DISPOSABLE & SINGLE-USE SYSTEMS

PDA TRAINING COURSE
EXTRACTABLES – LEACHABLES
Venice
21 – 22 March 2019

Koen Smets

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BIOPRODUCTION PROCESS

Bioproduction process

Fermentation

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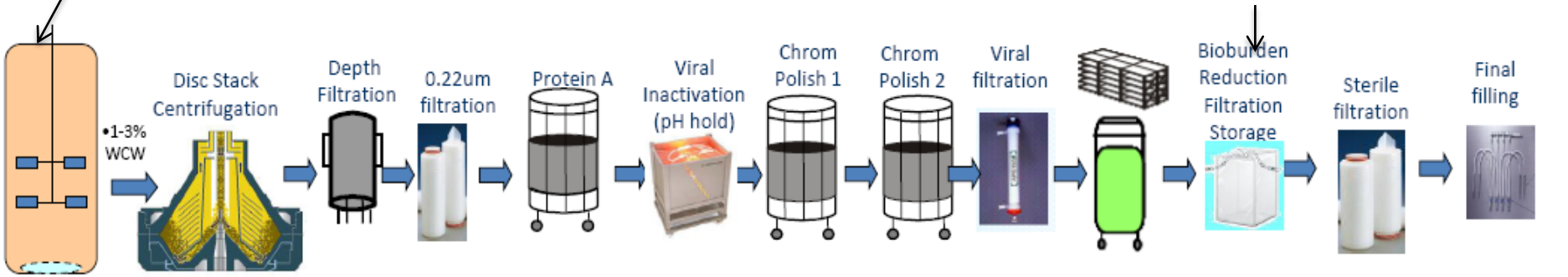


