DISPOSABLE & SINGLE-USE SYSTEMS

PDA TRAINING COURSE
EXTRACTABLES – LEACHABLES
Rome
01 – 02 March, 2018

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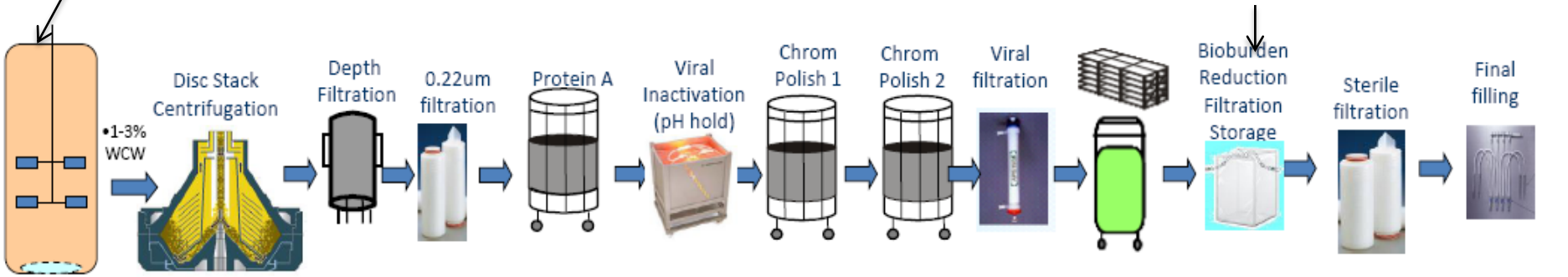
BIOPRODUCTION PROCESS

Bioproduction process

Fermentation

Storage of intermediate/bulk product



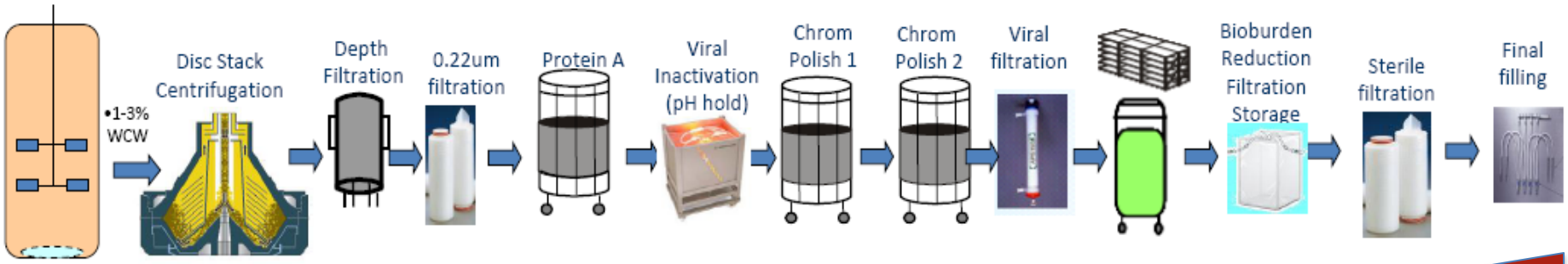
Product recovery / harvesting

Purification

Final formulation and filling

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.

Bioproduction process

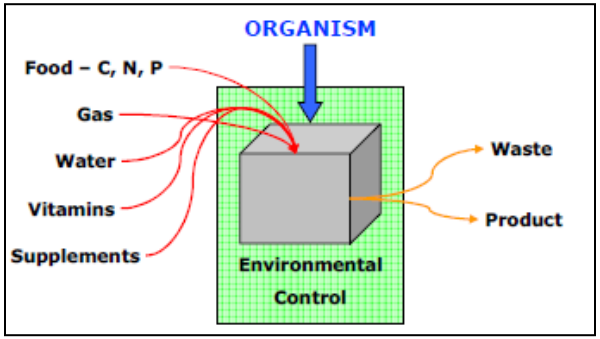


Leachables Impact on Toxicological Risk

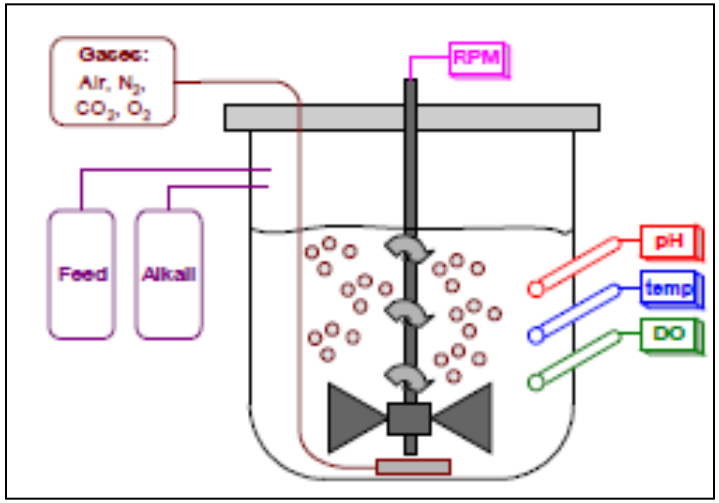
Bioproduction example from a slide from Presentation at IQPC Conference "Disposable Solutions", Munich, 18-20 FEB2014: "BPOG's Extractable Protocol Standardization Journey – Review 2013 Process ande Planning for 2014" Ken Wong (Sanofi-Pasteur), with permission of the Author.

Fermentation

Fermentation: Process where product is produced by mass culture of organisms



» **Fermentation process**
- growth medium and cell culture in fermentation tank (bioreactor)



» **Control parameters** for *optimized growth* and/or *production*
- Temperature
- pH
- Dissolved oxygen Tension
- Mixing
- Foam formation
- …

Fermentation

» In the past, traditional stainless steel bioreactors were used

» Over the past 10+ years, increasing implementation of single use & disposable bioreactors

- Elimination of **cleaning** & **sterilisation** proces
- Reduction of **energy cost** for steam generation
- Elimination of "**cleaning validation**" cost
- Reduced risk of **contamination**
- **Time saving** between production batches

Fermentation

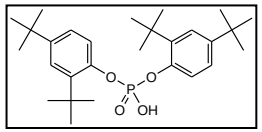
Evaluation of Extractables & Leachables

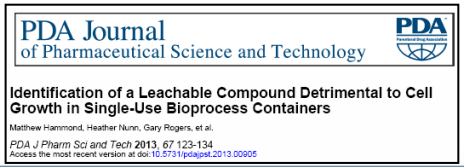
- Leachables introduced by the bioreactor might be **removed/diluted** by following process steps *(cell harvesting / purification / formulation)*
- For large batch volumes, the contact surface to volume ratio is low

→ **Toxicological risk** to the patient of leachables introduced by the bioreactor is in most cases **quite low**

- However, the **risk to product quality** caused by leachables introduced by the bioreactor might be very relevant

e.g. Bis(2,4-di-tert-butylphenyl)hydrogen phosphate (bDtBPP) causing inhibition of cell growth




PDA Journal of Pharmaceutical Science and Technology

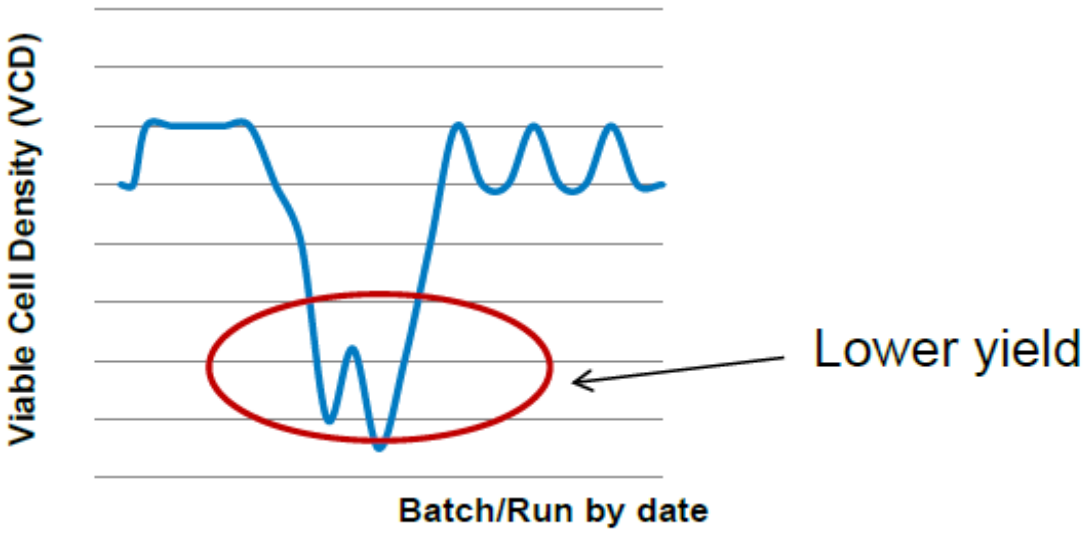
Identification of a Leachable Compound Detrimental to Cell Growth in Single-Use Bioprocess Containers

Matthew Hammond, Heather Nunn, Gary Rogers, et al.

PDA J Pharm Sci and Tech **2013**, 67 123-134

Access the most recent version at doi:10.5731/pdajpst.2013.00905

Cell Growth Inconsistency in SUBs



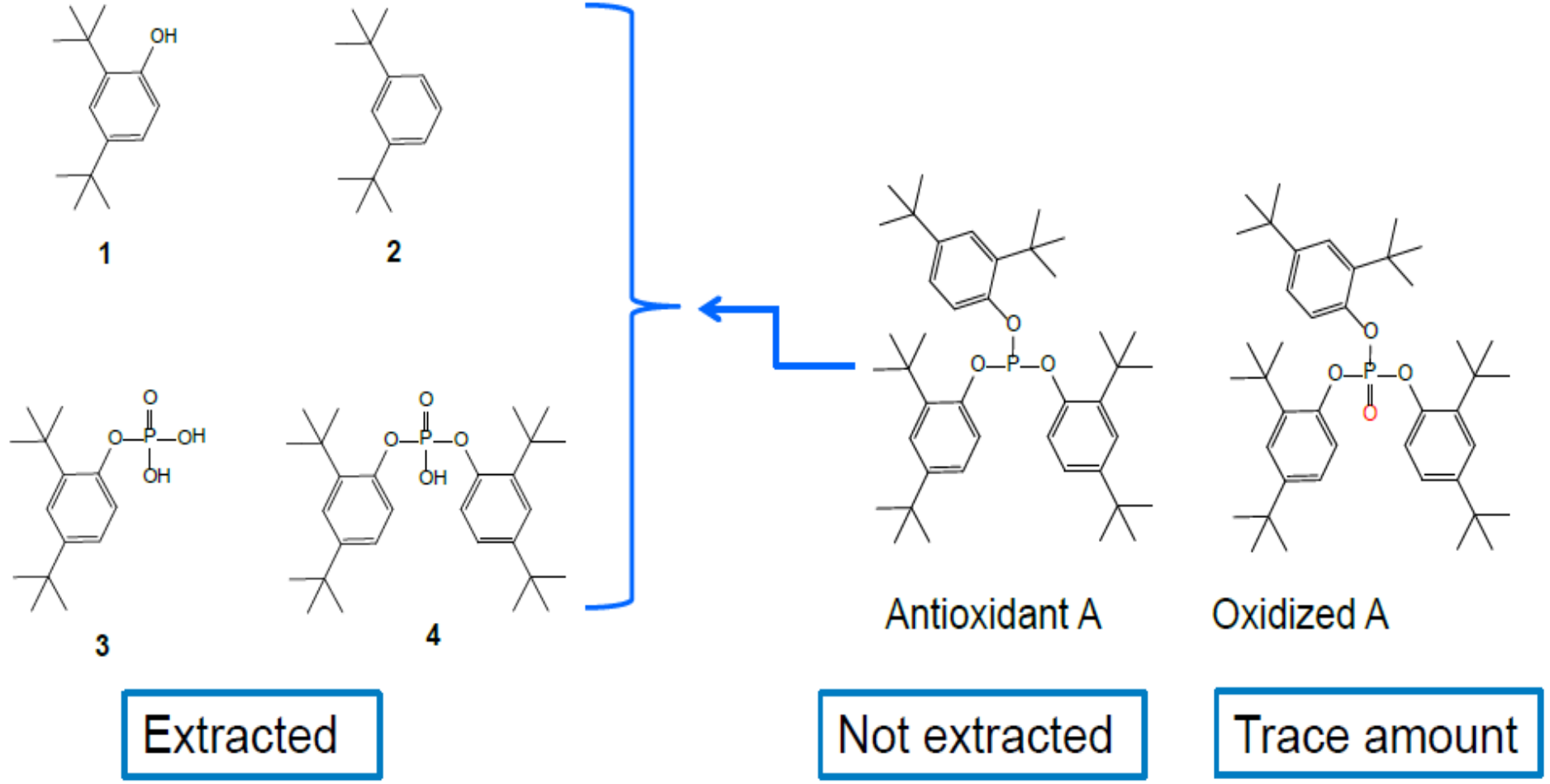
- Decreased yield = less profit
- Potential root cause(s)
  - Media
  - **Leached material from Bag**?
  - Innovative idea to non-Extractable people

