PDA Training Course Extractables & Leachables

25-26 April 2024

E&L TESTING OF SINGLE-USE SYSTEMS FOR PRODUCTION

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PDA Parenteral Drug Association

Nelson Labs A Sotera Health company

CONNECTING PEOPLE SCIENCE AND REGULATION®

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
4. Case study: E&L testing of a PET bottle

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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. Parts of production equipment that come into contact with the product must not be reactive, additive or absorptive to such an extent that it will affect the quality of the product ant thus present any hazard"*

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 and qualification of plastic components and systems used to manufacture pharmaceutical drug products and biopharmaceutical drug substances and products

Published ***IN DRAFT*** in Pharmacopeial Forum (PF) 43(3) [May – Jun. 2017]
Published ***UPDATED DRAFT*** in Pharmacopeial Forum (PF) 45(2) [Mar. – Apr. 2019]
Published ***2nd UPDATED DRAFT*** in Pharmacopeial Forum (PF) 46(5) [Sep. – Oct. 2020]
Published on USP website (May 2022): ***targeted offical date: 01 May 2026 (see next slide)***

1. REGULATORY REQUIREMENTS FOR SINGLE-USE SYSTEMS

STATUS UPDATE USP <665> (TO BE OFFICIAL: 2026)

(taken from USP website on 02 May 2022)

<665>Plastic Components and Systems Used to Manufacture Pharmaceutical Drug Products and Biopharmaceutical Drug Substances and Products

Type of Posting: Notice of Intent to Revise
Posting Date: 25–Feb–2022
Targeted Official Date: 01–May–2026, Revision Bulletin
Expert Committee: Packaging and Distribution Expert Committee

In accordance with the Rules and Procedures of the Council of Experts, this is to provide notice that the General Chapters–Packaging and Distribution Expert Committee intends to revise ⟨665⟩ *Plastic Components and Systems Used to Manufacture Pharmaceutical Drug Products and Biopharmaceutical Drug Substances and Products*.

As currently published, there are no requirements that are mandatory for compendial compliance purposes in this chapter. General Notices 3.10, *Applicability of Standards* states that a chapter below ⟨1000⟩ does not become an applicable general chapter unless referenced as such in General Notices, a monograph, or another applicable general chapter numbered below ⟨1000⟩. As none of these situations currently applies to ⟨665⟩, it is not an applicable general chapter. However, there have been inquiries around the applicability of the chapter and the current official date of May 1, 2022. To address these inquires and to give USP time to engage stakeholders regarding the advisability of making ⟨665⟩ an applicable general chapter and track the ICH Q3E development effort, USP intends to extend the official date for ⟨665⟩ to May 1, 2026.

It is anticipated that the revision will be posted as a Revision Bulletin April 29, 2022.

Should you have any questions, please contact Desmond G. Hunt, Scientific Liaison to the General Chapters–Packaging and Distribution Expert Committee (301–816–8341 or dgh@usp.org).

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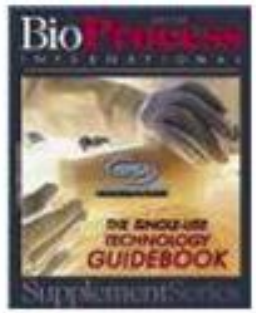
2. INTEREST GROUPS ON STANDARDIZATION

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)

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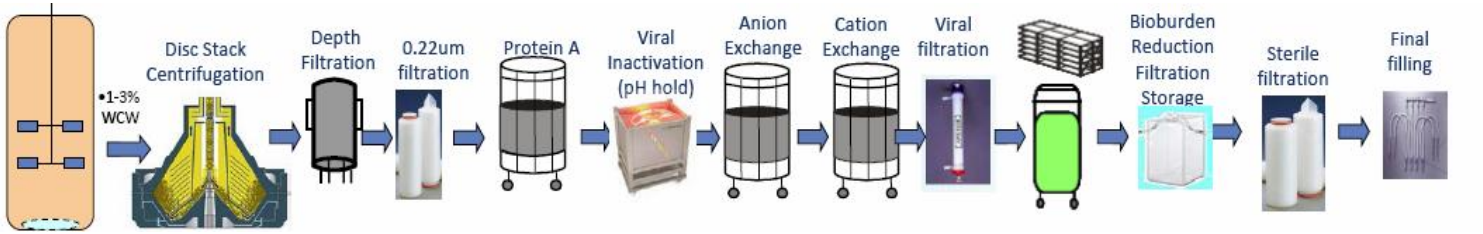
3.1 RISK ASSESSMENT: INTRODUCTION

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: INTRODUCTION

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

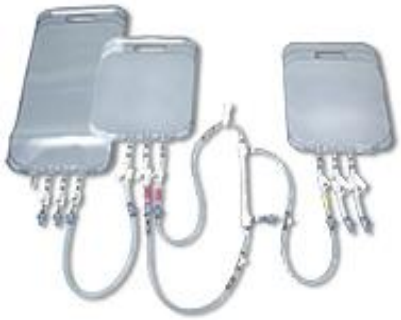
- GOAL?
  Select single-use components with greatest potential for objectable levels of leachables with regard to **safety** and **quality** of the final product, and **process performance**

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

3.1 RISK ASSESSMENT: INTRODUCTION

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: INTRODUCTION

“Guidance/recommendation documents”

USP risk assessment

- Cf. USP 1665 (informational chapter)
- Gives clear procedure of risk assessment but also mentions alternative risk evaluations as long as properly justified

BPOG risk assessment

- Model which companies can adapt to their requirements

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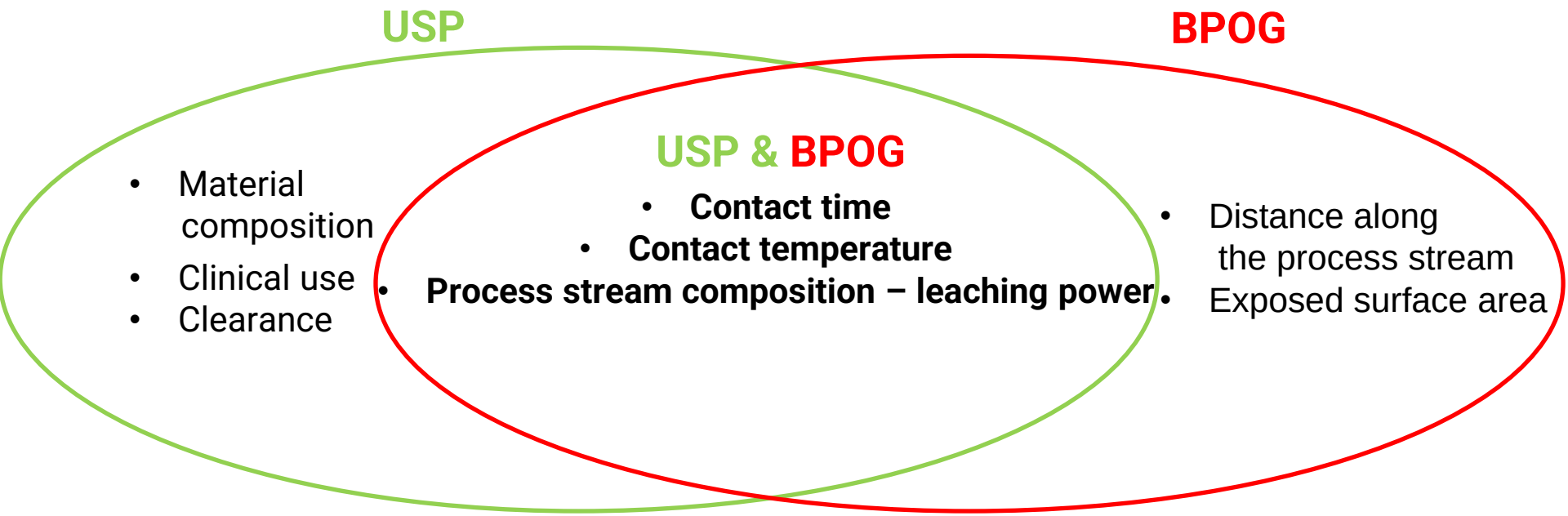
3.1 RISK ASSESSMENT: RISK FACTORS



3.1 RISK ASSESSMENT: RISK FACTORS

RISK FACTOR 1: Contact time (USP & BPOG)