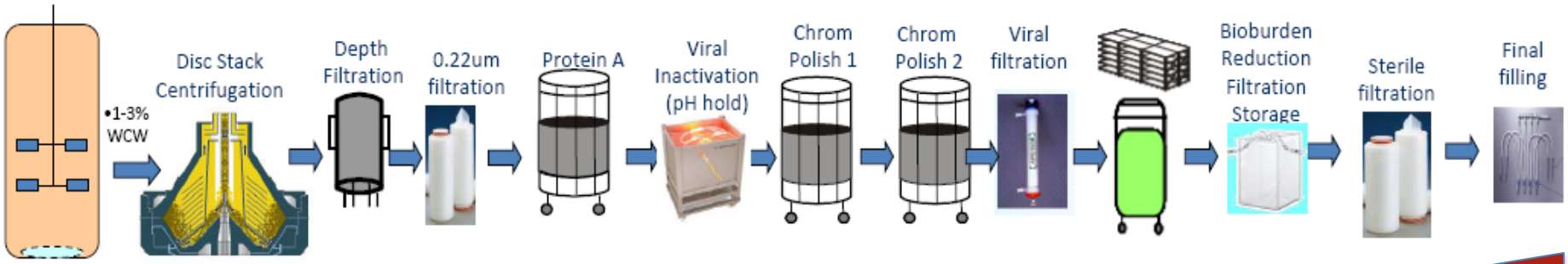
Product recovery / harvesting

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

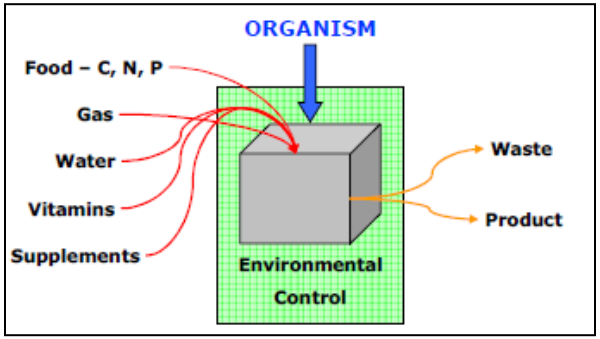


Leachables Impact on Toxicological Risk

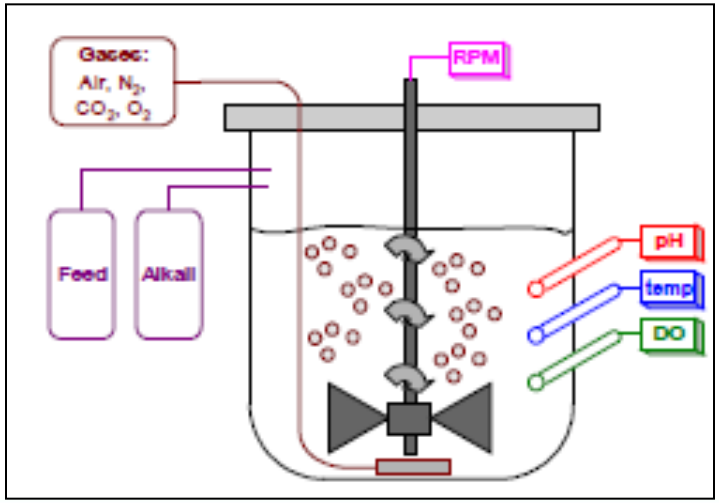
Bioproduction example from a slide from Presentation at IQPC Conference "Disposable Solutions", Munich, 18-20 FEB2014: "BPOG's Extractable Protocol Standardization Journey – Review 2013 Process ande Planning for 2014" Ken Wong (Sanofi-Pasteur), with permission of the Author.

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

Fermentation

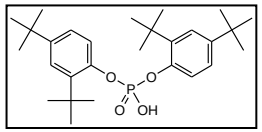
Evaluation of Extractables & Leachables

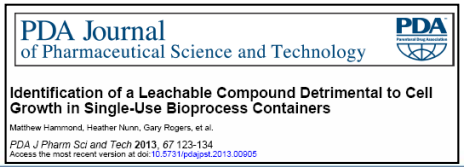
- Leachables introduced by the bioreactor might be **removed/diluted** by following process steps *(cell harvesting / purification / formulation)*
- For large batch volumes, the contact surface to volume ratio is low

→ **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

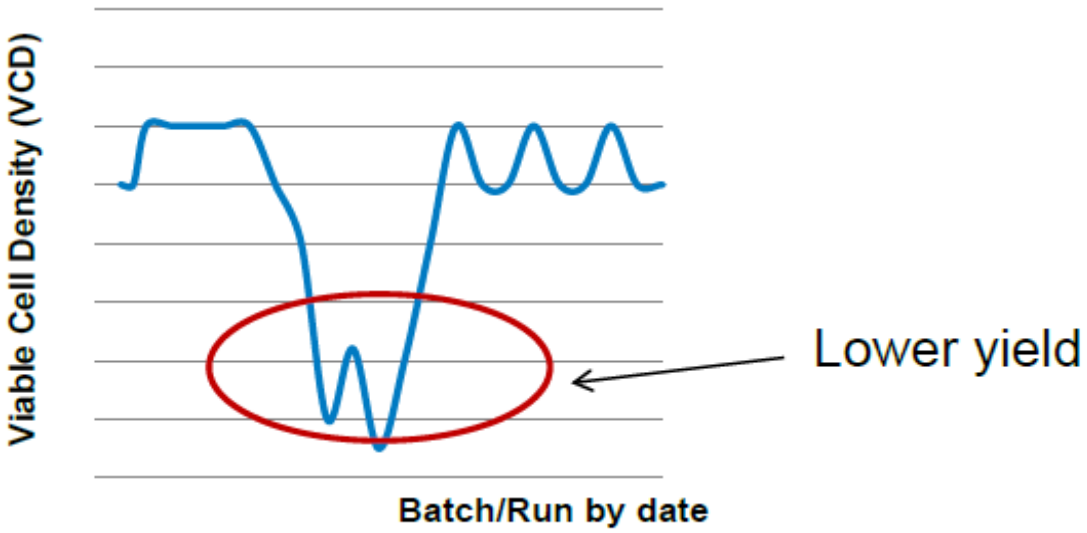
Identification of a Leachable Compound Detrimental to Cell Growth in Single-Use Bioprocess Containers