Hypothesis:
SUB Leachable(s) Inhibits Cell Growth

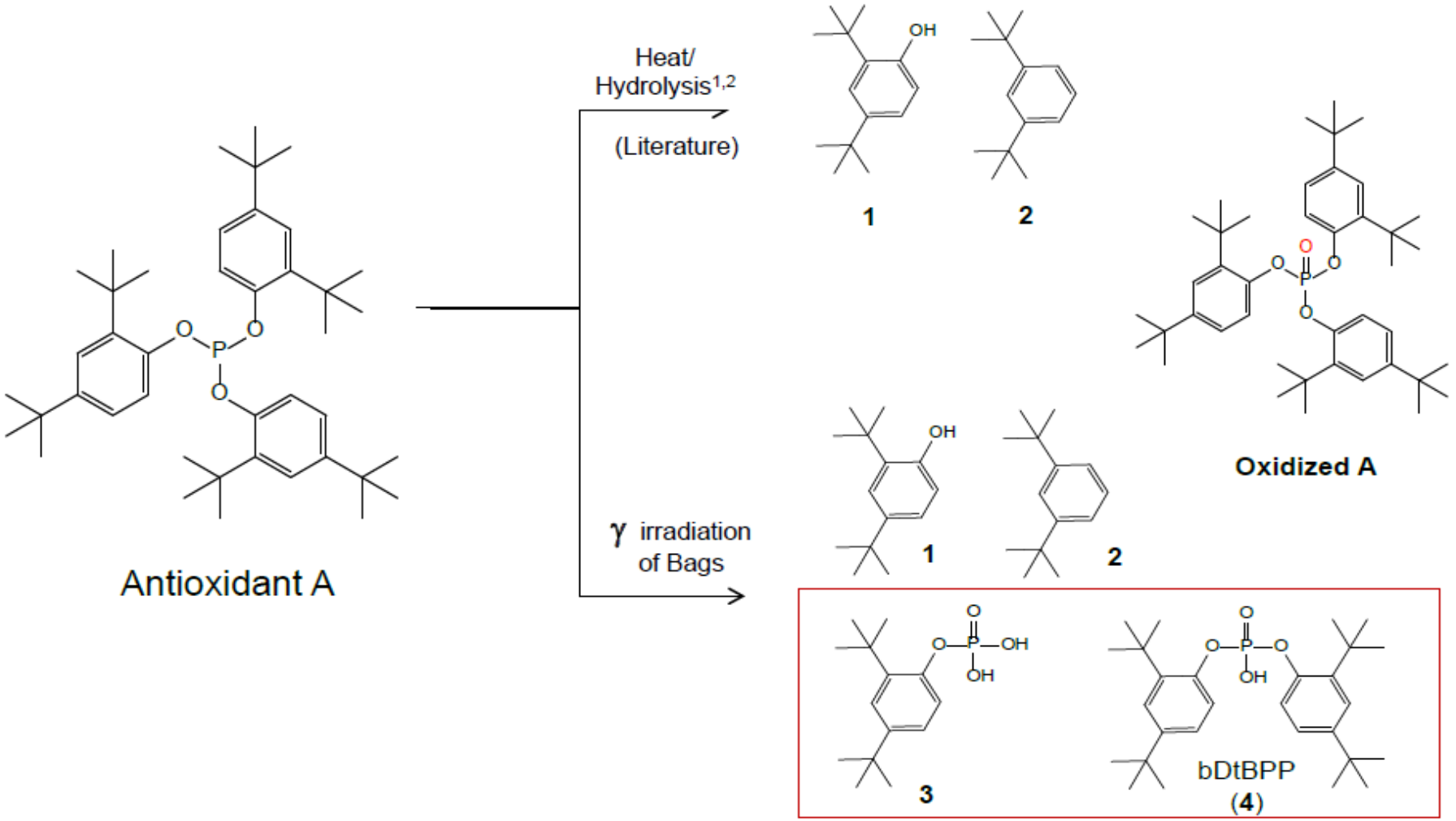
- Get information from vendor
- Perform Extractable study and ID Extractables
- Spike in individual water soluble Extractables into Cell Culture process using bags from “good” lots.
- Measure cell growth

*Vendor data/information from extractables testing

Tris(2,4-di-tert-butyl-phenyl)phosphite (A): Antioxidant in Polymer Film



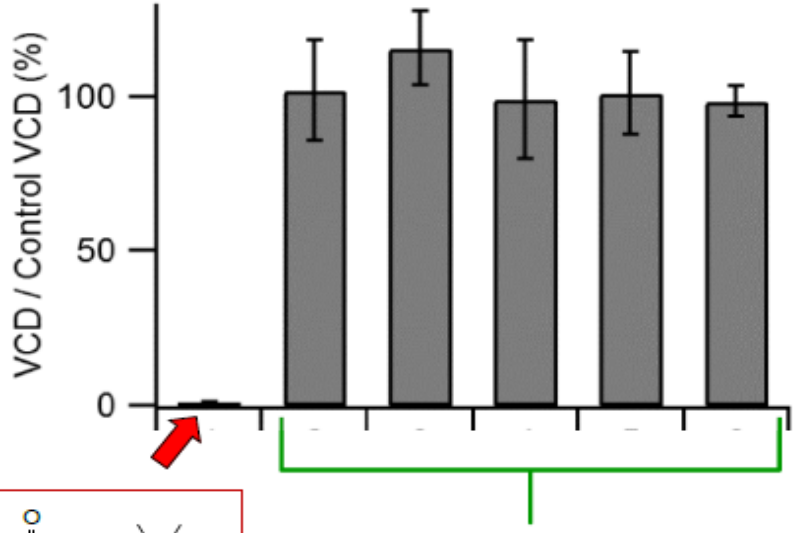
bDtBPP(4) Formation Due to Sterilization (gamma irradiation)

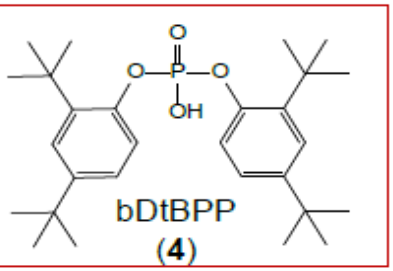


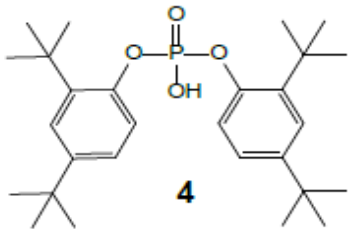
1. J. Sep. Sci. 2010, 33, p3463
2. *Packag. Technol Sci. 1999, 12, p119*

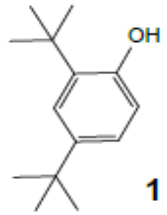
Extractable Detrimental Impact on Cell Culture

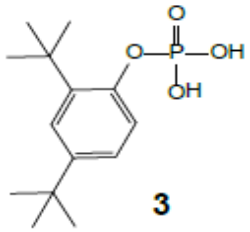
- Spike extractables at ~ 1ppm into cell culture medium

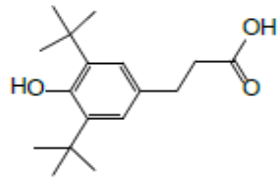


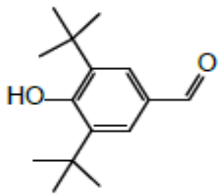


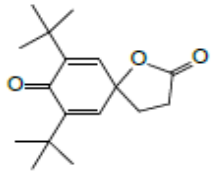










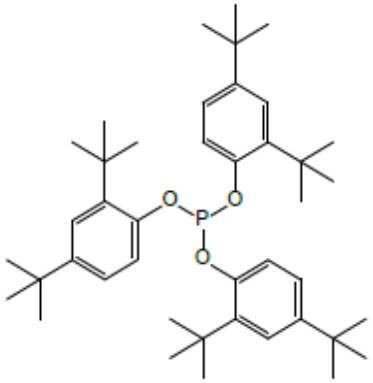


***b*D*t*BPP (4) is detrimental to cell growth**

PDA J Pharm Sci Tech 2013, 67(2) p123

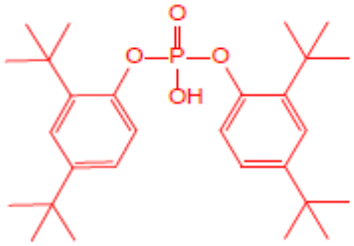
Summary/Conclusion

- Hypothesis: Extractable(s) impacts cell culture performance
- Extractables from intact bags were identified
- Poor cell culture performance correlated to an antioxidant tris(2,4-di-tert-butyl-phenyl)phosphite (A) degradant: Bis(2,4-di-t-butyl-phenyl)phosphate (bDtBPP)



Antioxidant A

Detrimental to cell growth

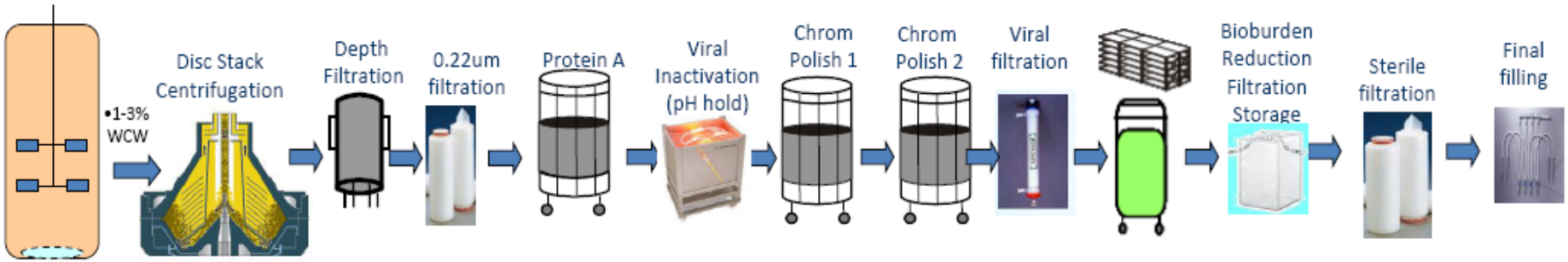


Antioxidant degradant: bDtBPP

- Currently, antioxidant A presents in many polymer films. Industry is now aware of bDtBPP.

