg for a 10" filter, which has a surface area of 0.54 m^2. However, the 5" device in use has a surface area of 0.23 m^2. The proportionately scaled contribution of this compound by the filter, then, is:

(0.23/0.54) 0.36 mg = 0.153 mg = 153 µg

Similarly, the extractables data for the bag must be scaled, as it is reported per cm^2. The 10 L bag has a surface area of 3,077 cm^2, so the bag's contribution is:

(2.81 µg/cm^2) 3,077 cm^2 = 8,646 µg

Note that the bag contributes the majority of the total 2,4-di-tert-butylphenol, which is 8,799 µg/assembly.

The next consideration is, how much of this will be delivered to patients? If the minimum process volume is 4 L, and a 1 L flush is added, the total volume is 5 L. The final concentration of 2,4-di-tert-butylphenol is:

8,799 µg/5,000 mL = 1.76 µg/mL

Aspects of posology such as dosage, frequency and route of administration are then used to calculate the actual patient exposure, as shown in Figure 6.

Patient Exposure

Posology

Maximum Drug Dosage	**100 mL**
Route of Administration	**Intravenous**
Frequency	**Once per week**
Expected Duration	**Lifetime**

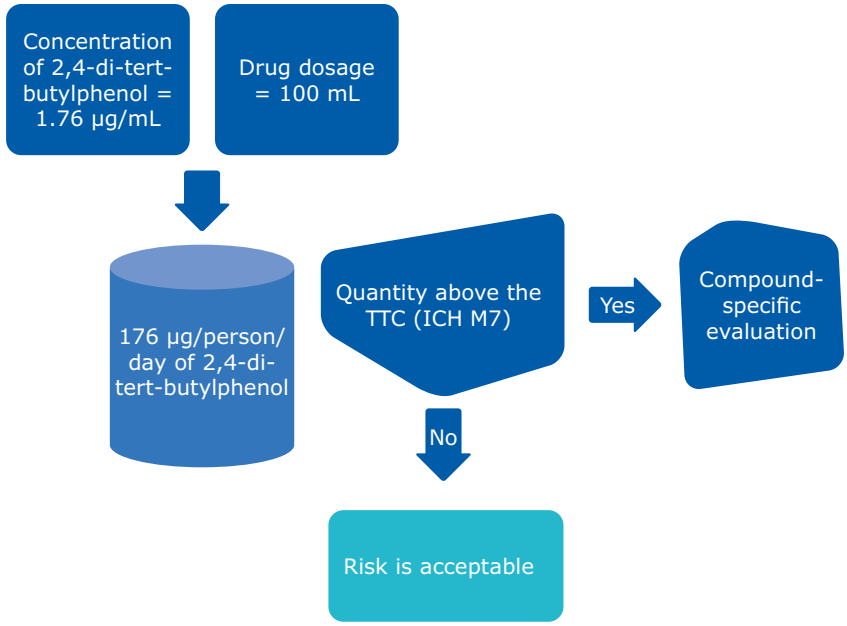


Figure 6. Patient exposure as a function of posology

The result is then assessed for risk. If the risk were acceptable in reference to the threshold of toxicological concern (TTC), that finding could be reported and no further investigation would be required. However, in this case, the exposure exceeds the TTC. Further analysis is needed to determine the compound-specific permitted daily exposure (PDE) for this particular compound. To do that, results from pre-existing rat toxicology studies are converted to equivalent human values using the ICH Q3C modifying factors for relating the data to humans, F1–F5. An additional bioavailability correction accounts for the incomplete absorption seen when drugs are administered orally, as they were in the rat study. The overall approach is shown in Table 3.

Table 3. Converting the result of an animal study of 2,4-di-tert-butylphenol toxicity to a compound-specific PDE for humans

Factors:		**Starting dosage: 75 mg/kg bw/day**	**No-observed-adverse-effect level (NOAEL) when orally administered to rats**
Interspecies extrapolation	F1	5	Rats -> humans
Intraspecies variability	F2	10	Default
Exposure duration	F3	10	4 weeks -> chronic
Severity of effects	F4	1	No severe effects
LO(A)EL -> NO(A)EL	F5	1	NOAEL available
Bioavailability correction	F6	10	Oral -> parenteral, no data
Overall safety factors		5,000	
Permitted daily exposure (PDE)		75 mg/kg bw/day / 5,000 = 15 µg/kg bw/day	
PDE for 50 kg adult human		**750 µg/day**	

The calculated PDE for 2,4-di-tert-butylphenol – 750 µg/day – can now be compared to the total exposure calculated above – 176 µg/day. The risk has now been shown to be acceptable and can be reported as such. If the risk were still too high, a method for mitigating risk elsewhere in the process would need to be applied. The overall process of risk evaluation is summarized in Figure 7.

Acceptable Risk

Patient Safety

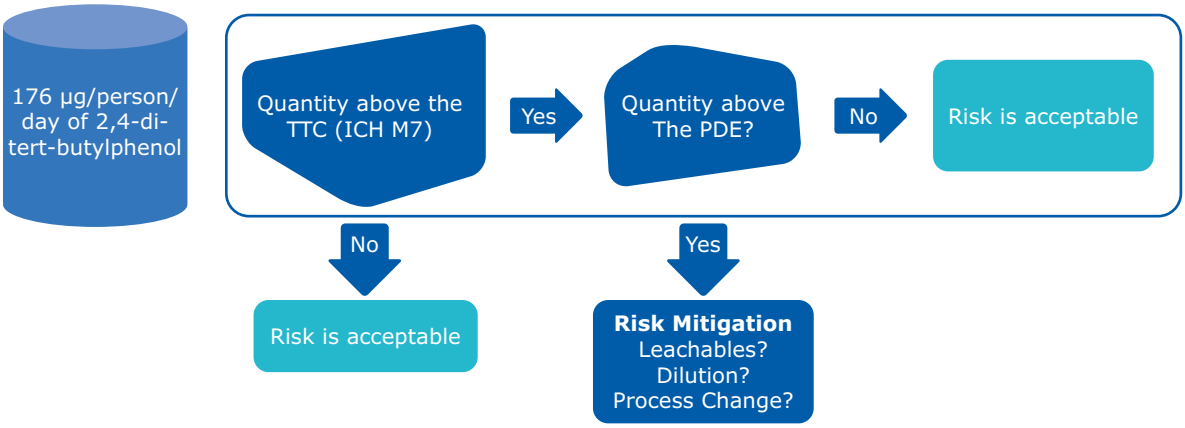


Figure 7. E&L risk evaluation decision tree

Summary

Regulatory expectation does require patient safety evaluations with supporting data for manufacturing components that directly come into contact with drug manufacturing process streams. Readily available extractables data can help manufacturers using single-use technology to accelerate product qualifications, risk assessments and process optimization. E&L risk analysis is a complex process, and expert guidance is helpful to ensure compliance and drug safety. While the task of analytical E&L test data interpretation and submission can be daunting, the right single-use system supplier can save time by providing single-use system E&L data in well-organized, easy-to-use formats.

Emprove® Program

Accelerating Drug Regulatory Approvals

The Emprove® Program entails organized, detailed product information and assistance to help customers with material qualification and risk assessment of biomanufacturing devices, as well as process optimization. By avoiding the need to develop test methods, search quality-related documents or evaluate scientific data, customers can reduce the time, effort and resources needed to prepare documents for drug approval submissions.

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