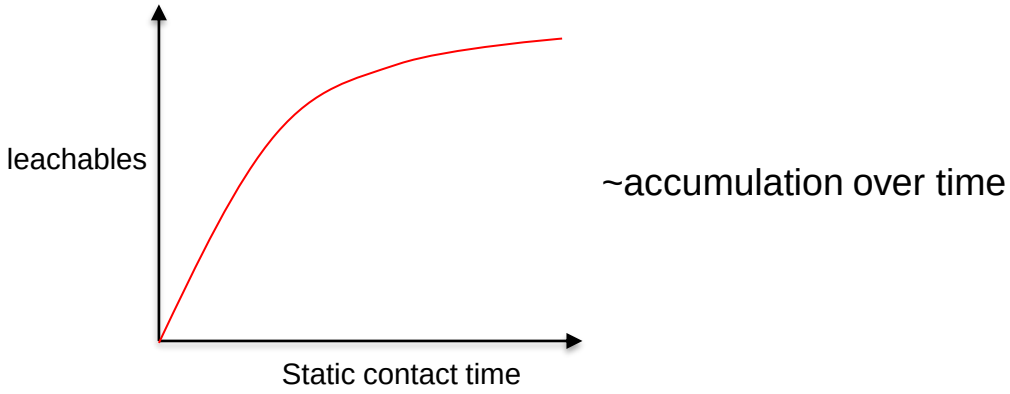
- Evidently, higher risk in case of longer times
  → more time for migration

USP	BPOG
	Transient
≤24 h	
	≤24 h
≤7 days	≤7 days
≥7 days	≥7 days

3.1 RISK ASSESSMENT: RISK FACTORS

RISK FACTOR 1: Contact time (USP & BPOG)

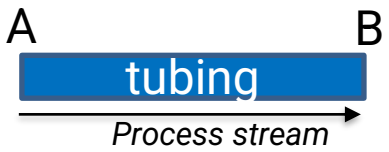
High risk: “long term static contact” DS storage bag, pooling bag,...



3.1 RISK ASSESSMENT: RISK FACTORS

RISK FACTOR 1: Contact time (USP & BPOG)

Low risk: “transient” contact: tubings, filters, gaskets,...
=> most materials of production process



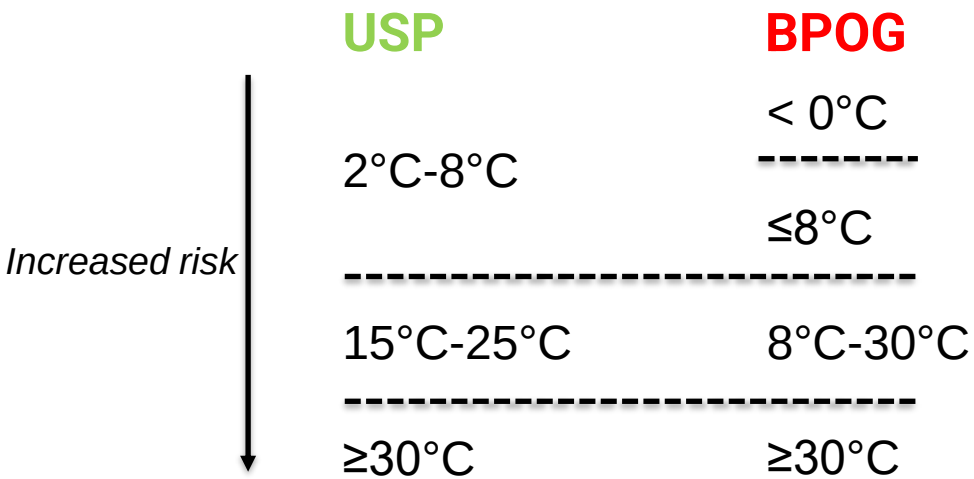
Time for leachable accumulation = Time from A to B:
-defined by length of material
-defined by flow rate (L/h)

Holding times also need to be considered!

3.1 RISK ASSESSMENT: RISK FACTORS

RISK FACTOR 2: Contact temperature (USP & BPOG)

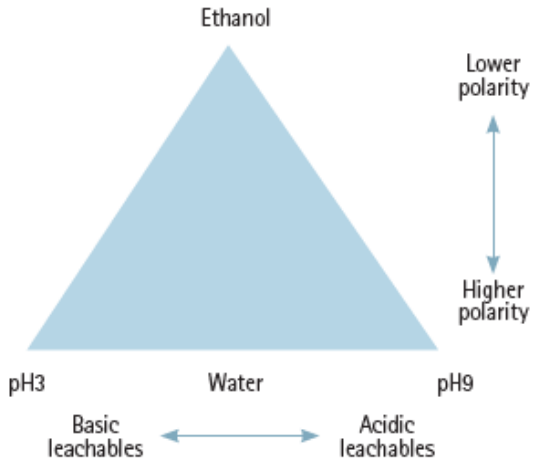
- Evidently, higher risk in case of higher temperatures
  → more rapid migration



3.1 RISK ASSESSMENT: RISK FACTORS

RISK FACTOR 3: Process stream composition – leaching power (USP & BPOG)

- Higher regulatory and safety concern for leachables in case of contact solutions with:
  - Low or high pH-values
  - High organic contents
  - Surfactants



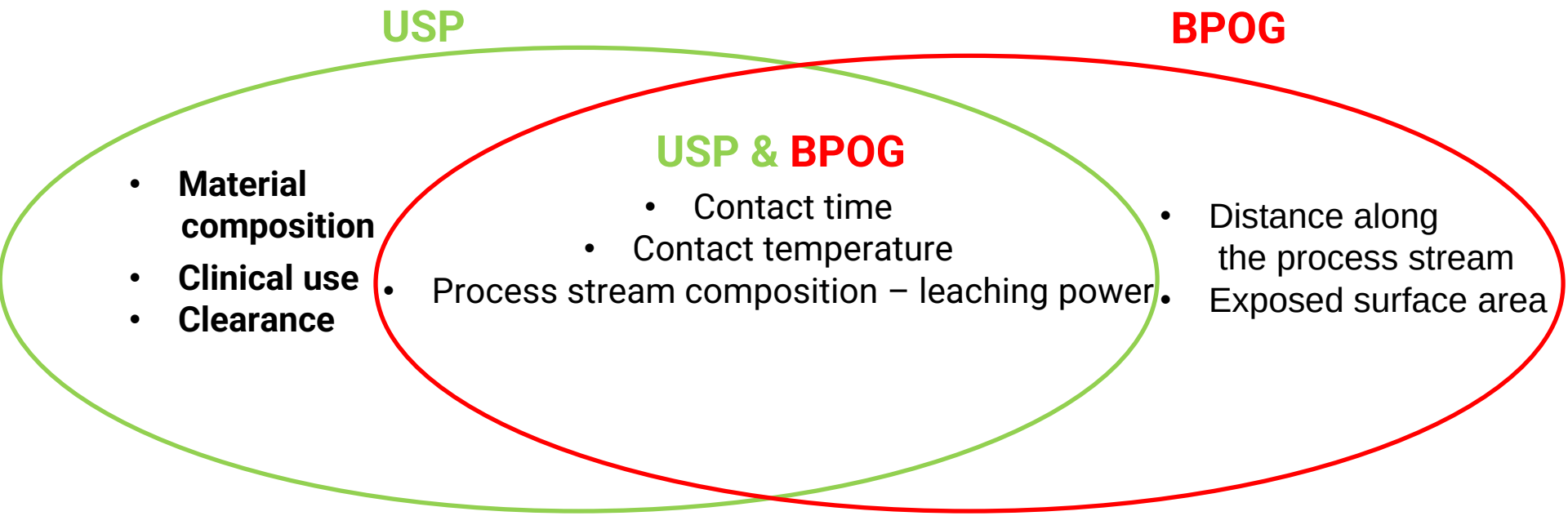
3.1 RISK ASSESSMENT: RISK FACTORS

RISK FACTOR 3: Process stream composition – leaching power (USP & BPOG)

Increased risk

USP	BPOG
pH 3-9 <5% org content <0.1% surfactants <1% blood products <1% lipids/proteins	Water Neutral solutions without organics
5-40% organics 0.1% -0.5% surfactants 1%-25% blood products 1%-5% lipids/proteins	Surfactants, low conc. organics, high/low pH without organics
>40% organics >0.5% surfactants >25% blood products >5% lipids/proteins	High/low pH with organics High conc. organics/surfactants

3.1 RISK ASSESSMENT: RISK FACTORS



3.1 RISK ASSESSMENT: RISK FACTORS

RISK FACTOR 4: Material composition (USP)

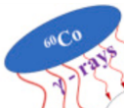
Materials with great number and/or level of additives
➔ greater total pool of potential leachables

- USP 1665
  - ➢ "low risk" component: total level of plastic additives in component is ≤0.1%
  - ➢ "intermediate risk" component: total level of plastic additives in component is >0.1% and ≤1%
  - ➢ "high risk" component: total level of plastic additives in component is >1%

} Supplier information available?