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



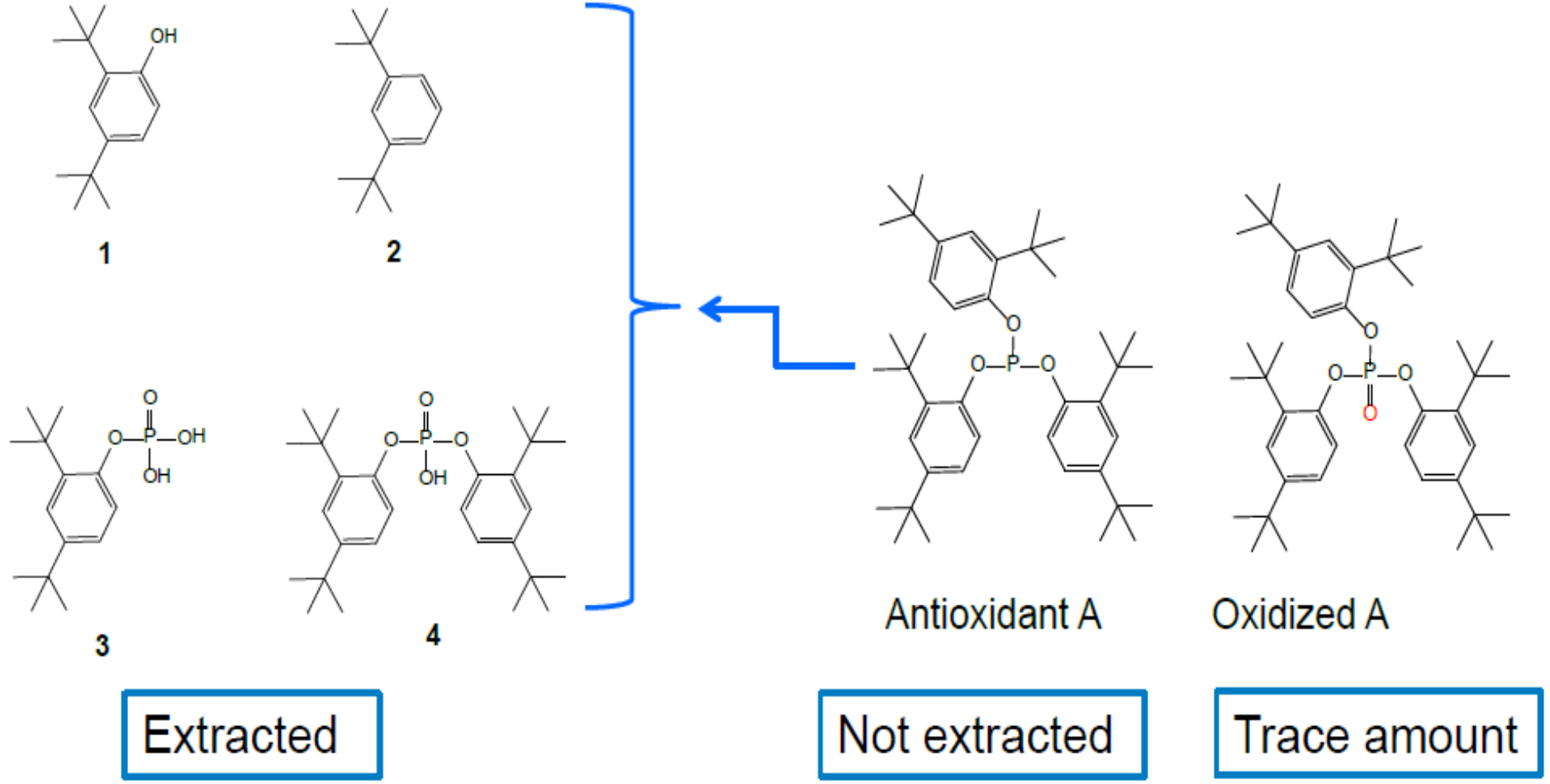
- Decreased yield = less profit
- Potential root cause(s)
  - Media
  - **Leached material from Bag**?
  - Innovative idea to non-Extractable people

