Nelson Labs™
A Sotera Health company

E&L TESTING OF SINGLE-USE SYSTEMS FOR PRODUCTION

SVP SYMPOSIUM

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29 MAR 2022

OVERVIEW

1. Regulatory requirements for SUS
2. Interest groups on standardization
3. How to set up extractables and leachables studies for SUS?
    - 3.1 Risk assessment of the materials used in the production process
    - 3.2 Gather extractables data
    - 3.3 Evaluation of extractables data
    - 3.4 Leachables study

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1. REGULATORY REQUIREMENTS FOR SINGLE-USE SYSTEMS

U.S.

Title 21 of the Code of Federal Regulations (CFR) 211.65 (1)

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

EUROPE

ICH Q7 – GMP Practice Guide

"...Equipment should not be constructed so that *surfaces that contact raw materials, intermediates or API's* ***do not alter the quality of the intermediates and API's*** *beyond the official or other established specifications..."*

EU – GOOD MANUFACTURING PRACTICES

"...Production Equipment ***should not present any hazard*** *to the products. The parts of the production equipment that come into contact with the product must not be reactive, additive... that it will affect the quality of the product..."*

1. REGULATORY REQUIREMENTS FOR SINGLE-USE SYSTEMS

OBSERVATIONS

- The CFR 211.65 and GMP's do ***not only*** refer to the ***impact on Safety***, but also on:
  - Quality
  - Purity
  - Strength (e.g. adsorptive behavior)
  - Reactive behavior
  - Additive behavior

- Reasoning of Regulators
  - Know your process
  - Know the impact of SUS on the quality of the product
  - Prove that you have made an assessment

1. REGULATORY REQUIREMENTS FOR SINGLE-USE SYSTEMS

- **United States Pharmacopeia <665>:**
  Plastic components and systems used to manufacture pharmaceutical drug products and biopharmaceutical drug substances and products

- **United States Pharmacopeia <1665>:**
  Characterization of plastic components and systems used to manufacture pharmaceutical drug products and biopharmaceutical drug substances and products

Official date: 1st of May 2022

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2. INTEREST GROUPS ON STANDARDIZATION

BPSA

BIO-PROCESS SYSTEMS ALLIANCE
Advancing Single-Use Worldwide

- Trade association of suppliers and users of single-use bioprocess technologies

- Publications:
  - Recommendations for Extractables and Leachables Testing (2008)
  - Recommendations for Testing and Evaluation of Extractables from Single-use Process Equipment (2010)

- Available at www.bpsalliance.org

2. INTEREST GROUPS ON STANDARDIZATION

BPOG (BioPhorum Operations Group)

- Global association of Biopharmaceutical manufacturers (end users)
- Publications:
  - Standardized Extractables Testing Protocol for Single-Use Systems in Biomanufacturing (Nov 2014)
  - Best Practices Guide for Evaluating Leachables Risk from Polymeric Single-Use Systems used in Biopharmaceutical Manufacturing (Mar 2017)

BioPhorum

- Global association of end users and suppliers
- Publications:
  - BioPhorum Best Practices Guide for Extractables testing of Polymeric Single-Use Components used in BioPharmaceutical Manufacturing (Apr 2020)
  - A Comprehensive Review of BioPhorum Standardized Extractables Testing Data: A Deep-Dive into Similarities, Differences and Trends Across Extraction Solvents and Time Points (Sep 2020)

www.biophorum.com

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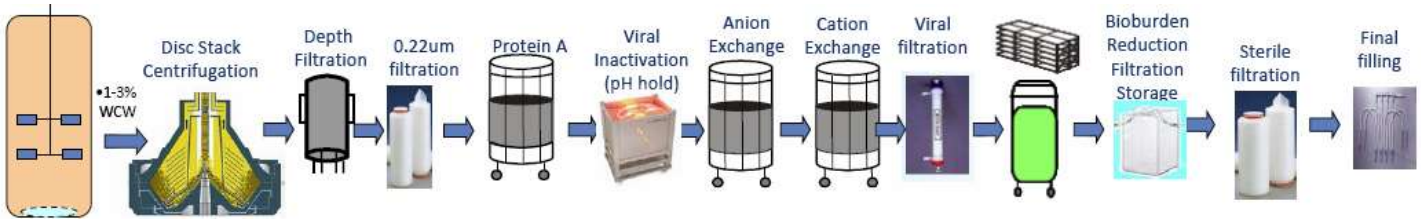
3.1 RISK ASSESSMENT

Why perform a risk assessment?