3.1 RISK ASSESSMENT: RISK FACTORS

RISK FACTOR 4: Material composition (USP)



High energy pretreatment ⟶ high risk

Adhesives, glues,... ⟶ Intermediate or high risk

➡ Pre-rinsing can reduce risk level

3.1 RISK ASSESSMENT: RISK FACTORS

RISK FACTOR 5: Clinical use (USP)

Risk factor not related to production process but to the drug product administration specifics

- oral administration
- Short duration of treatment (<7 days)
- Lower max daily dose volumes (<10 mL)

} = lower safety risk

3.1 RISK ASSESSMENT: RISK FACTORS

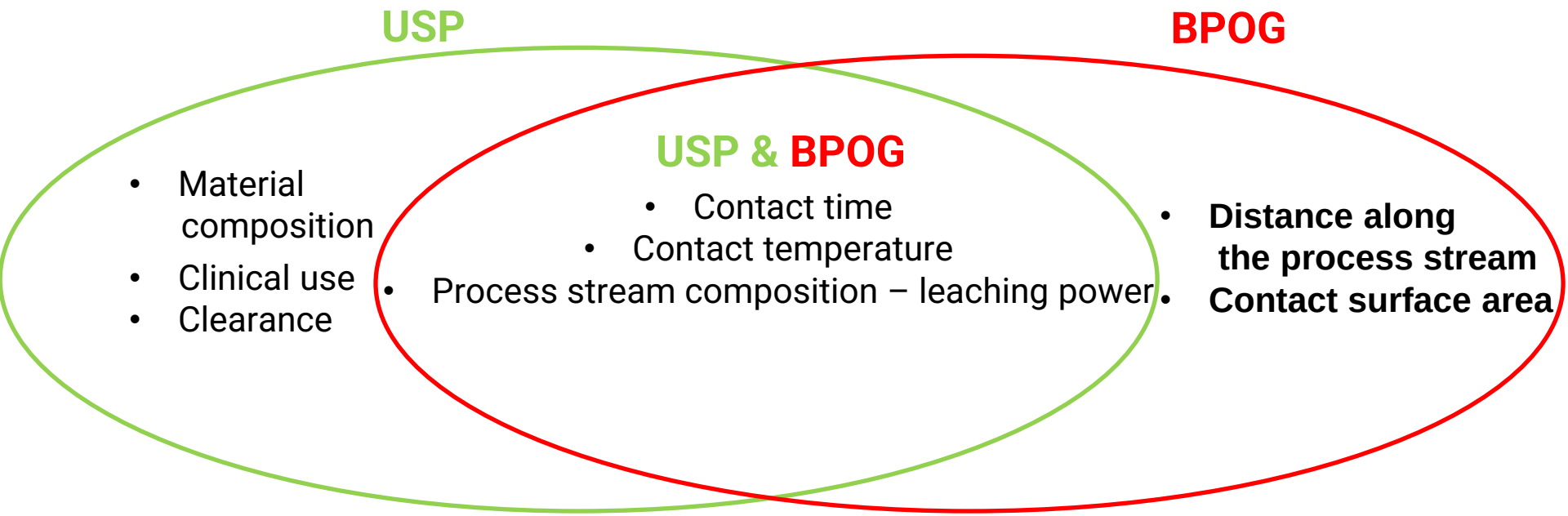
RISK FACTOR 6: Clearance steps (USP)

Possible remove migrated compounds from the process

- Ultrafiltration / diafiltration → removal of impurities?
- Lyophilization → removal of volatiles?

Possible dilution of migrated compounds from the process

3.1 RISK ASSESSMENT: RISK FACTORS



3.1 RISK ASSESSMENT: RISK FACTORS

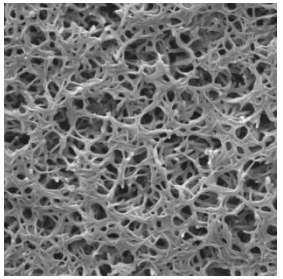
RISK FACTOR 7: Distance along the process stream (BPOG)

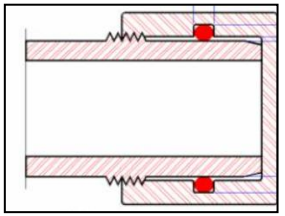
- Materials used in the final filling line have direct risk to the final product
- Locations upstream in the process MAY have reduced risk to the end product
- Partly similar as “risk factor 6: clearance steps”

3.1 RISK ASSESSMENT: RISK FACTORS

RISK FACTOR 8: Contact surface area (BPOG)

- The higher the surface area, the higher the risk!!
- High → Filters: porous structure leads to large internal surface area
- Low → O-ring seals
- Smaller process volumes are more critical





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3.1 RISK ASSESSMENT: HOW TO PERFORM

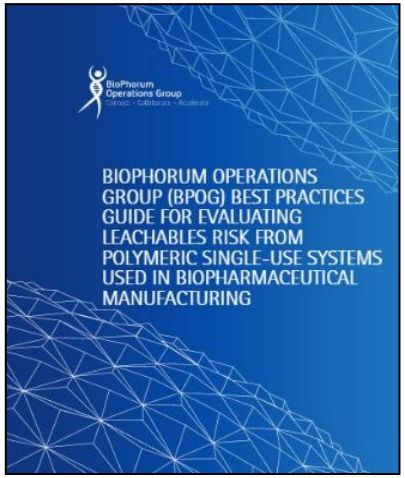
BPOG E/L Risk Assessment
Example of Proposed Risk Assessment

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	



(2017)

(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.

3.1 RISK ASSESSMENT: HOW TO PERFORM

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

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
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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	

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

3.1 RISK ASSESSMENT: HOW TO PERFORM

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

3.1 RISK ASSESSMENT: HOW TO PERFORM

USP<1665>: Example of a risk evaluation matrix

E.g. Sterilization filter:

- Step 1: Establish values for each risk dimension

	1	2	3	3
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: HOW TO PERFORM

USP <1665>: Example of a risk evaluation matrix

- E.g. Sterilization filter:
  Step 2: Link the 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

Temperature is level 2
➔ high risk (C)

[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.

Material composition has lower weight

3.1 RISK ASSESSMENT: HOW TO PERFORM

USP <1665>: Example of a risk evaluation matrix

- E.g. Sterilization filter:

  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?
    - Dosage form: solid oral, liquid oral or topical? ➔ No (no mitigation factor)
    - Duration <7 days ➔ yes
    - Dialy dose volume < 10 mL ➔ yes

  ➔ High risk (Level C) is reduced to intermediate risk (Level B)

Note: the “clearance” mitigation and the “clinical use” mitigation factors are additive.