Hypothesis:
SUB Leachable(s) Inhibits Cell Growth

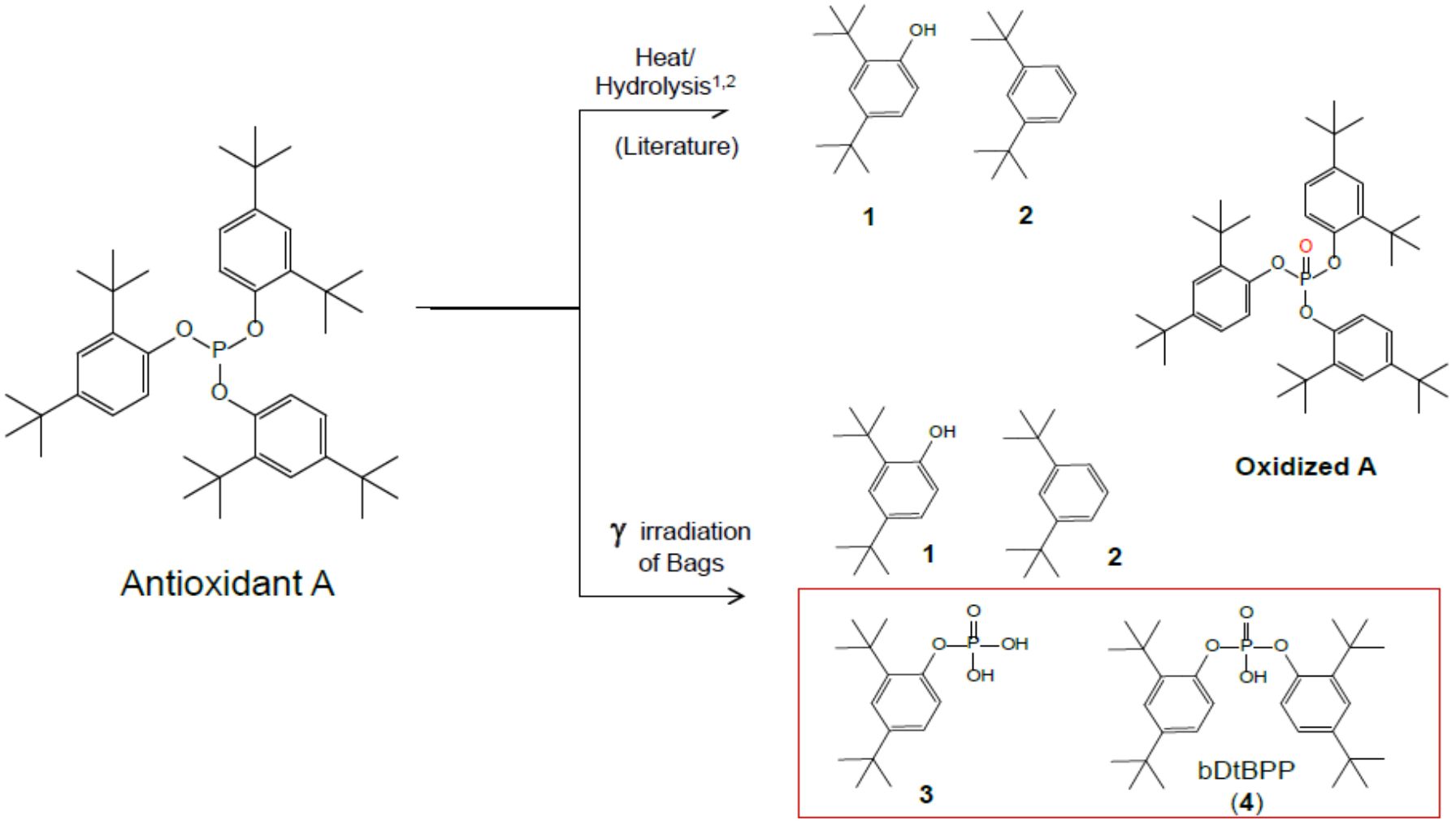
- Get information from vendor
- Perform Extractable study and ID Extractables
- Spike in individual water soluble Extractables into Cell Culture process using bags from “good” lots.
- Measure cell growth