- Bioproduction process may contain a lot of different SUS



Bioproduction example from a slide from Presentation at IQPC Conference "Disposable Solutions", Munich, 18-20 FEB2014: "BPOG's Extractable Protocol Standardization Journey – Review 2013 Process ande Planning for 2014" Ken Wong (Sanofi-Pasteur), with permission of the Author.

- Many SUS are custom made
  - Bag from Vendor A
  - Tubing from Vendor B
  - Filter from Vendor C
  - Connectors from Vendor D

- Complete E/L assessment for each component can be a challenging task

3.1 RISK ASSESSMENT

Perform a risk assessment

- Instead of testing every SUS for extractables, a **risk based approach** can be applied to focus on the materials with high impact

- GOAL?

  Select single-use components with greatest potential for objectable levels of leachables with regard to **safety** and **quality** of the final product, and **process performance**

- When?

  Best **performed early in the process development** when changes are more easily addressed

3.1 RISK ASSESSMENT

Create a list a "product contact materials"

- Understand your manufacturing process from start to finish!

- List any material with potential to leach into the final product through "product contact" with starting materials, intermediates, final DP,...

- Can include:
  tubing, bags, filters, connectors, O-rings, tangential flow cassettes, chromatographic resins, final bulk storage vessels,...





3.1 RISK ASSESSMENT

“RISK FACTORS” to consider for E/L assessment of “product contact materials”

1. Material compatibility
2. Proximity to final DP / distance along production stream
3. Composition of contact solution
4. Surface area to Volume ratio
5. Contact temperature and contact time
6. Pretreatment steps
7. Process performance

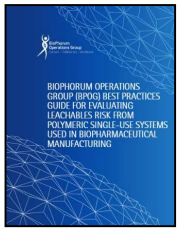
3.1 RISK ASSESSMENT

How to perform a risk assessment?

- Assign numerical values to different risk factors and convert to final risk score
- Risk assessment should be clear and well argumented towards the authorities
- Different company-specific approaches might be used
- Risk assessment based on ICH Q9 Quality Risk Management

3.1 RISK ASSESSMENT

- **BPOG:** Example of numerical values that indicate the risk level, including weight factors assigned to each risk factor



(2017)

BPOG E/L Risk Assessment
Example of Proposed Risk Assessment

Risk factors

Risk levels with rating

Weight factor

Consideration	Ratings [1]		Weight [2]
Distance along production stream (DAS)	1	Synthesis: Vial thaw, Inoculum, Expansion, Production, Harvest, Plasma	0.40
	3	Purification: Affinity chromatography, Viral inactivation, Ion exchange chromatography, Viral filtration, UF/DF	
	5	Bulk Drug Substance: Filtration, BDS storage	
	9	Final Formulation, Fill / Finish Potency adjustment, Sterile filtration Filling, Lyophilization, FDP Storage	
Exposure Temperature (ET)	1	Frozen	0.15
	3	0 C to <10 C	
	5	10 C to <30 C	
	9	> 30 C	
Exposure duration (ED)	1	Transient (i.e. ≤ 60minutes)	0.15
	3	Short (i.e.≤ 24 hours)	
	5	Medium (i.e.≤ 7 days)	
	9	Long (i.e. > 1 week or more)	
Process Fluid Interaction (PFI)	1	Non-solvent/No penetration of polymeric component	0.15
	3	Low solvation power or low penetration of polymeric component	
	5	Medium solvation power or medium penetration of polymeric component	
	9	High solvation power or high penetration of polymeric component	
Dilution ratio (DR)	1	< 1.E-03 m^2/L e.g. fittings, connectors, gaskets	0.15
	3	1.E-02 - < 1.E-03 m^2/L e.g. short/high diameter tubing	
	5	1.E-01 - < 1.E-02 m^2/L e.g. long low diameter tubing	
	9	> 1.E-01 m^2/L e.g. filters, final container	

(1): Parameter range definitions in this table represent an example. Individual companies should develop their specific range definitions according to their internal policies / SOPs.

(2): Weight levels used in the table represent an example. In this example, 0.40 is used for DAS rating and 0.15 is used for all other considerations. Individual companies may use equal weight distribution or may assign weight according to their internal policies.