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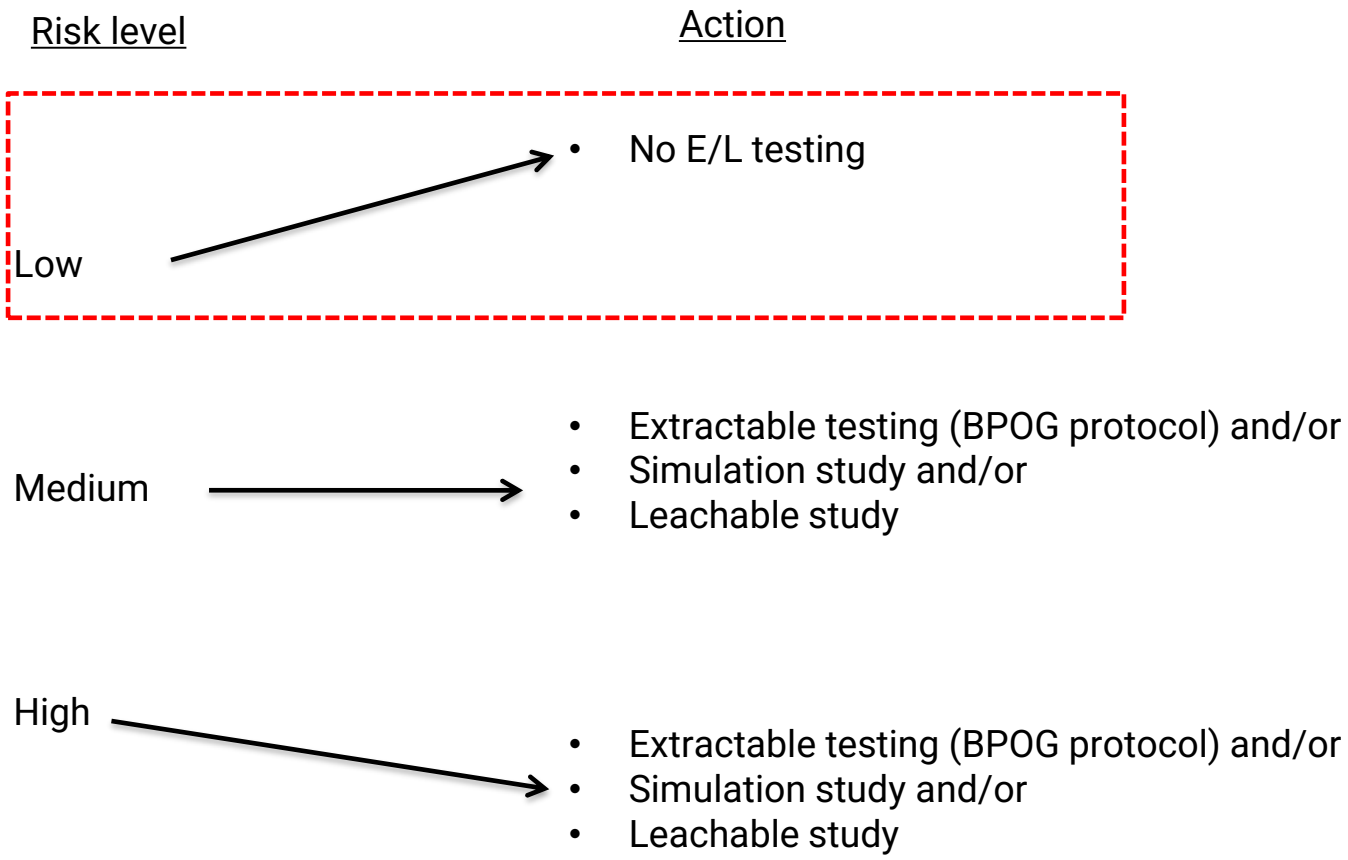
3.1 RISK ASSESSMENT: OUTCOMES

BPOG

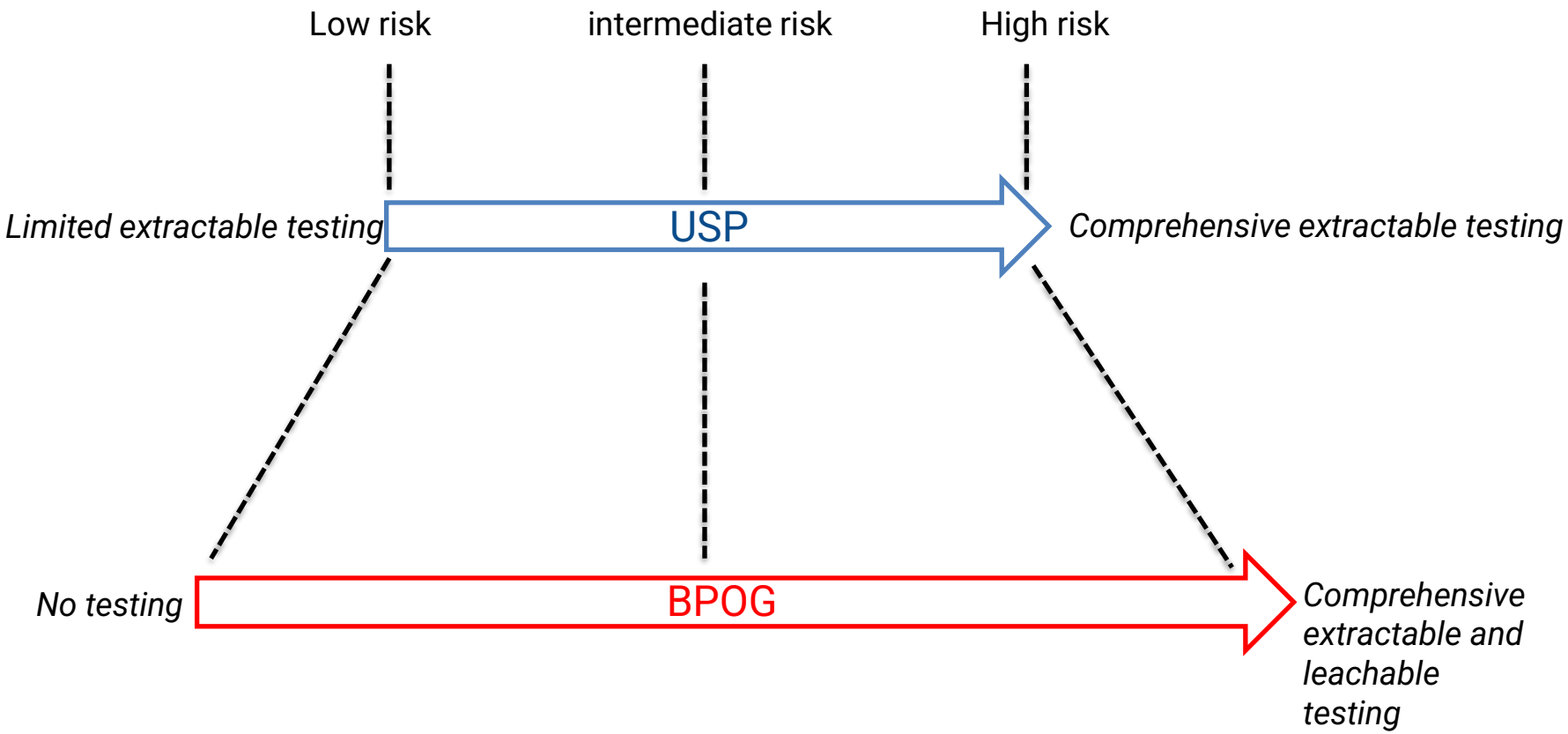


3.1 RISK ASSESSMENT: OUTCOMES

USP

Risk level	Action
Low (level A)	• Limited extractable testing: NVR and UV (50% ethanol)
Medium (level B)	• Extractable testing: full chemical profiling (50% ethanol)
High (level C)	• Comprehensive extractable testing: full chemical profiling (50% ethanol, low pH, high pH) (Standard Extraction Protocol (SEP))

3.1 RISK ASSESSMENT: OUTCOMES



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
        - 3.1.1 Introduction
        - 3.1.2 Risk factors
        - 3.1.3 How to perform a risk assessment
        - 3.1.4 Outcomes
        - **3.1.5 Notes**
    - 3.2 Gather extractables data
    - 3.3 Evaluation of extractables data
    - 3.4 Leachables study
4. Case study: E&L testing of a PET bottle

3.1 RISK ASSESSMENT: NOTES

Emphasis on "distance along the production stream" in BPOG risk assessment

⇒ process material in filling line always *at least medium risk*

Total score will be ***at least 4.0***

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

Comprehensive extractable and/or leachable testing

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	

3.1 RISK ASSESSMENT: NOTES

High impact of mitigation factors in USP risk assessment

=> Often applicable and result in downgrading of extractable testing

Step 1:

	1	2	1	3
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

Step 2: Numerical risk sequence = 1123 ⟶ Level B testing

Step 3: Use clinical use mitigation factor "daily dose < 10 ml" ⟶ Level A testing

3.1 RISK ASSESSMENT: NOTES

BPOG risk assessment: materials will often be classified as intermediate or even high risk. In case of low risk, no testing is required.

USP risk assessment: materials will often be classified as intermediate or even low risk. However, even for low risk materials, a certain degree of test is still required.