*Vendor data/information from extractables testing

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



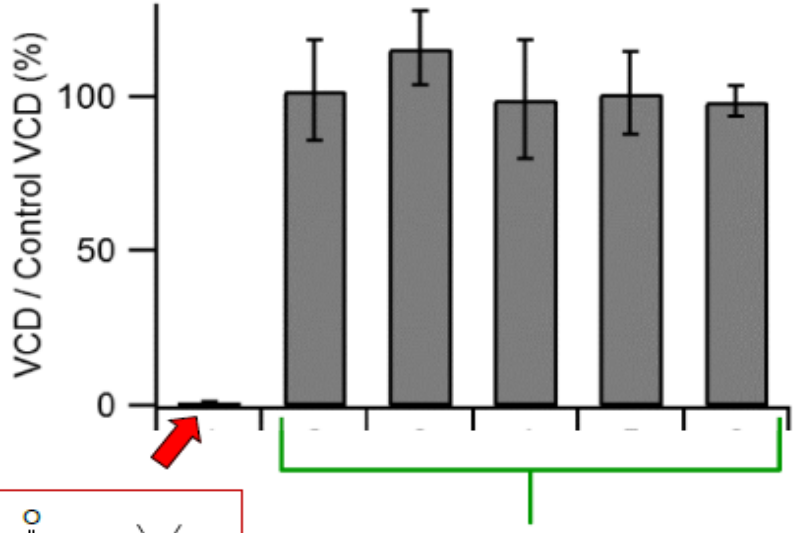
bDtBPP(4) Formation Due to Sterilization (gamma irradiation)

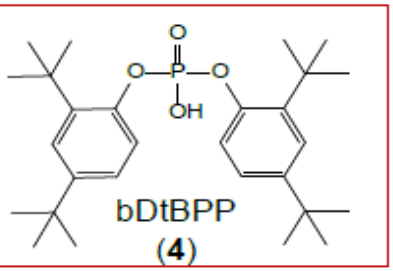


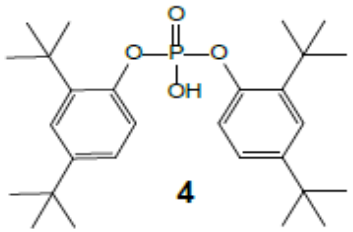
1. J. Sep. Sci. 2010, 33, p3463
2. *Packag. Technol Sci. 1999, 12, p119*

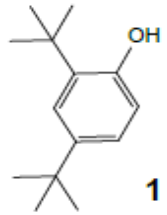
Extractable Detrimental Impact on Cell Culture

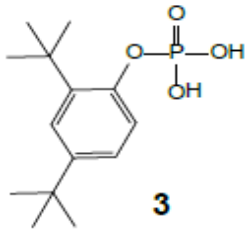
- Spike extractables at ~ 1ppm into cell culture medium