PharmaEd
9/15/2015

3.1 RISK ASSESSMENT

Example: Sterilization filter

Risk rating (EPR) =

(9 x 0.40)

+

(5 x 0.15)

+

(3 x 0.15)

+

(5 x 0.15)

+

(9 x 0.15)

=

6.9

E / L Propensity Rating (EPR)	Calculated Risk Rating	Risk Category
	6.3 – 9.0	High
	3.7 – 6.2	Medium
	1.0 – 3.6	Low

Filter should be tested

Consideration		Ratings [1]	Weight [2]
Distance along production stream (DAS)	1	Synthesis: Vial thaw, Inoculum, Expansion, Production, Harvest, Plasma	0.40
	3	Purification: Affinity chromatography, Viral inactivation, Ion exchange chromatography, Viral filtration, UF/DF	
	5	Bulk Drug Substance: Filtration, BDS storage	
	9	Final Formulation, Fill / Finish Potency adjustment, Sterile filtration Filling, Lyophilization, FDP Storage	
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	5	1.E-01 - < 1.E-02 m^2/L e.g. long low diameter tubing	
	9	> 1.E-01 m^2/L e.g. filters, final container	

3.1 RISK ASSESSMENT

USP<1665>: Example of a risk evaluation matrix

- **Risk evaluation matrix uses a 3-step process**:

  Step 1: Establish values for each risk dimension

  Step 2: Link the numerical risk sequence with a level of characterization

  Step 3: Use mitigating factors to adjust the characterization level

Risk Dimension	Duration of contact	Temperature of contact	Chemical Composition of the Process Stream	Chemical composition of the Component
Level 1	< 24 h	Frozen (<-10 °C)	Aqueous (≤5% organic v/v; pH ≥3 and pH ≤ 9)	Low risk
Level 2	1-7 days	Refrigerated (2 °C – 8 °C) Ambient (15 °C – 25°C)	Somewhat organic (<5% and ≤40% v/v)	Intermediate risk
Level 3	>7 days	Elevated (>30 °C)	Highly organic (>40% v/v) or aqueous, extreme pH (pH <3 or pH >9)	High risk

3.1 RISK ASSESSMENT

USP<1665> draft: Example of a risk evaluation matrix

- E.g. Sterilization filter:

  Step 1: Establish numerical risk sequence ➔ 3321

  Step 2: Link numerical risk sequence with a level of characterization

Table A-3. Linking the Numerical Risk Sequence with a Level of Characterization

If...	And...	Then the Characterization Level Is...
Four of the dimension scores are Level 3	There is no additional qualifier (3333)	Level C (High Risk)
Three of the dimension scores are Level 3	The other dimension score is Level 2 (3332)	Level C
	The other dimension score is Level 1 (3331)	Level C
Two of the dimension scores are Level 3	The other two dimension scores are both Level 2 (3322)	Level C
	One dimension score is Level 2 (3321)	Level B (Moderate Risk) or C[a, b]
	The other two dimension scores are Level 1 (3311)	Level A (Low Risk) or B[b, c]
One of the dimension scores is Level 3	All of the other dimension scores are Level 2 (3222)	Level B
	One of the other dimension scores is Level 1 (3221)	Level B
	Two of the other dimension scores are Level 1 (3211)	Level A or B[b, c]
	All of the other dimension scores are Level 1 (3111)	Level A
None of the dimension scores is Level 3	All of the dimension scores are Level 2 (2222)	Level B
	Not all of the dimension scores are Level 2	Level A

[a] If the Level 2 score is in temperature, solvent, or duration dimensions, then Level C; otherwise, Level B.
[b] In these cases the temperature, solvent, or duration dimensions have a greater influence on risk than do component composition.
[c] If one of the Level 1 scores is in the component composition dimension, then Level A; otherwise, Level B.

Temperature is level 2 score
➔ Level C (high risk)

3.1 RISK ASSESSMENT

USP<1665> draft: Example of a risk evaluation matrix

- E.g. Sterilization filter:

  Step 1: Establish numerical risk sequence ➔ 3321

  Step 2: Link numerical risk sequence with a level of characterization ➔ Level C

  Step 3: Use mitigating factors to adjust the characterization level

  - Clearance after contact processing step?
    - ➔ No (no mitigation factor)
  - Clinical use of the final DP?
    - ➔ "Duration < 7 days" and "dialy dose < 10 mL" (factor = 1)

➔ Level C testing is reduced to Level B testing

3.1 RISK ASSESSMENT

Table 2. Guidelines for Application of Chemical Component Tests as Established by Risk