AMGEN

Bioproduction process



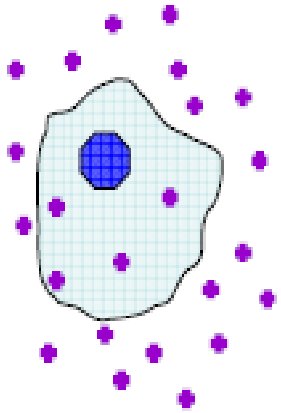
Product recovery / harvesting

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.

Product Recovery

Extracellular secreted product

» Mammal cells

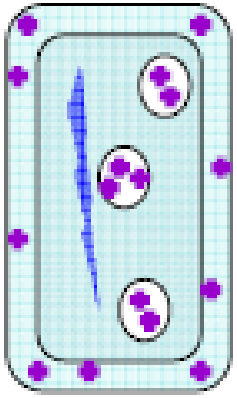


Intracellular product

» Bacteria

1. Cytoplasmatic expression
   (e.g. E.coli)

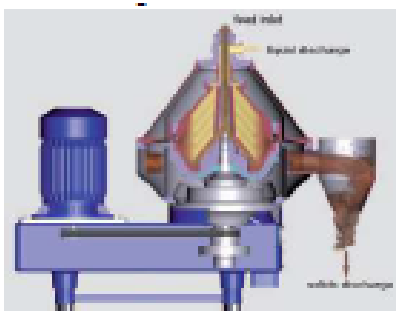
2. Periplasmatic expression
   (e.g. Gram-negative)

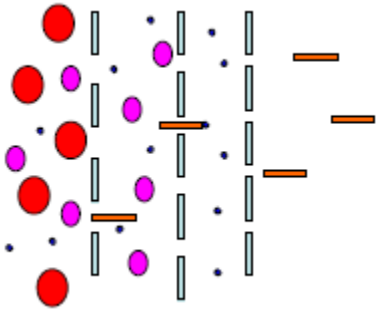


Product recovery: Extracellular Secretion

Step 1: removal of cells

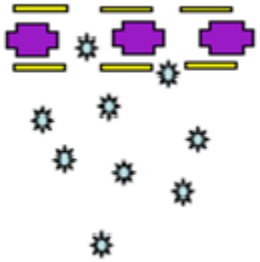
Centrifugation or Filtration

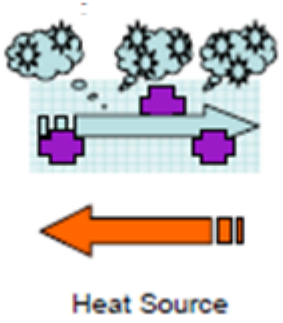


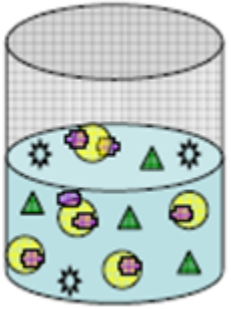


Step 2: volume reduction

Ultrafiltration or damping or batch adsorption







Product recovery:
Intracellular Secretion

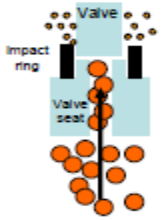
Step 1: Cell recovery

centrifugation

Step 2: **Cellular disruption**

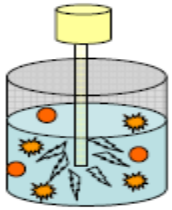
Mechanical

homogenisation



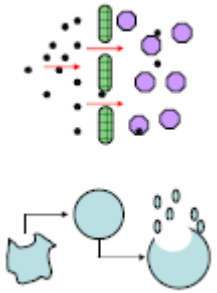
milling

sonication

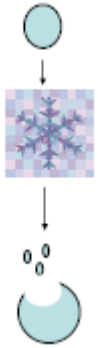


Non mechanical

osmotic shock



'freeze thaw'



enzymatic

lysozyme + EDTA

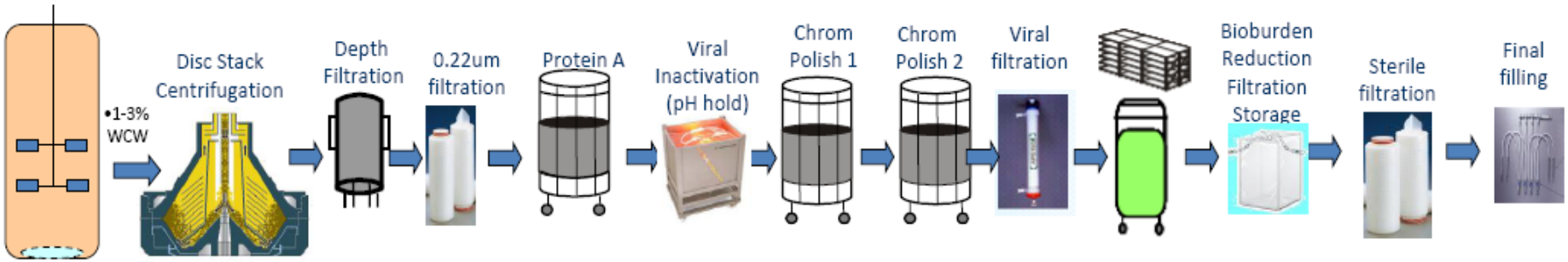
of solvents:
increase of celwand cell permeability

of detergents:
dissolution of membrane-fosfolipids

Step 3: Clarification

Step 4: Concentration

Bioproduction process



Purification

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Purification

THREE STEPS

Step 1

ISOLATION: **Transfer** product to an environment which **protects** the **activity** & **functionality**

Step 2:

INTERMEDIATE PURIFICATION: **Removal** of bulk impurities

e.g. DNA, guest cell proteïns, virusses, endotoxines

Step 3

POLISHING: Final purification to remove impurities similar to the product

Purification

Techniques used in Purification

» Chromatografic techniques:
- Affinity chromatografy
- Hydrofobic interaction chromatography
- Reverse phase chromatography
- Ion exchange chromatography

» Filtration
- Gel filtration
- Ultrafiltration
- Virus filtration (20 nm filters)

- Low pH treatment (viral inactivation)

Product Recovery & Purification

Evaluation of Extractables & Leachables

» Filters & chromatography resins have **high contact surface area** vs **solution volume**

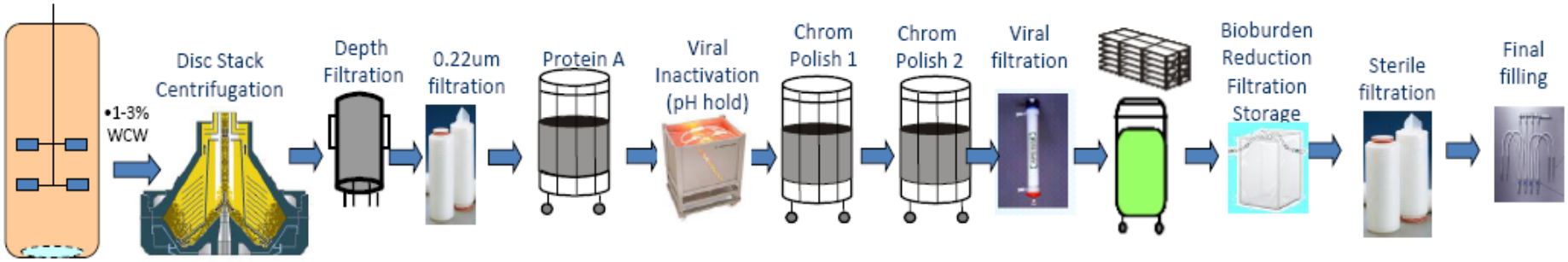
- Increased exposure amount
- Higher risk for leachables

» Subsequent process steps (such as *purification & formulation*) may ***remove/dilute*** leachables introduced during the *product recovery & purification*

However, no published data is currently avaialble

Bioproduction process

Storage of intermediate/bulk product



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Storage of Bulk Products

Storage of drug substance, buffer solutions, growth medium, etc...

Duration can be *weeks, months, years*...

Bulk Containers of different material types might be used

- PET(G)
- Polycarbonate
- Polypropylene
- High Density Polyethylene (HDPE)
- Flexible bags with multilayer films

Storage of Bulk Products

Evaluation of Extractables & Leachables

» Containers with **low filling volume** have **higher contact surface area vs solution volume** ratio

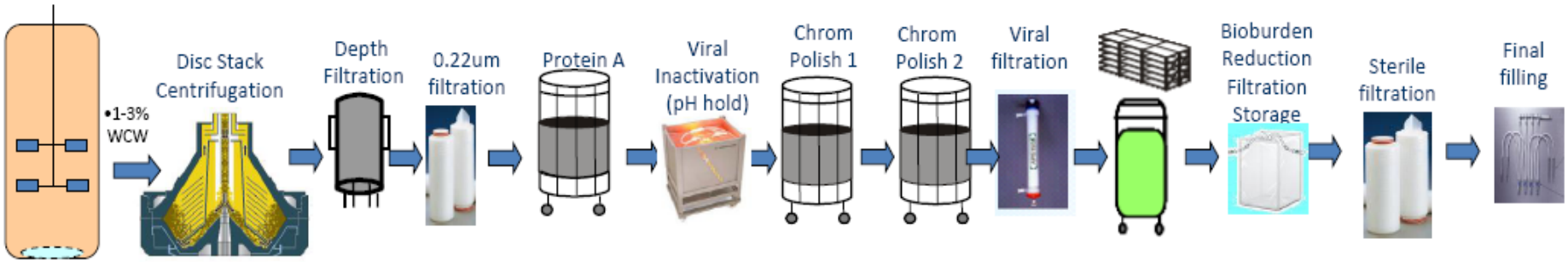
 - higher risk for leachables

» Impact of storage conditions:

↑ storage temperature: ↑ amount of leachables

↑ storage time: ↑ amount of leachables

Bioproduction process



Final formulation and filling

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Formulation & Filling

Adding excipients in order to obtain the **right stability & administration** composition

» Sterile filtration
» Filling in final packaging container via tubing
  - Pharmaceutical grade tubings:
    - Silicone: Pt-cured or peroxide cured
    - TPE (thermoplastic elastomer)
    - PTFE coated
    - ...
» not only used in bioproduction, but also relevant for conventional small molecule drug products

Formulation & Filling

Evaluation of Extractables & Leachables

» Filters have a **high contact surface area** to **solution volume ratio**

» Filling equipment makes direct contact with the final drug product

→ all leachables will end up in the final product
(no longer any *dilution/purification steps*)

FDA 1999 "Container/Closure Guidance": also applicable for storage of Drug Substance

Processing Materials

1. Bioproduction process typically contains a lot of individual process components

2. Many of the systems are custom configs *(of components)*
   - Bag from *Vendor A*
   - Tubing from *Vendor B*
   - Filter from *Vendor C*
   - Connectors from *Vendor D*