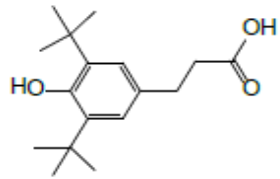


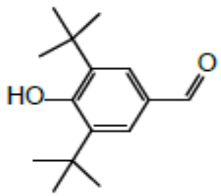


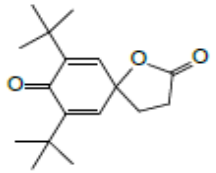










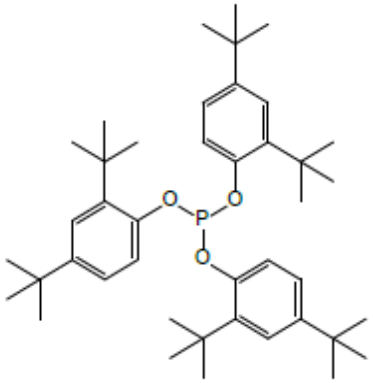


***b*D*t*BPP (4) is detrimental to cell growth**

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

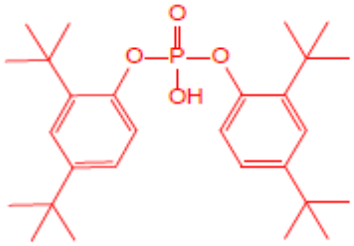
Summary/Conclusion

- 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

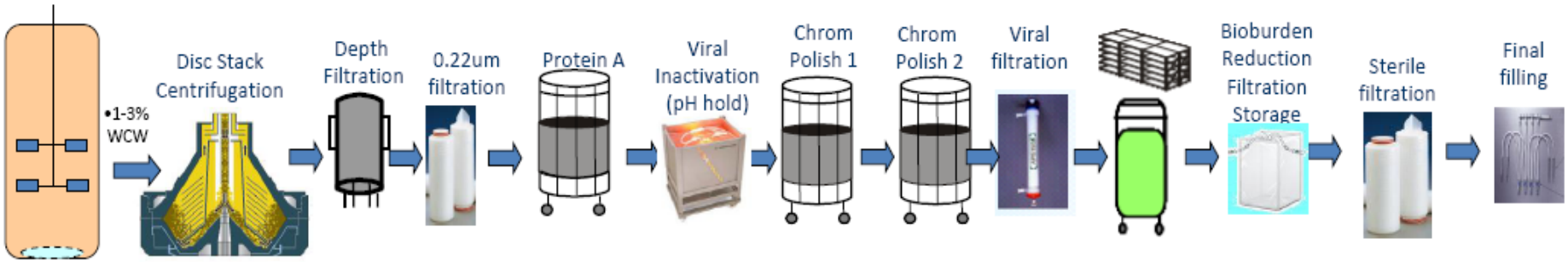


Antioxidant degradant: bDtBPP

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

AMGEN

Bioproduction process



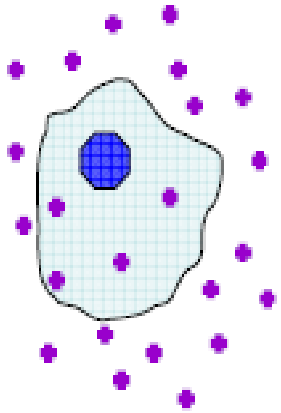
Product recovery / harvesting

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

» Mammalian cells

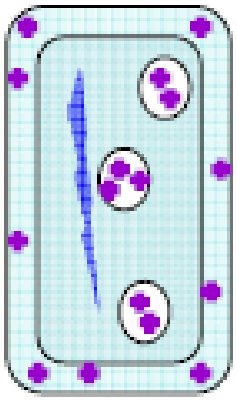


Intracellular product

» Bacteria

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

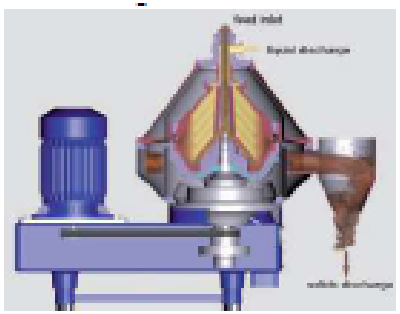
2. Periplasmatic expression
   (e.g. Gram-negative)