Risk Level		Extraction Solutions for Chemical Testing	Chemical Testing of Extracts
Low	(A)	*Solution C1*	• Non-volatile residue • UV absorbance
Moderate	(B)	*Solution C1*	• Organic extractables profiling
High	(C)	*Solution C1, Solution C2, and Solution C3*	• Organic extractables profiling • Extracted elements (as necessary and appropriate)[a]

[a] The relevance of extractable elements testing should be considered by the component's potential user. Should such testing be deemed necessary, it is the user's responsibility to establish and justify the means by which testing is accomplished, taking into account extraction conditions, target elements, and reporting requirements.

C1: 50% EtOH in UPW
C2: UPW pH 3 (HCl/KCl)
C3: UPW pH 10 (phosphate buffer)

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3.2 GATHERING EXTRACTABLES DATA

- Extractables data from the supplier:

  Is the data suitable for the intended application(s)?
  - Composition of extraction solvents: organic content, pH, polarity
  - Extraction conditions: time and temperature
  - Pretreatments steps: sterilization
  - Analytical techniques: screening, combination of different techniques
- Can extractables data generated by different suppliers be compared?
  - Outcome of extractables study is higly dependent upon the set-up
- Increasing demand for standardized extractables protocol for extractables testing performed by the supplier
  - Cover the majority of the biopharmaceutical applications
  - Easily compare data from different suppliers

3.2 GATHERING EXTRACTABLES DATA

- BPOG extractables protocol (2014)

	SOLVENTS						TIME				
	50% Ethanol	1% PS-80	5M NaCl	0.5N NaOH	0.1M Phosphoric acid	WFI[a]	Time 0 (≤ 30 min)	24 hrs	7 days	21 days	70 days
							Temperature				
							25°C	40°C			
Storage, Mixing, and Bioreactor Bags	X	X	X	X	X	X	X	X		X	X[b]
Tubing	X	X	X	X	X	X	X	X		X	X[b,c]
Tubing Connectors & Disconnectors	X	X	X	X	X	X	X	X		X	
Aseptic Connectors & Disconnectors	X	X	X	X	X	X	X	X	X		
Sterilizing-Grade / Process Filters	X	X	X	X	X	X	X	X	X		
TFF Cassettes	X	X	X	X	X	X	X	X		X	
Sensors and Valves	X	X	X	X	X	X	X	X		X[d]	
Molded Part of Mixers	X	X	X	X	X	X	X	X		X	
Chrom. Columns; Elastomer Parts; Wetted Polymeric Surfaces of Positive Displacement Pumps	X	X	X	X	X	X	X	X			
Filling Needles	X	X	X	X	X	X	X	X			

[a] If WFI is not available, use deionized water [b] Necessary to support 3-year storage time at 0°C [c] Tubing is integrated with bag during storage
[d] The 21-day time-point only applies to sensors used with bioreactor (e.g., DO and pH)

Reference: Presentation at 'Bioproduction 2015', Dublin, 14 Oct 2015, presented by D. Buckley and A.Sexton

Rationale for updating BPOG protocol -> cf. BioPhorum Best Practices Guide for Extractables Testing of Polymeric Single-Use Components (2020)

3.2 GATHERING EXTRACTABLES DATA

- BioPhorum extractables protocol (2020)

Component type	Solvents				Time			
	50% ethanol	0.5N NaOH	0.1M phosphoric acid	WFI*	24 hours	7 days	21 days	70 days
					Temperature			
					40 °C			
Bag film, bottles, and carboys intended for long-term storage	X	X	X	X	X		X	X
Tubing intended for storage bags	X	X	X	X	X		X	X
Bag ports intended for storage bags	X	X	X	X	X		X	X
Molded stoppers	X	X	X	X	X		X	X
Bag film, bottles, and carboys	X	X	X	X	X		X	
Bag ports	X	X	X	X	X		X	
Impellers (e.g. in bioreactors, mixers)	X	X	X	X	X		X	
TFF cassettes intended for perfusion/continuous processing	X	X	X	X	X		X	
Tubing	X	X	X	X	X		X	
Tubing connectors and disconnectors, fittings, overmolded junctions	X	X	X	X	X		X	
TFF cassettes	X	X	X	X	X			
Aseptic connectors and disconnectors	X	X	X	X	X	X		
Sterilizing-grade filters/process filters	X	X	X	X	X	X		
Filling needles	X	X	X	X	X			
Chromatography column housing	X				X			
Small parts (e.g. sensors, O-rings, gaskets, check valves, diaphragms, septa)	X				X			