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

A good risk assessment to define critical process steps/components is important

REGULATORY REQUIREMENTS FOR SINGLE USE SYSTEMS

Production Components/Materials

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..."*

Production Components/Materials

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

Disposable Production is fairly new, may trigger additional questions

How to address:
REGULATORY REQUIREMENTS

UNIQUE CHALLENGES OF BIOLOGICS

- Administration by injection is among those of highest concern
- Likelihood of interaction between packaging component and injectable dosage is high
- Biologics are complex
  - ✓ Large molecular weights
  - ✓ Abundance of binding sites on the surface (hydrophilic & hydrophobic)
  - ✓ Heterogeneous mixtures
- Biologics are sensitive to structural modifications
  - ✓ Safety considerations (immunogenicity)
  - ✓ Efficacy considerations (loss of activity, formation of neutralizing antibodies)
  - ✓ Quality considerations (protein aggregates, stability)

I. *Markovic (2014) regulatory Perspective on Extractables & Leachables in Biologics, ASTM E55 Workshop, May 21, 2014*
II. *Kim Li (2016) Predicting the risk of extractables and leachables (E&L) interacting with Therapeutic proteins, presentation at PEPTALK 2016*

How to address:
REGULATORY REQUIREMENTS

E&L STRATEGY FOR BIOLOGICS MUST ADDRESS BOTH SAFETY & QUALITY CONCERNS

- The strategy can be applied to drug containers, drug delivery systems & single-use systems
- It should incorporate key ICH Q9 concepts, science- and risk based
- It should be phase appropriate, progressing from screening and selection of critical components to life cycle management of drug products

Evaluation of E/L should provide understanding of toxicity profile and likelihood of interaction with drug, excipient and/or package

I. *Kim Li (2016) Predicting the risk of extractables and leachables (E&L) interacting with Therapeutic proteins, presentation at PEPTALK 2016*

How to address:
REGULATORY REQUIREMENTS

E&L STRATEGY FOR BIOLOGICS MUST ADDRESS BOTH <u>SAFETY</u> & <u>QUALITY</u> CONCERNS

- For **Safety Evaluations**, one can **rely in well described risk based approaches**
  - ✓ E.g. Extrapolation of the PQRI Threshold approach to Single-Use Systems
  - ✓ ICH M7 for Genotoxic Impurities
  - ✓ In depth Toxicological Evaluation (see other presentation)

- However, what about **thresholds – *or acceptance criteria* – for the evaluation of <u>leachable impact</u> on Drug Prudct QUALITY**?
  - ✓ Not yet described
  - ✓ Not clear on “how low to go” from a quality perspective

How to address: REGULATORY REQUIREMENTS

Guidance for Industry

Immunogenicity Assessment for Therapeutic Protein Products

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

Consequences for EFFICACY – some of the concerns:

Development of "***Neutralizing Antibodies***" *(e.g. through chemically modified therapeutic protein product)* can **block the efficacy** of therapeutic protein products

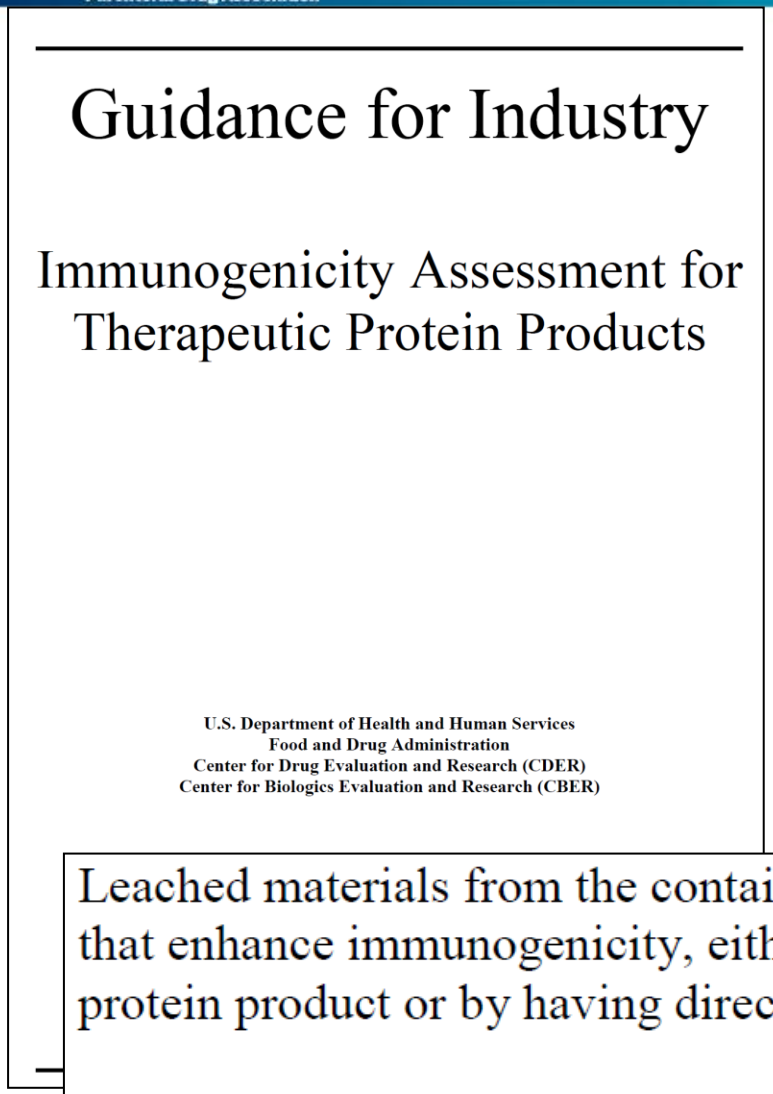
May also change the Pharmacokinetics

- Enhancing Clearance
- Or Prolonging Product Activity

Leached materials from the container closure system may be a source of materials that enhance immunogenicity, either by chemically modifying the therapeutic protein product or by having direct immune adjuvant activity.

FDA Guidance for Industry, 2014

How to address: REGULATORY REQUIREMENTS


Guidance for Industry

Immunogenicity Assessment for Therapeutic Protein Products

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

Consequences for SAFETY – some of the concerns: *(e.g. "...through chemically modified therapeutic protein product...")*

- Anaphylaxis (serious, accute allergenic reaction)
- Cytokine Release Syndrome
- "Infusion Reactions"
- Non-Acute Reactions
- Cross-reactivity to Endogeneous Proteins

Leached materials from the container closure system may be a source of materials that enhance immunogenicity, either by chemically modifying the therapeutic protein product or by having direct immune adjuvant activity.

FDA Guidance for Industry, 2014

AMGEN

Extractable Compound List
Identity & Quantity

Gather Information on Properties
ClogP, pKa

Classify Potential Interactions

Highest risk of structural modifications of proteins

COVALENT

Organic
Reactive as:
Michael acceptor
Schiff base formers
Acylating agents
SN1
SN2

Inorganic
Catalysts of Oxidation
Disulfide Formation
Halogens
Leaving group

NON COVALENT

Organic
AMPHIPHILIC
Anionic
Cationic
Non-Ionic

PHENOLIC
BzOH
Cresols

Inorganic
POLYOXOMETALA-TES
W, V, Mo,,,,

SILICONE OIL
Si

Other
Rapidly reversible

Known Adjuvant?

Likely to interfere with analytics?
UV absorbance
Cytotoxicity

Li, K., Rogers, G., Nashed-Samuel, N., Lee, H., Mire-Sluis, A., Cherney, B.,... Markovic, I., (2015). Creating a Holistic Extractables and Leachables 'E&L) Program for Biotechnology Products. PDA Journal of Pharmaeutical Science and Techniology 69(5), 590-619
Kim Li (2016) Predicting the risk of extractables and leachables (E&L) interacting with Therapeutic proteins, presentation at PEPTALK 2016

Examples of Extractables that may form covalent binding with protein

- Michael acceptors
  - ✓ Acrylic acid, Methacrylic acid, 1,6-hexanediol diacrylate, dibutylmaleate
  - ✓ Schiff base formers
  - ✓ BHT-related structures (BHT-OH, BHT-aldehyde, BHT-quinone, BHT-quinone methide)
- Acylating agents
  - ✓ Phthalic anhydride
- Transition Metals
  - ✓ Cr, Cu, Fe, Mn, Ni, W, Zn

Li, K., Rogers, G., Nashed-Samuel, N., Lee, H., Mire-Sluis, A., Cherney, B.,... Markovic, I., (2015). Creating a Holistic Extractables and Leachables 'E&L) Program for Biotechnology Products. PDA Journal of Pharmaeutical Science and Techniology 69(5), 590-619
Kim Li (2016) Predicting the risk of extractables and leachables (E&L) interacting with Therapeutic proteins, presentation at PEPTALK 2016

PDA Technical Report 26: "Sterilizing Filtration of Liquids"

"...It is the user's responsibility to demonstrate that the product does not contain objectable levels of extractables from the filter..."

"...The Filter user is responsible for obtaining the extractable data for the drug product formulation..."

TR26 is in Revision

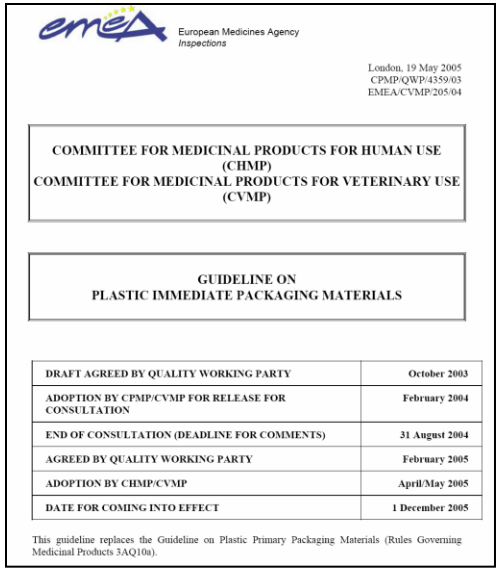
USP <1664> Table 1. Modified FDA/CDER/CBER Risk-Based Approach to Consideration of Leachables

Examples of Packaging Concerns for Common Classes of Drug Products			
Degree of Concern Associated with Route of Administration	**Likelihood of Packaging Component-Dosage Form Interaction**		
	High	**Medium**	**Low**
Highest	Inhalation Aerosols and Sprays	Injections and Injectable Suspensions; Inhalation Solutions	Sterile Powders and Powders for Injection; Inhalation Powders
High	Transdermal Ointments and Patches	Ophthalmic Solutions and Suspensions; Nasal Aerosols and Sprays	—
Low	Topical Solutions and Suspensions; Topical and Lingual Aerosols; Oral Solutions and Suspensions	—	Oral Tablets and Oral (Hard and Soft Gelatin) Capsules; Topical Powders; Oral Powders

While this table provides a convenient overview of the general level of regulatory concern with various dosage forms regarding leachables, it should not be inferred that “low-risk” dosage forms (e.g., oral tablets) by that definition carry no risk for leachables issues.