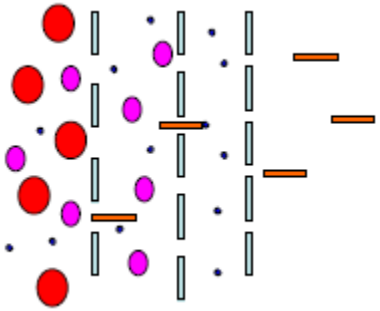


Product recovery: Extracellular Secretion

Step 1: removal of cells

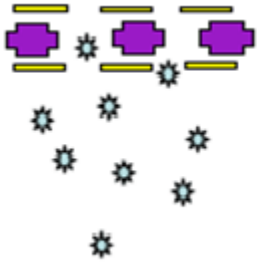
Centrifugation or Filtration

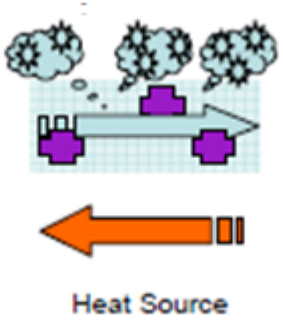


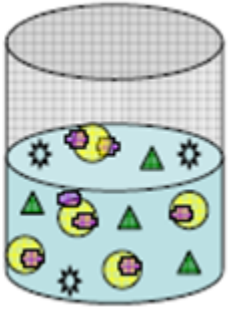


Step 2: volume reduction

Ultrafiltration or damping or batch adsorption







Product recovery:
Intracellular Secretion

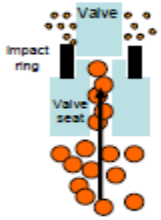
Step 1: Cell recovery

centrifugation

Step 2: **Cellular disruption**

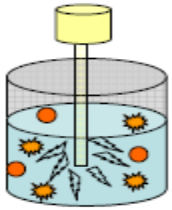
Mechanical

homogenisation



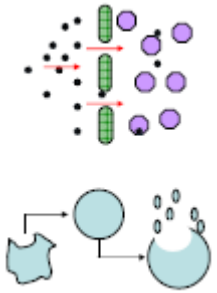
milling

sonication

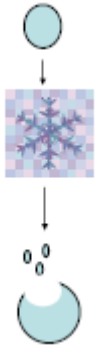


Non mechanical

osmotic shock



'freeze thaw'



enzymatic

lysozyme + EDTA

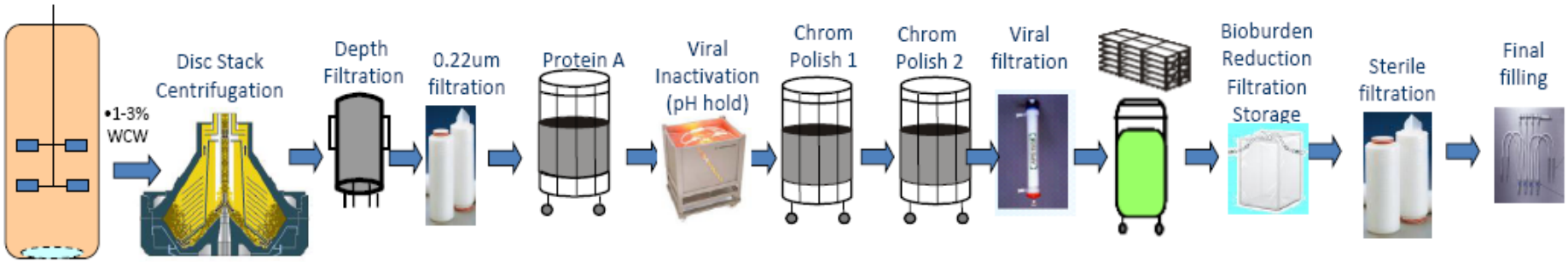
of solvents:
increase of celwand cell permeability

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 chromatography
- 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**