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)


Standardized Extractables Testing Protocol for Single-Use Systems in Biomanufacturing

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) (2022)

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[a]	X	-	-
Containers (bags, bottles, carboys) not intended for storage (such as mixing bags or bioreactors)[b]	X	-	-
Filling needles	X	-	-
Filters (process, sterilizing, and virus)	X	-	-
Filtration cassettes (tangential flow)	X	-	-
Impellers and molded parts for bio-reactors and mixers[b]	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	-	-
Aseptic connectors and disconnectors[a]	-	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[c]	-	-	X

T: 40 °C

UPW pH 3 (HCl/KCl)
UPW pH 10 (PO4 buffer)
50% EtOH in UPW

3.2 GATHERING EXTRACTABLES DATA

- What if no supplier data are available or suitable?
  - ➔It is the **responsibility of the end user** to demonstrate that the single-use system is **suitable** for his end application and that it does not alter the quality or safety of his end product

Overview

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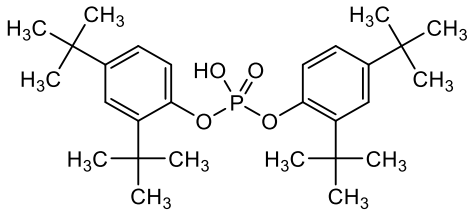
    - 3.1 Risk assessment of the materials used in the production process
    - 3.2 Gather extractables data
    - **3.3 Evaluation of extractables data**
    - 3.4 Leachables study
4. Case study: E&L testing of a PET bottle

3.3 EVALUATION OF EXTRACTABLES DATA

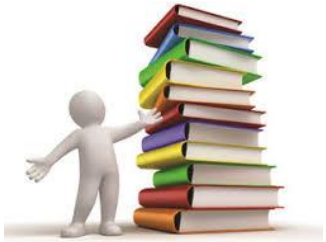
- Impact on **process performance:**
  - e.g. Bis(2,4-di-tert-butylphenyl)hydrogen phosphate (bDtBPP) causing cell growth inhibition



- Impact on the **final product**:
  - **Safety impact**: related to the toxicity of 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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4. Case study: E&L testing of a PET bottle

3.4 LEACHABLES STUDY: GENERAL CONSIDERATIONS

MOST CASES:

- Concentration extractable compounds << final AET
  → no leachable study required

When to perform a subsequent leachable study:

- Extractable compounds > final AET
- Filling line
- Storage applications (e.g. storage bag for DS)

3.4 LEACHABLES STUDY: GENERAL CONSIDERATIONS

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
    => lab prepared blank could be an option
  - 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

Overview

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    - 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
4. **Case study: E&L testing of a PET bottle**

STEP 1: EXTRACTABLES / SIMULATION STUDY – SET-UP

Sponsor info:

- 5-L PET bottle with HDPE cap (filling volume = 4 L)
- Used for storage of drug substance
- Composition contact solution/drug substance:
  - Blood protein (2.4%),
  - buffer (contains Na^+, K^+, phosphate) (pH 3.0-4.0)
- Contact time & temperature: 12 months at 2- 8 ℃

STEP 1: EXTRACTABLES / SIMULATION STUDY – SET-UP

Extractables study set-up (USP<665>):

- Filling and shaking incubation (inverted) of 125-mL bottles (filling volume = 100 mL)
- 21 days at 40 ºC
- Extraction solvents:
  - 50% ethanol in UPW (C1)
  - UPW pH 3 (KCl/ HCl) (C2)
  - UPW pH 10 (phosphate buffer) (C3)
- Analytical techniques:
  - HS-GC/MS screening → VOC
  - GC/MS screening → SVOC
  - HRAM-UPLC/MS screening → NVOC
  - ICP/OES → elements
  - ICP/MS → Hg

STEP 1: EXTRACTABLES / SIMULATION STUDY – SET-UP

AET calculation:

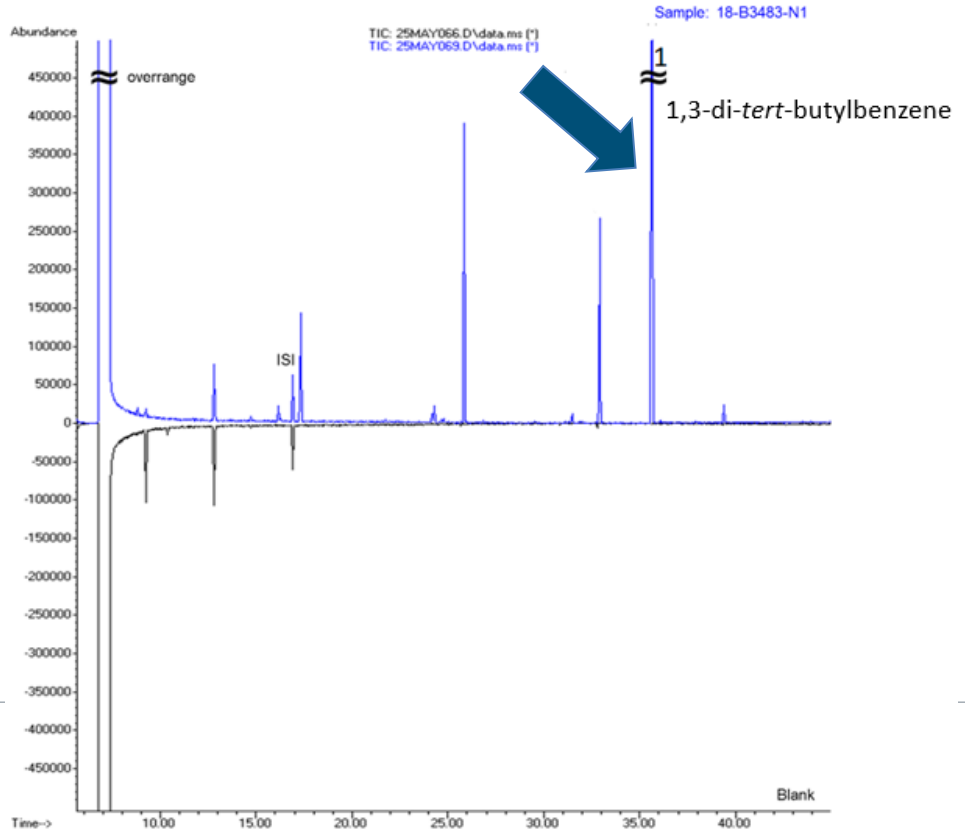
Safety concern threshold (PQRI)	1.5 µg/day
Safety concern threshold (non-chronically (ICH M7, PQRI))	**5 µg/day**

Safety concern threshold (non-chronically (ICH M7, PQRI))	**5 µg/day**
Max. daily dose (drug substance)	0.078 L/day
AET in drug substance (5 µg/day/0.078 L/day)	64 µg/L
Surface area of 5 L PET bottle	0.1842 m^2
AET in µg/m^2 (64 µg/L * 4 L/0.1842 m^2)	1400 µg/m^2
Final AET in µg/m^2 (incl. 50% uncertainty for screening analysis)	**690 µg/m^2**

STEP 1: EXTRACTABLES / SIMULATION STUDY – RESULTS

HS-GC/MS screening analysis:

- UPW pH 3: no compounds > final AET of 690 µg/m^2
- UPW pH 10: no compounds > final AET of 690 µg/m^2
- 50% ethanol extract: 1 compound > final AET of 690 µg/m^2



50% ethanol

STEP 1: EXTRACTABLES / SIMULATION STUDY – RESULTS

GC/MS screening analysis:

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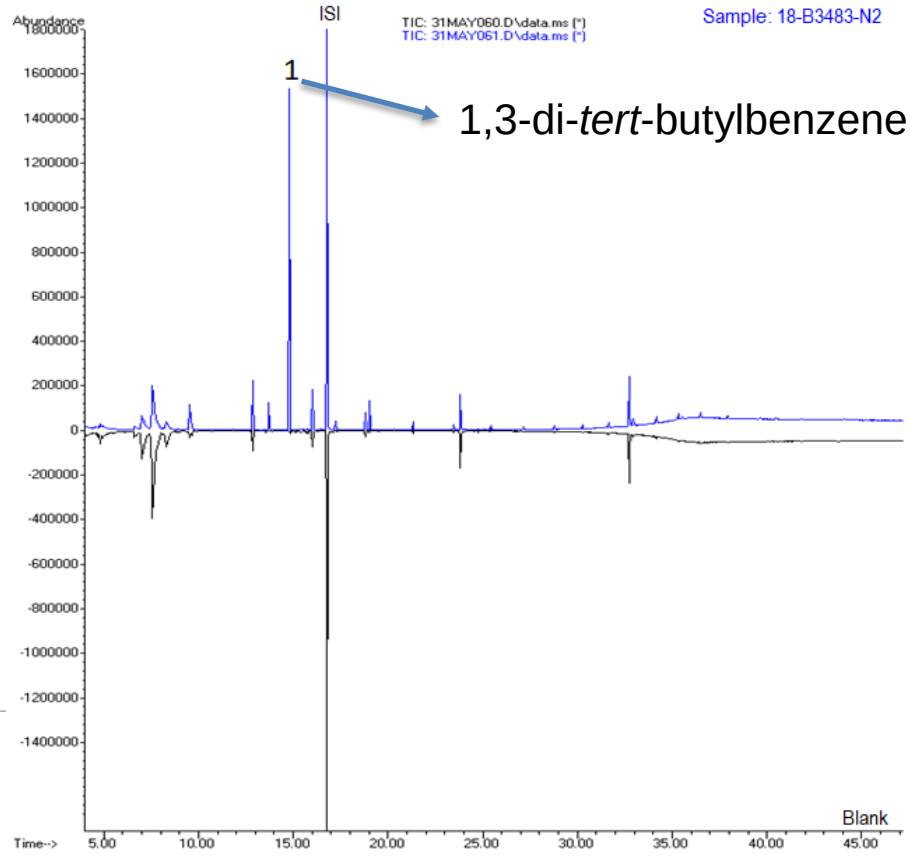