EMA Plastic Immediate Packaging materials (2005)

- Applicable to Active Substances or Drugs
- "Packaging materials intended to be in contact with the active substances or medicinal products"



European Medicines Agency
Inspections

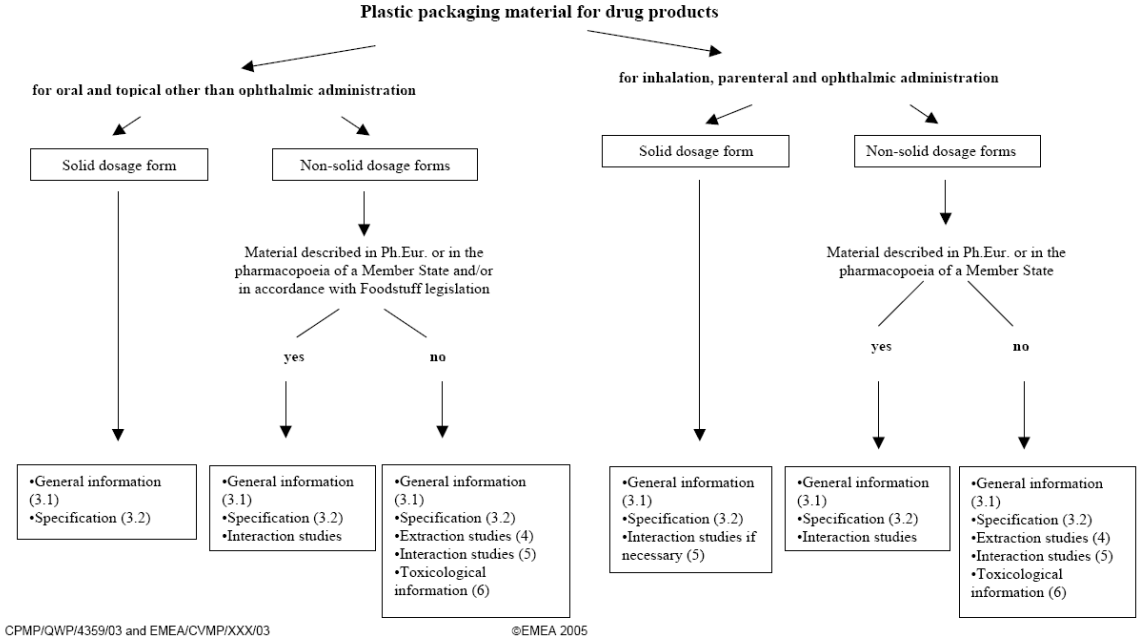
London, 19 May 2005
CPMP/QWP/4359/03
EMEA/CVMP/205/04

COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE (CHMP)
COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE (CVMP)

GUIDELINE ON
PLASTIC IMMEDIATE PACKAGING MATERIALS

DRAFT AGREED BY QUALITY WORKING PARTY	October 2003
ADOPTION BY CPMP/CVMP FOR RELEASE FOR CONSULTATION	February 2004
END OF CONSULTATION (DEADLINE FOR COMMENTS)	31 August 2004
AGREED BY QUALITY WORKING PARTY	February 2005
ADOPTION BY CHMP/CVMP	April/May 2005
DATE FOR COMING INTO EFFECT	1 December 2005

This guideline replaces the Guideline on Plastic Primary Packaging Materials (Rules Governing Medicinal Products 3AQ10a).



CPMP/QWP/4359/03 and EMEA/CVMP/XXX/03 ©EMEA 2005

INTEREST GROUPS, TRADE ASSOCIATIONS AND STANDARDIZATION ORGANIZATIONS FOR SINGLE USE SYSTEMS

ON THE WAY TO HARMONISATION

INTEREST GROUPS, TRADE ASSOCIATIONS
STANDARIZATION ORGINIZATIONS

1. **Bio-Process Systems Alliance (BPSA)**
2. **Biophorum Operations Group (BPOG)**
3. **ASME-BPE** ***(only mentioned) – In Preparation***
   ASME: American Association for Mechanical Engineers
   BPE: BioProcessing Equipment
4. **ISPE – BPOG – ASTM** ***– In Preparation***
   ISPE: International Society for Pharmaceutical Engineering
5. **USP <665>**

Bio-Process Systems Alliance (BPSA)

Selected slides with permission of the Author from Presentation at IQPC Conference "Disposable Solutions", Munich, 18-20 FEB2014: "Collaborative Efforts to Standardise Supplier's Extractable Data for Single-Use Components", Jerold Martin (BPSA, Chairman, Pall Life Sciences)

BPSA

Bio-Process Systems Alliance

Bio-Process Systems Alliance

- Trade association of suppliers and users
- Facilitates implementation of single-use
  - Networking opportunities
  - Safe harbor for dialogue among suppliers
  - End-user / supplier forums
  - Best practice guides

www.bpsalliance.org

Bio-Process Systems Alliance (BPSA)

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BPSA Extractables Guides (2008, 2010)

- Recommendations for Extractables and Leachables Testing (2008)
  - Part 1: Introduction, Regulatory Issues, and Risk Assessment
  - Part 2: Executing a Program

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

- Available at www.bpsalliance.org

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PALL Life Sciences

Bio-Process Systems Alliance (BPSA)

Selected slides with permission of the Author from Presentation at IQPC Conference "Disposable Solutions", Munich, 18-20 FEB2014: "Collaborative Efforts to Standardise Supplier's Extractable Data for Single-Use Components", Jerold Martin (BPSA, Chairman, Pall Life Sciences)

Extraction Conditions

- Test Articles
- Pre-conditioning
  - Autoclave, irradiate, flush
- Solvents
  - Water, ethanol, high/low pH, low polarity, surfactants
- Temperature
- Time
- Dynamics (e.g. Agitation)
- Surface area : volume ratio
- Component types
  - Biocontainer/Bioreactor, Filter, Connector, Tubing, Mixing Bag, Integrated System

* *BPSA Recommendations for Extractables and Leachables from Single-Use Process Equipment (2010)*

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Analytical Methods

Analytical Techniques

- FTIR
- GC/FID
- GC/MS
- HPLC/DAD
- HPLC/MS
- HS/GC/MS
- IC
- ICP
- NVR
- Conductivity
- pH
- TOC

Characteristics

- Identification
- Overview
- Category/Classification
- Sensitivity (LOD)
- Detectable Species
- Sample Preparation
- Strengths
- Limitations

* *BPSA Recommendations for Extractables and Leachables from Single-Use Process Equipment (2010)*

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Selected slides with permission of the Author from Presentation at IQPC Conference "Disposable Solutions", Munich, 18-20 FEB2014: "Collaborative Efforts to Standardise Supplier's Extractable Data for Single-Use Components", Jerold Martin (BPSA, Chairman, Pall Life Sciences)

Summary Recommendations

- Extractables data = potential leachables
- Perform extractions with at least two solvents
  - Water and low MW alcohol
  - Low MW organic or pH extremes where applicable
- Use exaggerated time, temperature, surface area/volume ratio and pretreatment steps
- Apply analytical methods to characterize, identify and quantify
- Supplier data is acceptable where applicable
- Perform risk assessment – impact on final drug?

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BioPhorum Operations Group (BPOG)

Slides Selected with permission of the Author 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)

Who are We - BioPhorum Operations Group (BPOG) ?

- BPOG is a global collaboration of biopharmaceutical manufacturers. Since 2008 it has grown to...
  - 23 member companies
  - 750 active representatives working in 12 workstreams including Disposables
  - Extractables is the largest sub group in the Disposables workstream – it started up in late 2012, involves 19 member companies and a mix of analytical chemists and process engineers/scientists who are subject matter experts.
- BPOG mission
  - To accelerate the rate of the journey to industrial maturity.
- BPOG is not a standards body or representative of suppliers
  - BPOG enables companies to collaborate, build and share solutions to the most significant common challenges they face.
  - BPOG works with and through other bodies to realise change.

BioPhorum Operations Group (BPOG)

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Standard Extractable Studies – Subset of Sample Preparation Table

Single Use Component Type	Recommended Sample Extraction Conditions
Storage / Mixing / Bioreactor bags	Use a small bag (≤ 5L) - meet 6:1 (cm^2/mL) surface area to volume ratio. Studies performed with 2D bags with the same MOC (represent 3D bags). Shaking on an orbital shaker is recommended. Express analytical results in µg/cm^2.
Tubing	Use tubing with ½'' ID - meet 6:1 (cm^2/mL) surface area to volume ratio. Record and report the length and ID of the tubing. Shaking on an orbital shaker is recommended. Express analytical values in µg/cm and µg/cm^2.
Sterilizing-grade/ Process Filters	Use filter with effective filtration area (EFA) equal to or greater than 0.1 m^2 (if possible) for study and maintain at least 1:1 (cm^2/mL) EFA to volume ratio. Either recirculating solvent through the filter or filling the filter and shaking on an orbital shaker is recommended. Express the analytical values in µg/cm^2.

6:1 ratio can be adjusted down with justification

1:1 ratio is the minimum. Higher is desirable

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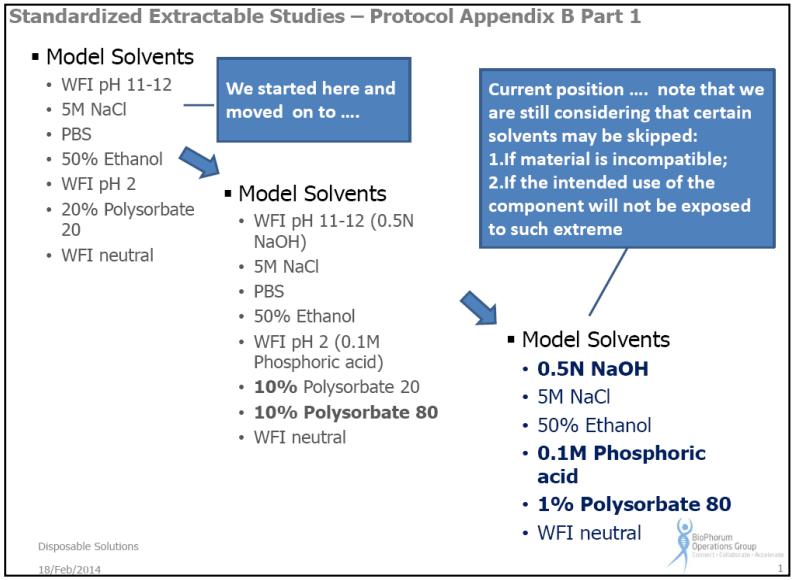