Reference: BioPhorum Best Practices Guide for Extractables Testing of Polymeric Single-Use Components (2020)

3.2 GATHERING EXTRACTABLES DATA

- USP<665>: Standard Extractables Protocol (SEP)

Table 1. List of Components Typically Encountered in Pharmaceutical and Biopharmaceutical Manufacturing Systems

Components	Extraction Duration (days)		
	1 day (24 ± 1 h)	7 days (168 ± 4 h)	21 days (504 ± 8 h)
Chromatography column housing	X	–	–
Connectors, disconnectors, fittings, overmolded junctions for tubing	X	–	–
Containers (bags, bottles, carboys) not intended for storage (such as mixing bags or bioreactors)[a]	X	–	–
Filling needles	X	–	–
Filters (process, sterilizing, and virus)	X	–	–
Filtration cassettes (tangential flow)	X	–	–
Impellers and molded parts for bioreactors and mixers[a]	X	–	–
Ports on containers not intended for storage (such as mixing bags or bioreactors)	X	–	–
Small components (O-rings, gaskets, check valves, diaphragms, septa, polymer pump surfaces, sensors)	X	–	–
Tubing attached to containers not intended for storage	X	–	–
Connectors and disconnectors, aseptic	–	X	–
Closures (e.g., molded stoppers) for storage containers	–	–	X
Containers (bags, bottles, carboys) intended for storage	–	–	X
Ports on containers intended for storage	–	–	X
Tangential flow modules for perfusion or continuous processing	–	–	X
Tubing attached to containers intended for storage	–	–	X
Tubing for fluid transport[b]	–	–	X

[a] These items can be used in several different m… the circumstances of use during manufacturing. If warranted and justified, longer extraction durations …

[b] Tubing for fluid transport can be used in sever… nsistent with the circumstances of use during manufacturing. If warranted and justified, shorter extraction durations of 1 or 7 days can be used.

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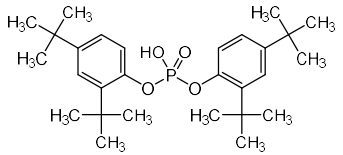
3.3 EVALUATION OF EXTRACTABLES DATA

- Impact on **process performance**
  - e.g. Bis(2,4-di-tert-butylphenyl)hydrogen phosphate (bDtBPP) causing cell growth inhibition



- Impact on the **final product**:
  - **Safety impact**: related to the toxicity of the extractables (potential leachables)
    - Is there a safety risk towards the patient?
    - e.g. Mutagenic compounds ending up in the final product administered to the patient
  - **Quality impact**:
    - e.g. Compounds promoting the formation of protein aggregates
  - **Efficacy impact**:
    - e.g. Compounds altering the tertiary structure of the protein causing loss of activity

3.3 EVALUATION OF EXTRACTABLES DATA

- Safety evaluation based on the toxicity of the compound

  - literature data often very limited or non-existent:
    - *polymer oligomers*
    - *polymer degradation compounds*
    - *polymer additive degradation compounds*
    - *reaction products*

  - (Q)SAR ((Quantitative) Structure Activity Relationship) software packages might assist in assessing the safety risk of extractables

    E.g. Derek Nexus, Sarah Nexus, MultiCase, Leadscope

- PQRI: Product Quality Research Institute
  - safety concern thresholds dependent on the administration route of the final product

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3.4 LEACHABLES STUDY: GENERAL CONSIDERATIONS

MOST CASES:

- Conc. extractable compounds << final AET

  => no leachable study

When to perform a subsequent leachable study:

- Extractable compounds > final AET
- Filling line

  (Worst-case final AET approximation: all potential filling line leachables end up in 1 dose)
- Storage applications (e.g. storage bag for DS)

3.4 LEACHABLES STUDY

Set-up:

- **Before and after the process step**
- **Integrated in the container leachables study**
  - Blank reference should not have been in contact with the process materials
  - Sometimes not possible to generate a true blank, since the DS is manufactured in single-use
  - Use placebo solution as a blank, but cause differential peaks originating from the DS

Final leachables results to be subjected to thorough **toxicological assessment** to classify the SUS as safe for use in the bioproduction process

Thank you

Questions?

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