50% ethanol

STEP 1: EXTRACTABLES / SIMULATION STUDY – RESULTS

HRAM-UPLC/MS screening analysis:

- UPW pH 3: no compounds > final AET of 690 µg/m^2
- UPW pH 10: no compounds > final AET of 690 µg/m^2
- 50% ethanol extract: no compound > final AET of 690 µg/m^2

STEP 1: EXTRACTABLES / SIMULATION STUDY – RESULTS

Results for ICP/OES

Elements¤	Results·¶ (µg/m²)¤	
	Blank¤	**UPW,·pH·3¤**
Aluminum·(Al)¤	<10¤	<10¤
Antimony·(Sb)¤	[10]*¤	<10¤
Arsenic·(As)¤	<30¤	<30¤
Barium·(Ba)¤	<5¤	<5¤
Beryllium·(Be)¤	<2¤	<2¤
Boron·(B)¤	<10¤	<10¤
Cadmium·(Cd)¤	<5¤	<5¤
Calcium·(Ca)¤	[20]¤	**60¤**
Chromium·(Cr)¤	<5¤	<5¤
Cobalt·(Co)¤	<2¤	<2¤
Copper·(Cu)¤	<10¤	<10¤
Indium·(In)¤	<20¤	<20¤
Iron·(Fe)¤	<10¤	<10¤
Lead·(Pb)¤	<10¤	<10¤
Lithium·(Li)¤	<2¤	<2¤
Magnesium·(Mg)¤	<10¤	<10¤

Elements¤	Results·¶ (µg/m²)¤	
	Blank¤	**UPW,·pH·3¤**
Manganese·(Mn)¤	<2¤	<2¤
Molybdenum·(Mo)¤	<10¤	<10¤
Nickel·(Ni)¤	<10¤	<10¤
Palladium·(Pd)¤	<100¤	<100¤
Platinum·(Pt)¤	<20¤	<20¤
Potassium·(K)¤	N/A¤	N/A¤
Selenium·(Se)¤	<40¤	<40¤
Silicon·(Si)¤	<100¤	<100¤
Silver·(Ag)¤	<5¤	<5¤
Sodium·(Na)¤	**100¤**	**190¤**
Strontium·(Sr)¤	<5¤	<5¤
Sulfur·(S)¤	<100¤	<100¤
Thallium·(Tl)¤	<20¤	<20¤
Tin·(Sn)¤	<40¤	<40¤
Titanium·(Ti)¤	<5¤	<5¤
Vanadium·(V)¤	<10¤	<10¤
Zinc·(Zn)¤	<5¤	<5¤

No further follow-up required in leachable study

STEP 1: EXTRACTABLES / SIMULATION STUDY – RESULTS

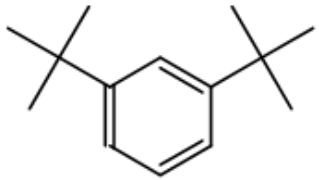
Results for ICP/MS

Element	Results (µg/ m^2)		Reporting Limit (µg/m^2)
	Blank	**UPW, pH 3**	
Mercury (Hg)	<2	<2	2

No Mercury detected

STEP 2: EVALUATION OF EXTRACTABLES DATA

Selected target compound

Chemical name; synonyms [CAS No./ToxID] formula mol. wt.	Structure
1,3-Di-*tert*-butylbenzene [1014-60-4] $C_{14}H_{22}$ 190.32	

→ Used as target in Method Suitability Test (HS-GC/MS and GC/MS)

STEP 3: LEACHABLES STUDY – SET-UP

Storage under real conditions

- Contact sample:
  - 125 mL bottles filled with 100 mL drug substance (DS)
  - Storage under inverted conditions at 5 °C

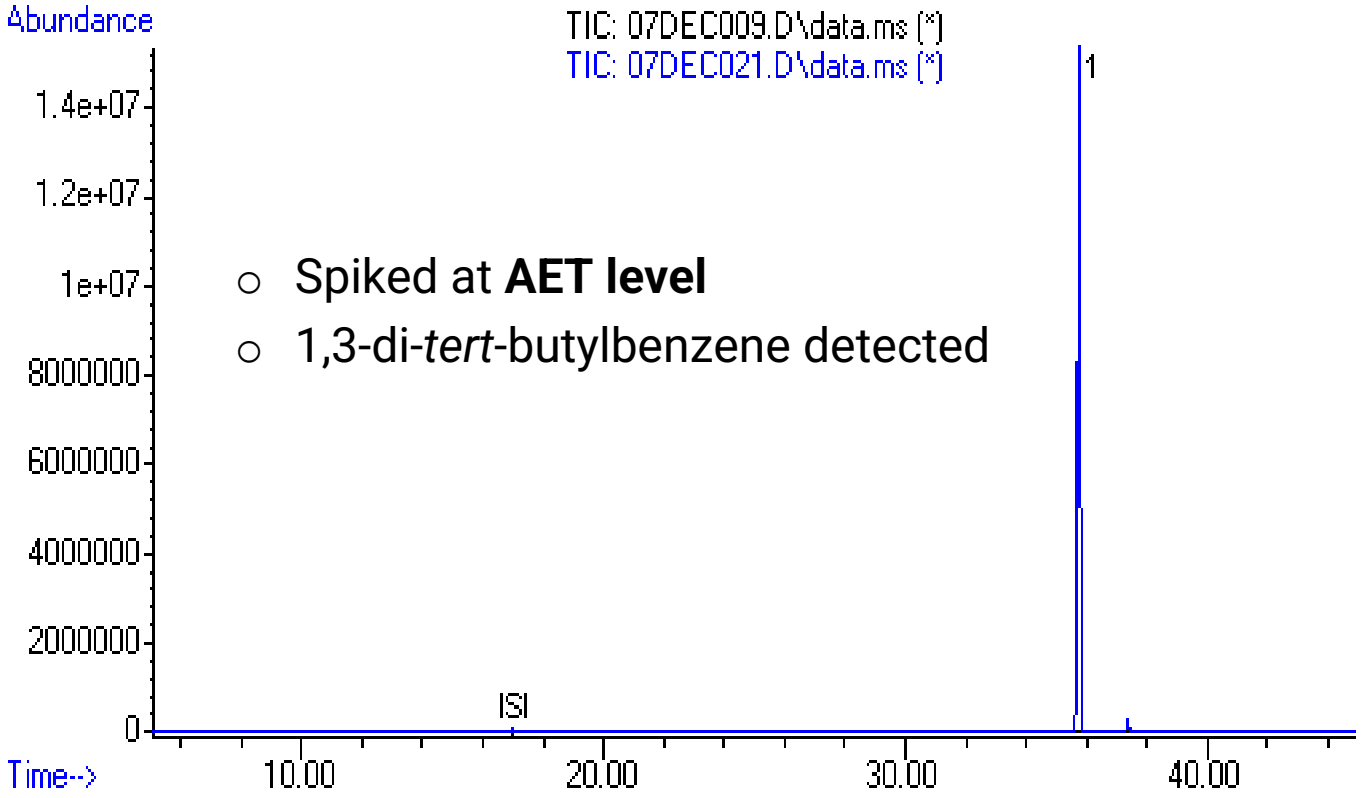
- Blank solution:
  - DS in inert glass botte stored at 5 °C

T0 & T12 months

Final AET: 690 µg/m² or lower (cf. Extractables study)

STEP 3: LEACHABLES STUDY– RESULTS

HS-GC/MS – MST result for 1,3-di-tert-buylbenzene:

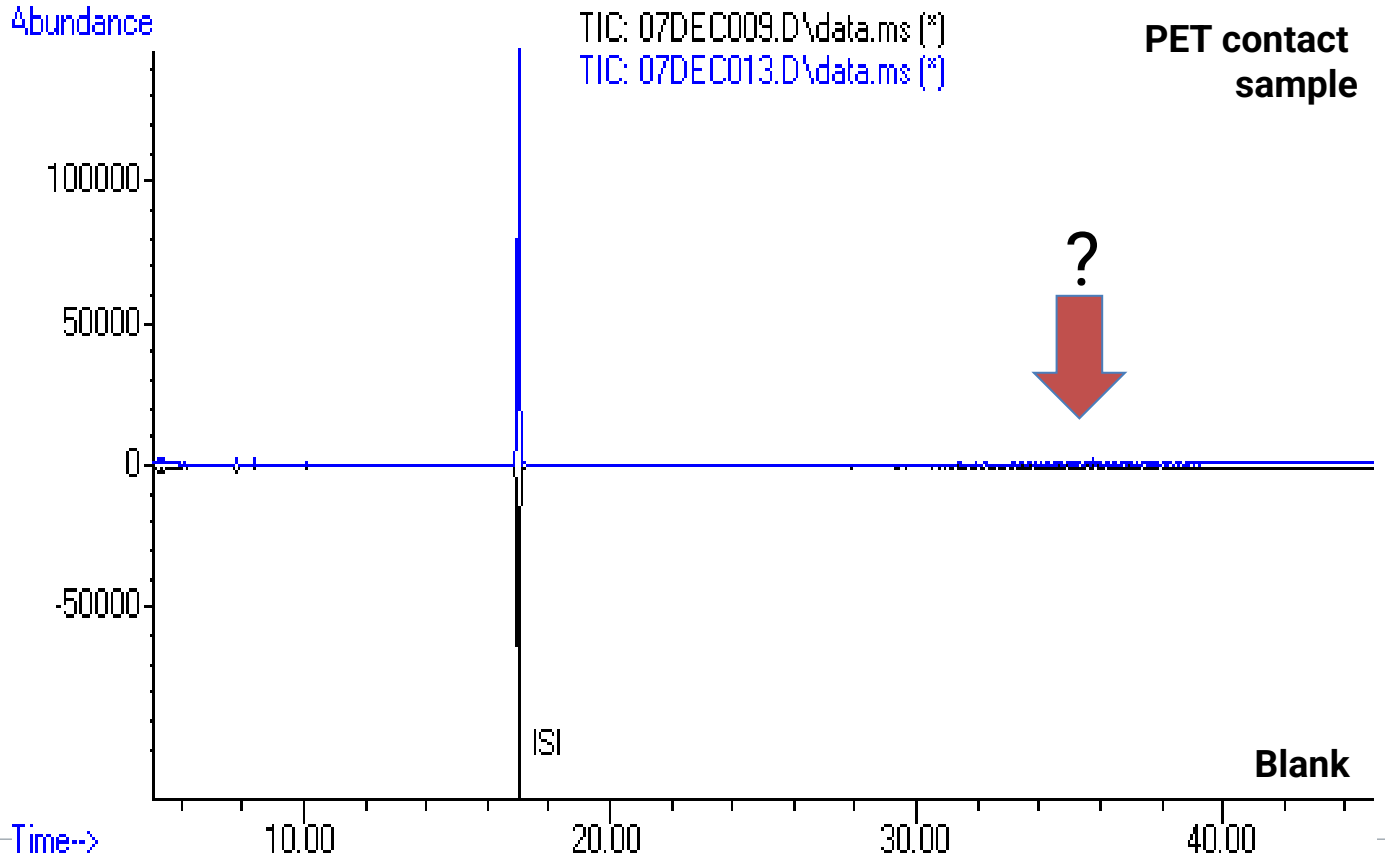


- Spiked at **AET level**
- 1,3-di-*tert*-butylbenzene detected

STEP 3: LEACHABLES STUDY– RESULTS

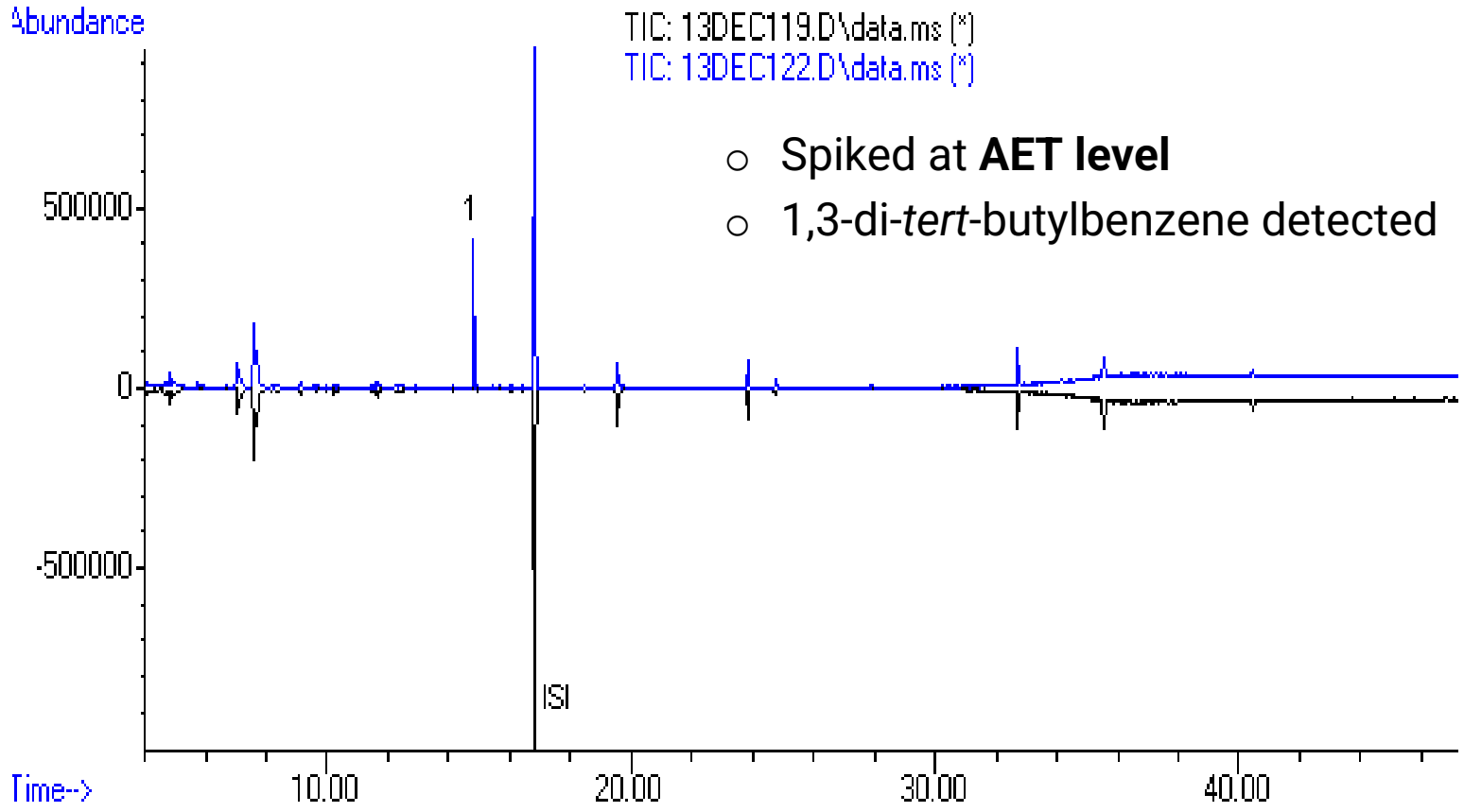
HS-GC/MS contact sample:

- No compounds detected > final AET of 690 µg/m² for T0 & T12M



STEP 3: LEACHABLES STUDY– RESULTS

GC/MS – MST result for 1,3-di-tert-buylbenzene:

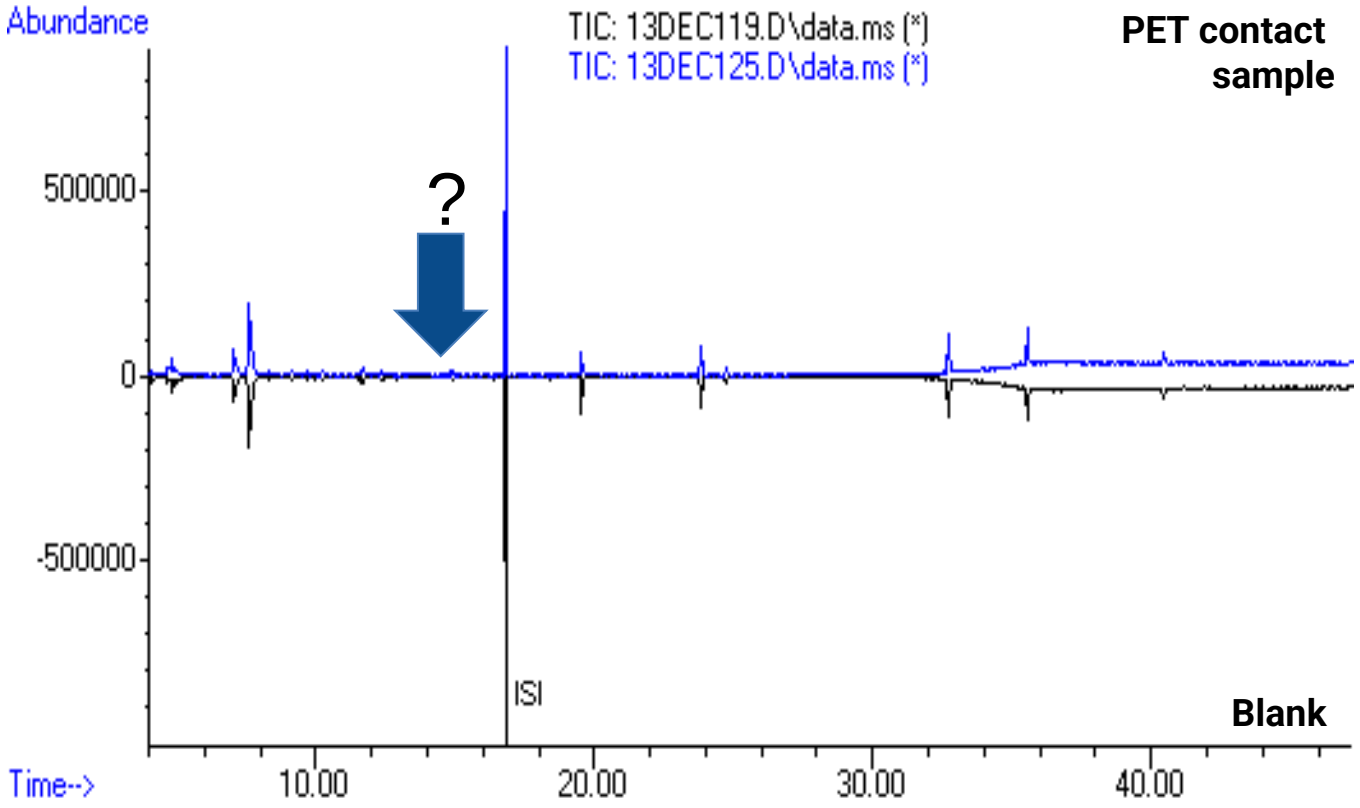


- Spiked at **AET level**
- 1,3-di-*tert*-butylbenzene detected

STEP 3: LEACHABLES STUDY– RESULTS

GC/MS contact sample:

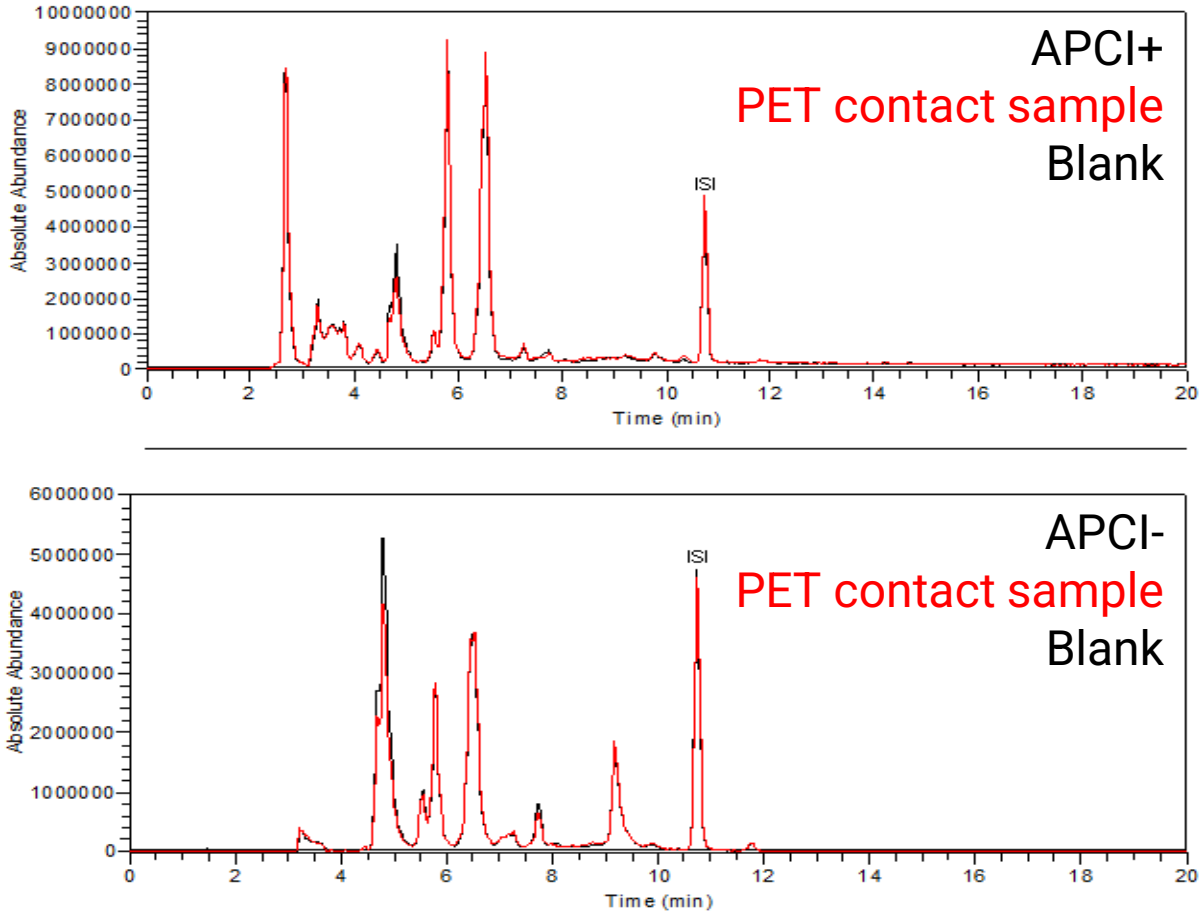
- No compounds detected > final AET of 690 µg/m² for T0 & T12M



STEP 3: LEACHABLES STUDY– RESULTS

HRAM-UPLC/MS contact sample:

- No compounds detected > final AET of 690 µg/m² for T0 & T12M



QUESTIONS?

REFERENCES

- Title 21 of the Code of Federal Regulations (CFR), Sec. 211.65 Equipment construction (April 1, 2021).
- ICH Q7: Good Manufacturing Practice for Active Pharmaceutical Ingredients (November 1, 2000).
- EU GMP EudraLex – Volume 4 – Part I – Basic Requirements for Medicinal Products – Chapter 3 – Premise and Equipment (March 1, 2015).
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- USP <1665>: Characterization and qualification of plastic components and systems used to manufacture pharmaceutical drug products and biopharmaceutical drug substances and products (2022).
- “Recommendations for Extractables and Leachables Testing” by the Extractables and Leachables Subcommittee of the Bio-Process System Alliance (2008)
- “Recommendations for Testing and Evaluation of Extractables from Single-Use Process Equipment” Bio-Process Systems Alliance (2010)
- “Standardized Extractables Testing Protocol for Single-Use Systems in Biomanufacturing” by BPOG (Nov 2014)
- “Best Practices Guide for Evaluating Leachables Risk from Polymeric Single-Use Systems used in Biopharmaceutical Manufacturing” by BPOG (Mar 2017)

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- "BioPhorum Best Practices Guide for Extractables testing of Polymeric Single-Use Components used in BioPharmaceutical Manufacturing" by BioPhorum (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" by BioPhorum (Sep 2020)