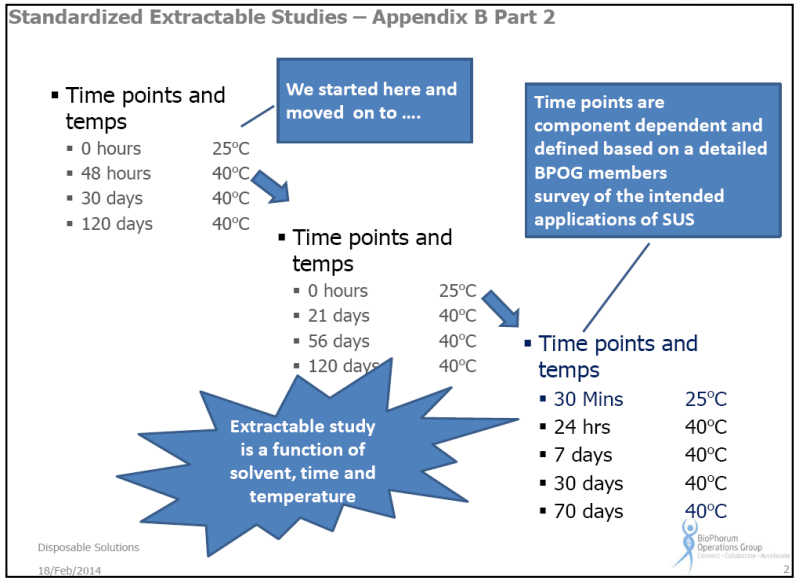

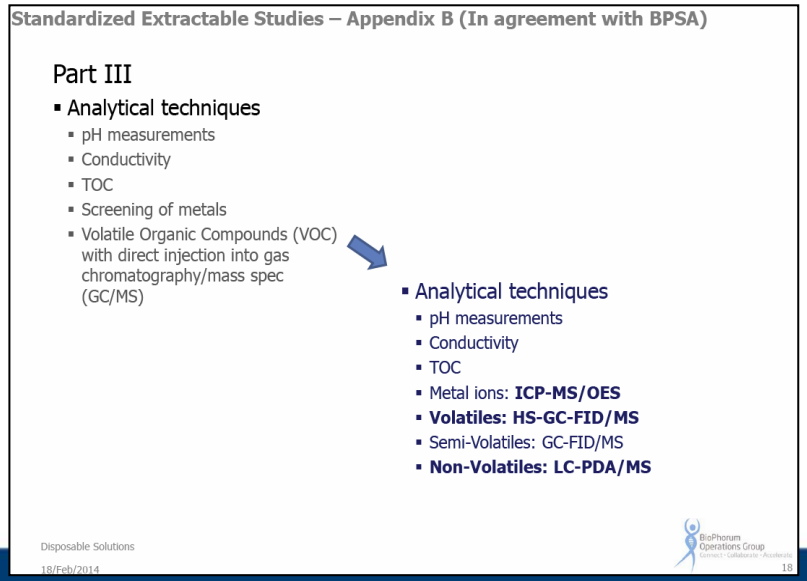

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SUS Category	SOLVENTS[1]						TIME				
	50% Ethanol	1% PS-80	5M NaCl	0.5N NaOH	0.1M Phosphoric acid	WFI neutral	Time 0 (≤ 30mins)	24 hrs	7 days	30 days	70 days
							25°C	40°C			
Storage bags	X	X	X	X	X	X	X	X		X	X
Mixing bags / mixing device	X	X	X	X	X	X	X	X		X	
Bioreactor bags	X	X	X	X	X	X	X	X		X	X
Tubing, Liquid injection materials	X	X	X	X	X	X	X			X	
Process (UF/DF) filters	X	X	X	X	X	X	X		X		
Bioreactor Sensors	X	X	X	X	X	X	X			X	
Other Sensors	X	X	X	X	X	X	X		X		
Sterile (~0.2µm) and viral filters	X	X	X	X	X	X	X	X			
Aseptic/non-aseptic tubing dis/connectors	X	X	X	X	X	X	X			X	
Prepacked column body	X	X	X	X	X	X	X				X
Filling manifold	X	X	X	X	X	X	X	X			

[1] *Certain solvent may be skipped:*
If material is incompatible;
If the intended use of the component will not be exposed to such extreme

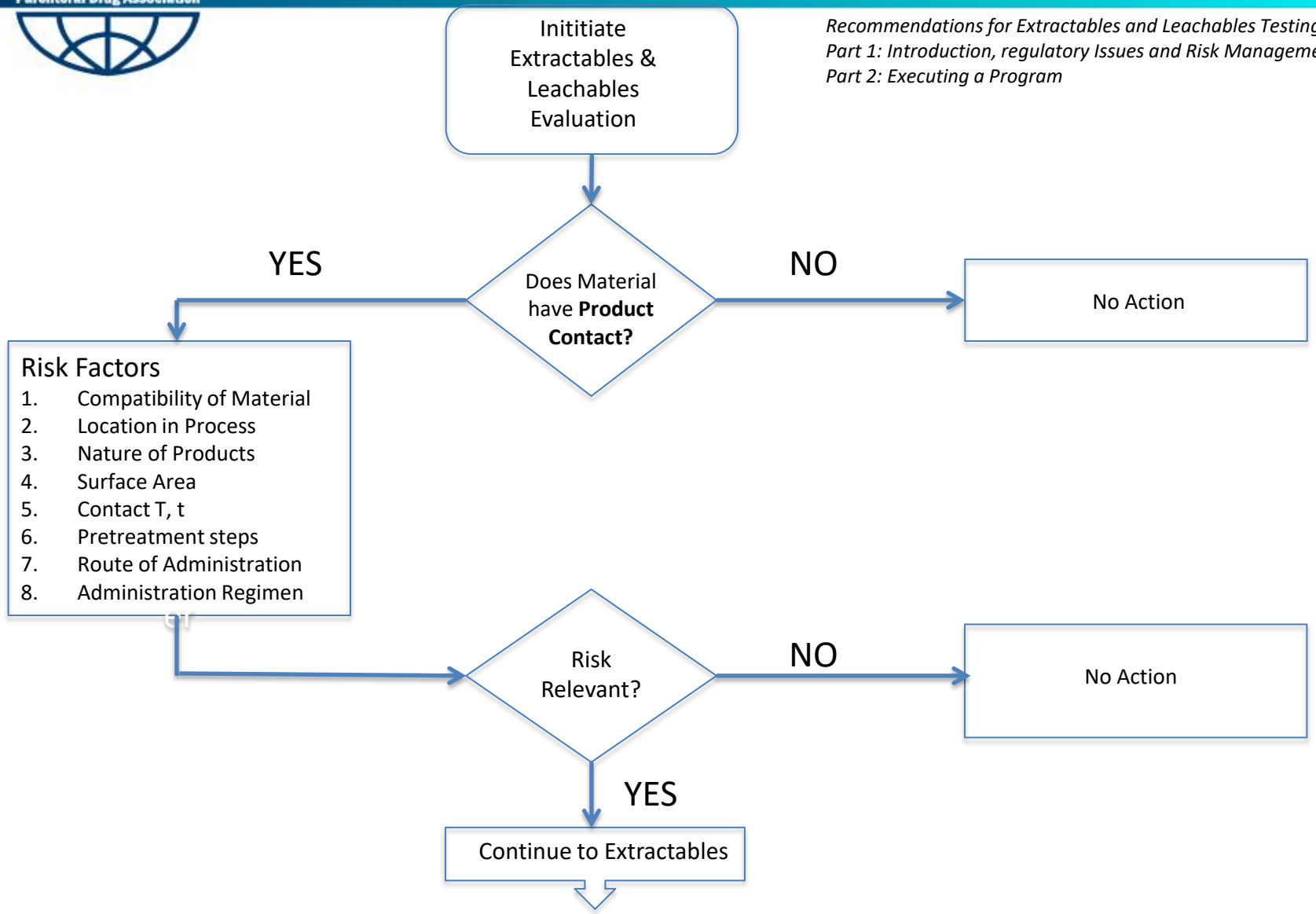
BIOPRODUCTION PROCESS

THE *BPSA RISK ASSESSMENT APPROACH*

Recommendations for Extractables and Leachables Testing (2008)
Part 1: Introduction, regulatory Issues and Risk Management
Part 2: Executing a Program

BPSA Flow Chart

Recommendations for Extractables and Leachables Testing (2008)
Part 1: Introduction, regulatory Issues and Risk Management
Part 2: Executing a Program



Create a list of Product Contact Materials

- Any Material that has the potential to migrate into the final product
- List begins UPSTREAM with starting Buffers
- List Finishes with Materials used directly before the final fill & finish of containers
- Can include: *Tubing, Bags, Filters, Connectors, O-rings, Tangential Flow Cassettes, Syringes, Chromatographic resins, Final Bulk Storage vessels,…*

Recommendations for Extractables and Leachables Testing (2008)
Part 1: Introduction, regulatory Issues and Risk Management
Part 2: Executing a Program

Perform Risk Assessment

- **GOAL**: to determine the product contact materials that have the greatest potential for an objectable level of leachables
- Must be performed using criteria that are specific to the end user – cannot be generalized between applications
- Best Performed early in the process development when changes are more easily addressed

Recommendations for Extractables and Leachables Testing (2008)
Part 1: Introduction, regulatory Issues and Risk Management
Part 2: Executing a Program

RISK FACTOR 1: Material Compatibility

- Most biopharmaceutical products are aqueous and therefore are compatible with many materials
- Most biopharmaceutical materials **PASS** USP<87> or USP<88> testing
- First, obtain manufacturers recommended operating parameters, such as pH, temperature, pressure…
- Check to be sure the material is being used within the recommended normal operating procedures

Recommendations for Extractables and Leachables Testing (2008)
Part 1: Introduction, regulatory Issues and Risk Management
Part 2: Executing a Program

RISK FACTOR 2: Proximity to Final Product

- Location directly upstream of final fill has direct risk to final product
- Location upstream in process MAY have reduced risk
- This is true if there are steps where contaminants can leave the process
  - Diafiltration – diafiltrate volume can be 100x the process volume
  - Lyophilization – volatiles may be removed
- Ideally, supporting data should be obtained

Recommendations for Extractables and Leachables Testing (2008)
Part 1: Introduction, regulatory Issues and Risk Management
Part 2: Executing a Program

RISK FACTOR 3: Solution Composition

- Extreme pH
- High organic or alcohol content
- Surfactants

Recommendations for Extractables and Leachables Testing (2008)
Part 1: Introduction, regulatory Issues and Risk Management
Part 2: Executing a Program

RISK FACTOR 4: Surface-to-Volume ratio

- The higher the ratio, the higher the risk!!
- Filters – porous structure leads to area much larger than filtration area
- Smaller process volume usually has higher surface-to-volume ratio's

Recommendations for Extractables and Leachables Testing (2008)
Part 1: Introduction, regulatory Issues and Risk Management
Part 2: Executing a Program

RISK FACTOR 5: Contact time and temperature

EVIDENTLY:

- The longer the contact time, the higher the risk
- The higher the temperature, the higher the risk

Recommendations for Extractables and Leachables Testing (2008)
Part 1: Introduction, regulatory Issues and Risk Management
Part 2: Executing a Program

RISK FACTOR 6: Pretreatment steps

- STERILIZATION (e.g. gamma, EtO, autoclave) tends to change, and possibly increase, leachables
- RINSING prior to product contact tends to lower leachables
  - E.g. Preflush for filters

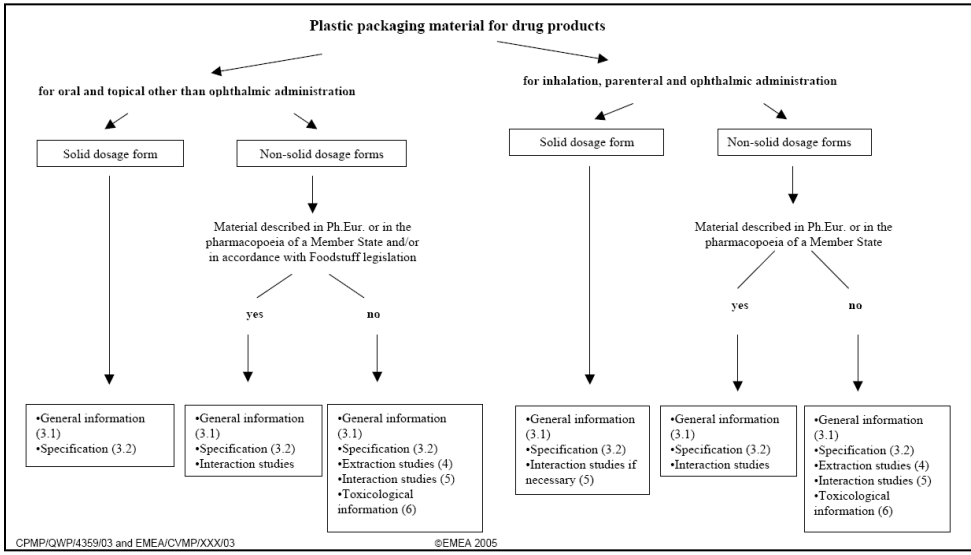
Recommendations for Extractables and Leachables Testing (2008)
Part 1: Introduction, regulatory Issues and Risk Management
Part 2: Executing a Program

RISK FACTOR 7: Route of Administration

- The Classification, presented in the FDA-Guidance (Table 1) and the EMEA-Guideline (Decision Tree), is also valid for the concern on impurities (leachables) introduced in the (bio)pharmaceutical production!!

Table 1
Examples of Packaging Concerns for Common Classes of Drug Products

Degree of Concern Associated with the Route of Administration	Likelihood of Packaging Component-Dosage Form Interaction		
	High	**Medium**	**Low**
Highest	Inhalation Aerosols and Solutions; Injections and Injectable Suspensions[a]	Sterile Powders and Powders for Injection; Inhalation Powders	
High	Ophthalmic Solutions and Suspensions; Transdermal Ointments and Patches; Nasal Aerosols and Sprays		
Low	Topical Solutions and Suspensions; Topical and Lingual Aerosols; Oral Solutions and Suspensions	Topical Powders; Oral powders	Oral Tablets and Oral (Hard and Soft Gelatin) Capsules



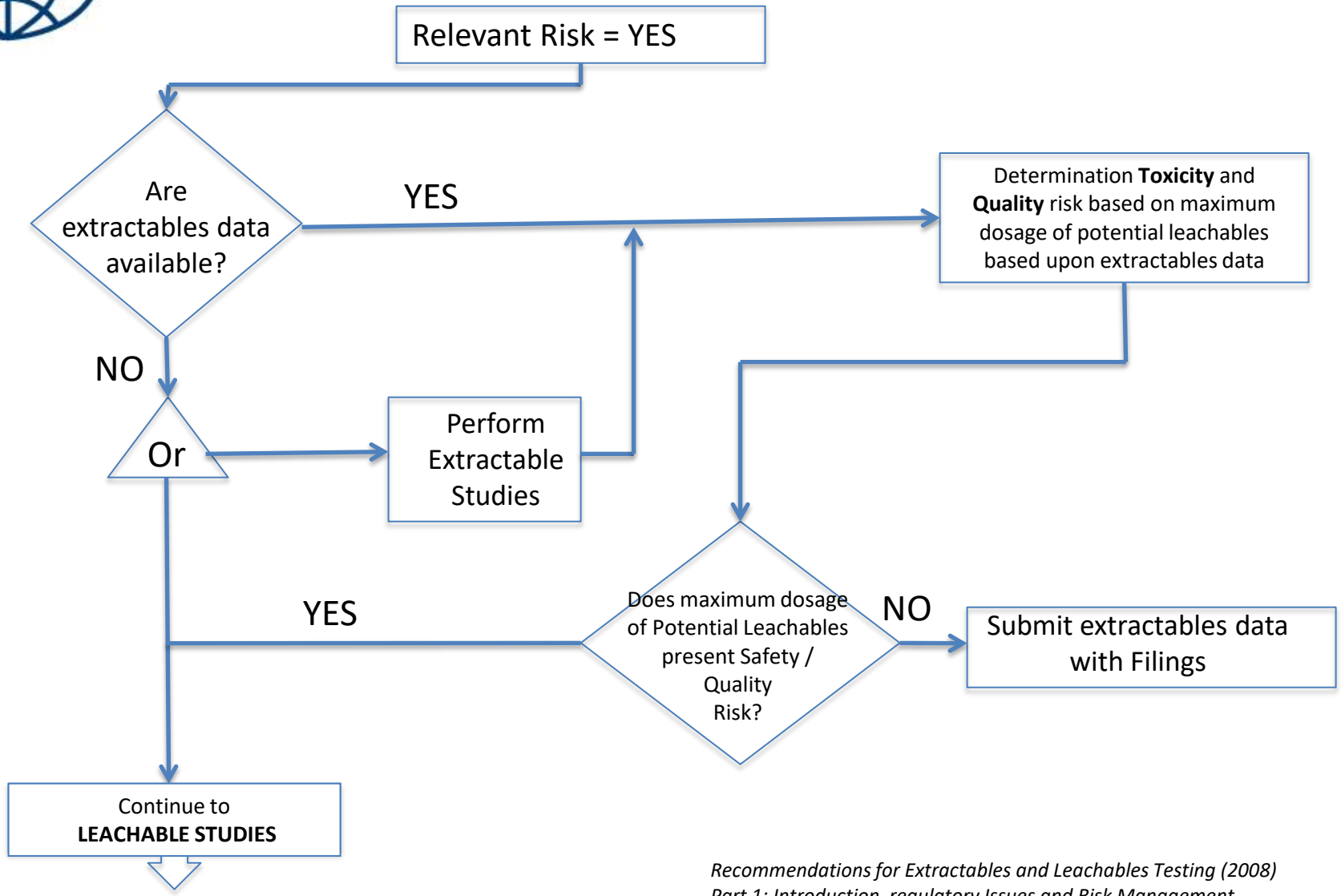
BioProcess System Alliance (BPSA)

Recommendations for Extractables and Leachables Testing (2008)
Part 1: Introduction, regulatory Issues and Risk Management
Part 2: Executing a Program

What to do with RISK FACTORS?

- Create priorities for testing
  - If a change is needed, determine early
- Weight according to end-user specific criteria
  - EXAMPLE: the presence of surfactants may be considered a high risk automatically requiring more testing for a particular end-user
- Although the Use of Numbers to assess risk (e.g. 1 to 10) is discouraged, it is often performed in this manner
  - If numerical risk values are utilized, first determine supporting data… because this potentially leads to a pseudo-scientific conclusion based on arbitrarily assigned numbers
- If it is determined there is no relevant regulatory or safety risk for a specific product contact/material interaction, then submit vendor information for regulatory filings
- If there is relevant risk, then proceed to extractables evaluation

BPSA Flow Chart (continued)



Recommendations for Extractables and Leachables Testing (2008)
Part 1: Introduction, regulatory Issues and Risk Management
Part 2: Executing a Program

Extractable Studies

- To Determine the conditions of Sample Prep:
  Look at the evaluation of the SUS and the product(s) that will be in contact to determine the right conditions

- BPSA-testing Protocol
- BPOG-testing Protocol

- Analytical Techniques
  Compound Specific:
  Headspace GC/MS, GC/MS, UPLC/HRAM, ICP-MS, IC
  Not Compound Specific:
  pH, Conductivity, TOC, NVR, FTIR on NVR...

Assess toxicity based on worst-case extractables data

Many processing material applications have a high dilution factor

- **Extractable** studies are conducted with **sufficiently high surface-to-volume** ratio
- Process Materials can have **in-use surface-to-volume** ratios **1,000 times lower** than common extraction studies
- Relatively **high concentration** of extractable **may be acceptable** when converted to dosage
- Must be evaluated **case by case**

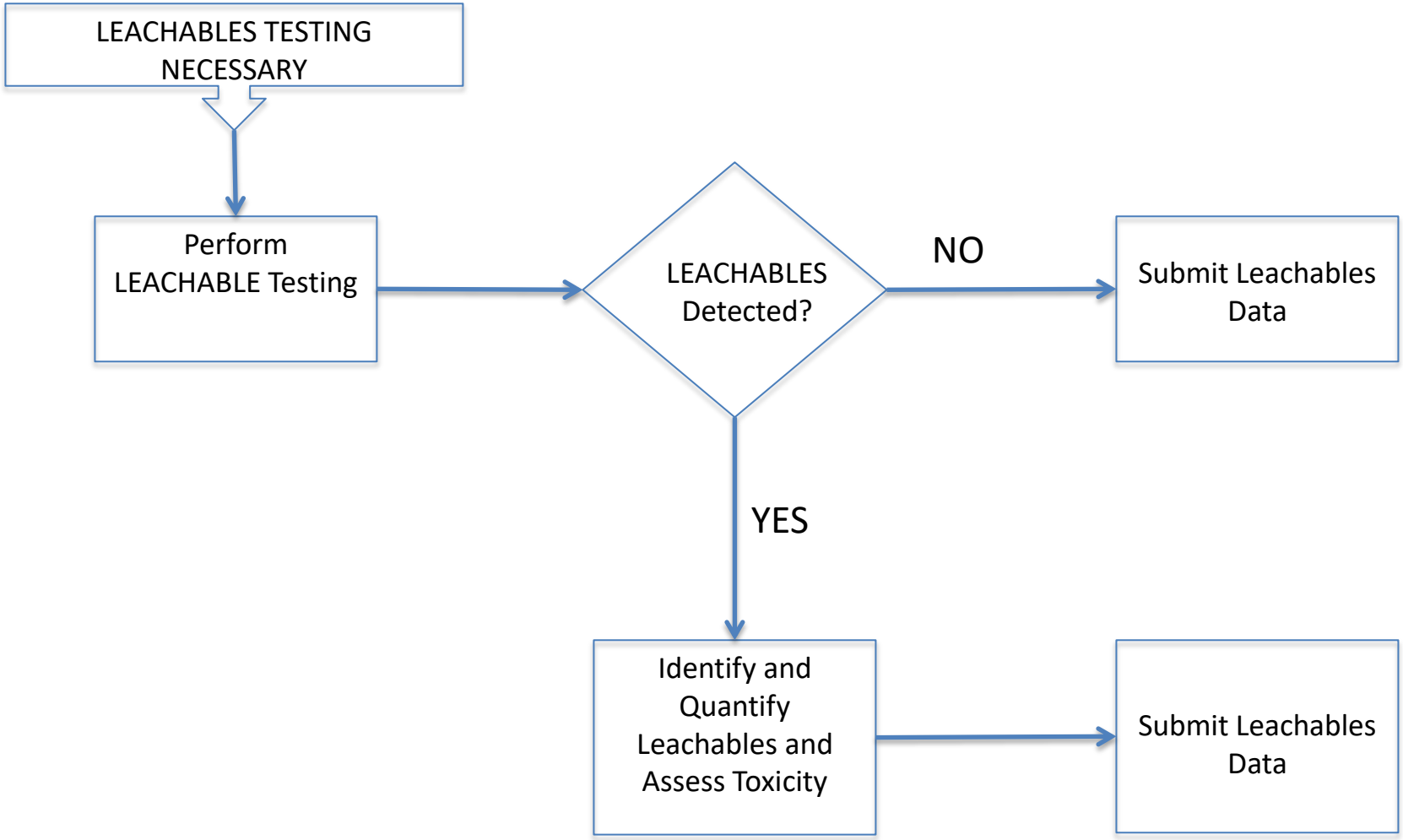
- *Determine if extractables **data** is available **from vendor** or other reference source*

- *The most useful extractables data leads to a comprehensive list of potential leachables.*

- ***GOAL**: to identify as many potential leachable compounds as possible*

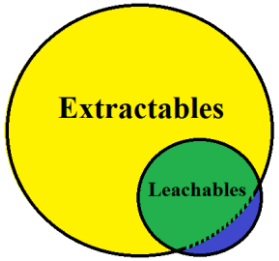
- *A vendor who performs high quality extractables testing and identifies many extractables should be admired and not punished!*

BPSA Flow Chart (continued)



REMARKS

1. The BPSA Flow Chart holds the **assumption that Leachables are a Subset of Extractables, which is not always the case**!



2. **Immediate step towards Leachables Tersting** (with skipping Extractables Evaluation), as proposed in the BPSA Flow Chart, can be cumbersome, as it is not always clear what to look for. **Need for Excellent Screening Methodologies in LEACHABLE STUDIES!!**

3. There is **more and more a trend towards Leachables testing, backed by Suppliers Extractable Data**, where the actual interaction between the product stream and the SUS is studied.

“SAFETY EVALUATION” OF A BIOPROCESS, BASED UPON E/L DATA

EXTRAPOLATION OF PQRI APPROACH

SCT: SAFETY CONCERN THRESHOLD

"Threshold below which a leachable would have a dose so low as to present negligible safety concerns from carcinogenic and non-carcinogenic toxic effects"

PQRI for **OINDP's**: SCT = 0,15 µg/day

The SCT is not a Control Threshold, it is not a TTC

AET: ANALYTICAL EVALUATION THRESHOLD

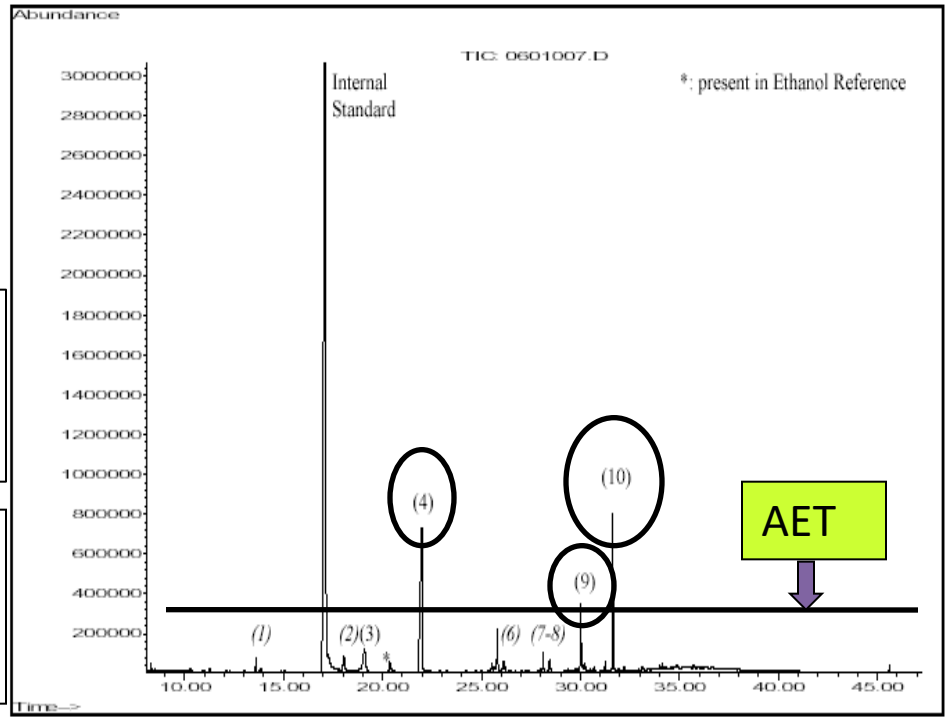
Translate SCT

into Analytical Thresholds

for Extractable Studies

Taking into account:

- *Total N° of doses / packaging*
- *Max. N° of doses administered / day*



PQRI: SUGGESTED THRESHOLDS FOR **PARENTERAL** & OPHTHALMIC APPLICATIONS (PQRI-PODP) – current status

	Class I	Class II	Class III
Threshold Level (µg/day)	50 ~~Under Evaluation~~ **SET**	5	1.5

Class I: class of compounds which are **no** sensitizers, irritants, genotoxicants or carcinogens.
Class II: class of compounds which are known or expe`cted to have sensitizing or irritating properties, but do not have any indications of genotoxicity or carcinogenicity.
Class III: class of compounds which are known or expected to be genotoxic or carcinogenic.

AET: **A**NALYTICAL **E**VALUATION **T**HRESHOLD

Example:

Filter is used to produce 1000 vials

Maximum Daily Intake: 1 vial

Evaluation of Filter

Extraction ratio: 1 Filter is filled with 2 L an Extraction Solution that Substantially Exaggerates the worst case use

EXTRACTABLES:

Threshold Class I: 50 µg/day: final AET level: 75.000 µg/Filter

Threshold Class II: 5 µg/day: final AET level: 2.500 µg/Filter

Threshold Class III: 1,5 µg/day: final AET level: 750 µg/Filter

AET: **A**NALYTICAL **E**VALUATION **T**HRESHOLD

Formula used (see PQRI recommendations):

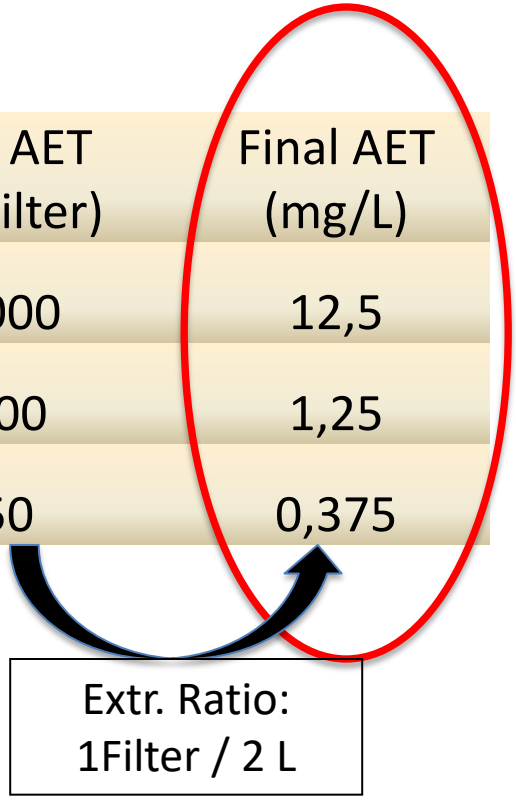
$$\text{Est. AET} = \frac{\text{Threshold}}{\text{dose/day}} \cdot \frac{\text{total dose}}{\text{Filter}}$$

$$\text{Class I: Est. AET} = \frac{50\ \mu g / day}{1\,dose\ /\ day} \cdot \frac{1000\,dose}{Filter} = 50.000\ \mu g\ /\ Filter$$

$$\text{Final AET} = 25.000\ \mu g / Filter$$ **50% uncertainty for screening methods**

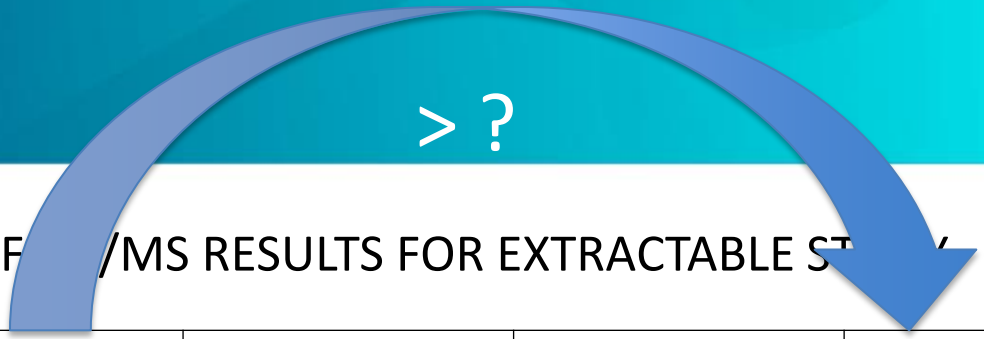
Further Calculations will give the following AET levels for the respective Classes:

	Threshold (µg/day)	Final AET (µg/Filter)	Final AET (mg/L)
Class I	50	25000	12,5
ClassII	5	2500	1,25
Class III	1,5	750	0,375



Typical Results for an Exhaustive Extraction on a Filter Unit

	EXT result mg/L extract	EXT result µg / Filter
COMPOUND #1	0,1	200
COMPOUND #2	0,2	400
COMPOUND #3	1,25	2500
COMPOUND #4	2	4000
COMPOUND #5	0,4	800
COMPOUND #6	0,25	500
COMPOUND #7	13	26000
COMPOUND #8	0,1	200
COMPOUND #9	47	94000
COMPOUND #10	0,4	800
COMPOUND #11	0,1	200
COMPOUND #12	5,5	11000
COMPOUND #13	32,5	65000
COMPOUND #14	1,2	2400
COMPOUND #15	0,35	700



EXAMPLE OF /MS RESULTS FOR EXTRACTABLE ST Y

	EXT result mg/L	Class	Threshold for Class (µg/day)	FINAL AET for Class (mg/L)
COMPOUND #1	0,10	Class I	50	12,5
COMPOUND #2	0,20	Class I	50	12,5
COMPOUND #3	**1,25**	**Class III**	**1,5**	**0,375**
COMPOUND #4	2,00	Class I	50	12,5
COMPOUND #5	0,40	Class II	5	1,25
COMPOUND #6	0,25	Class I	50	12,5
COMPOUND #7	**13,00**	**Class II**	**5**	**1,25**
COMPOUND #8	0,10	Class III	1,5	0,375
COMPOUND #9	**47,00**	**Class I**	**50**	**12,5**
COMPOUND #10	0,40	Class II	5	1,25
COMPOUND #11	0,10	Class III	1,5	0,375
COMPOUND #12	5,50	Class I	50	12,5
COMPOUND #13	**32,50**	**Class III**	**1,5**	**0,375**
COMPOUND #14	1,20	Class I	50	12,5
COMPOUND #15	0,35	Class II	5	1,25

Conclusion of the Threshold Evaluation (Safety):

- ❑ Exaggerated/Exhaustive Extraction Results indicate that – if all would come out – these compounds would be detected as leachable above their respective threshold level

- ❑ Were Compounds 3, 7, 9 and 13 identified?
  In some cases, further attention to additional identification needs to be given

- ❑ Analytical methods for compounds 3, 7, 9 and 13 will need to be validated for the subsequent leachable study

- ❑ The validation range will be different for the 4 compounds as a result of:
  - ➢ The **concentration** level of the compound, found in the Filter
  - ➢ The **different classess** for the respective compounds:
  - ➢ The **validation range** should always **include the AET** level for the respective compound, as a minimum

- ❑ Presence of **other compounds** may be **monitored** (semi-quantitatively) in Leachable Study, using **screening methodology**

Footmark:

- The **Threshold Approach only evaluates "Safety Aspects**" of the leachables

  Other Concerns, like *QUALITY PURITY, STRENGTH, REACTIVE or ADDITIVE BEHAVIOR* are not assessed via the Threshold Approach

  Nor are IMMUNOGENICITY concerns addressed

- Even if an evaluation of a Single-Use System (SUS)
  - Based open the initail (paper) risk assessment
  - Based upon the analytical data

  Shows no concern

*Even then it may (need to) be considered to document **impact** of the **SUS contact** on the **impurities profile** of the product stream*

CONCLUSION

1. When looking at a Bioproduction Process, **- potentially – a lot of materials, components and/or systems may need to be evaluated**

2. The **"BPSA Risk Evaluation"** of a Bioproduction Process may be a good guidance to determine what to **focus** on in a subsequent E/L efforts

3. Both the **BPSA & BPOG Protocol** *(later on, ~~USP<661.3> & new(?) ASTM standard~~ USP <1665>)* give very good guidance and indications on how to put together a E/L-testing programme

4. **Optimize the BPSA & BPOG protocol** to the actual gaps in the documentation

5. Perform E/L testing

6. Perform a Risk Assessment
   - Quality
   - Safety (extrapolated PQRI PODP Approach)