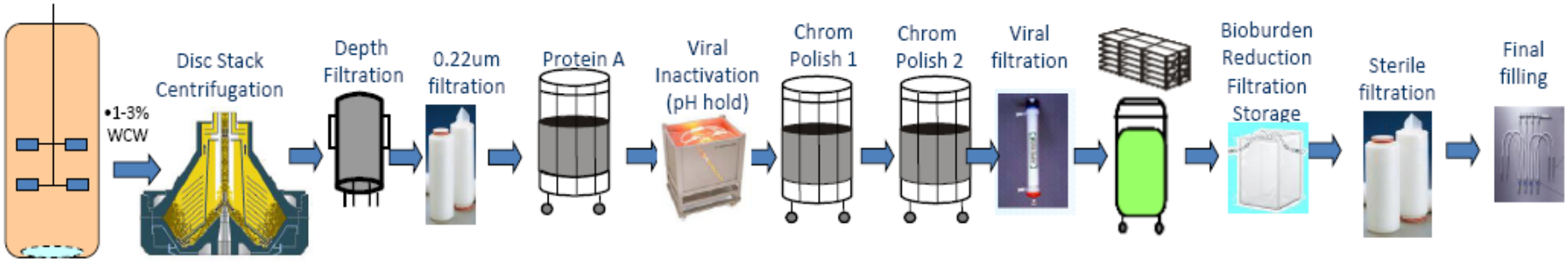
- Increased exposure amount
- Higher risk for leachables

» 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

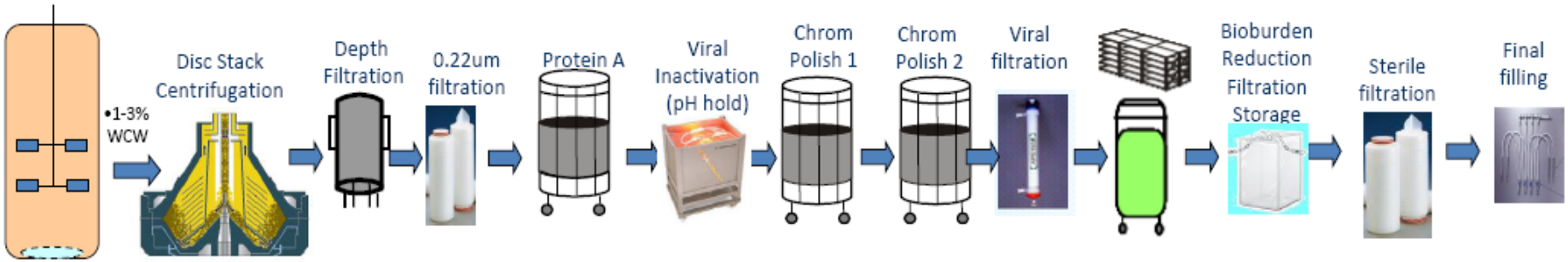
 - higher risk for leachables

» 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 & Tubing have **high 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

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 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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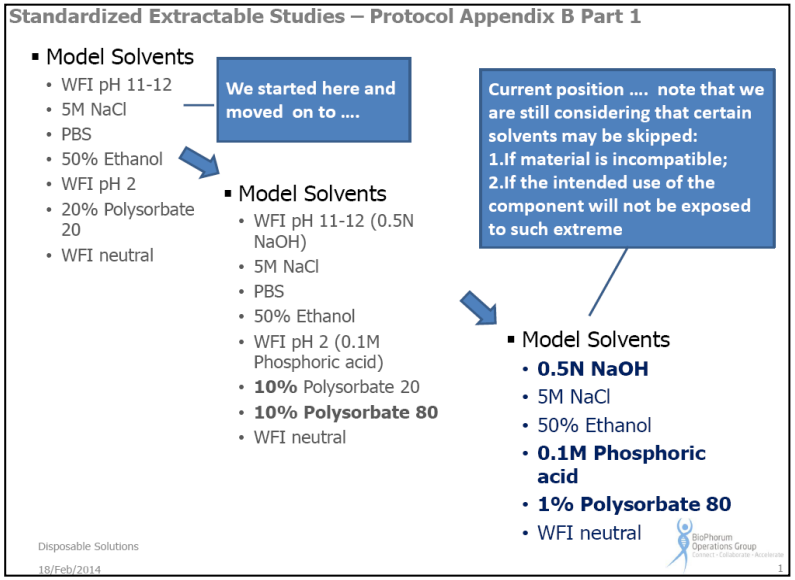
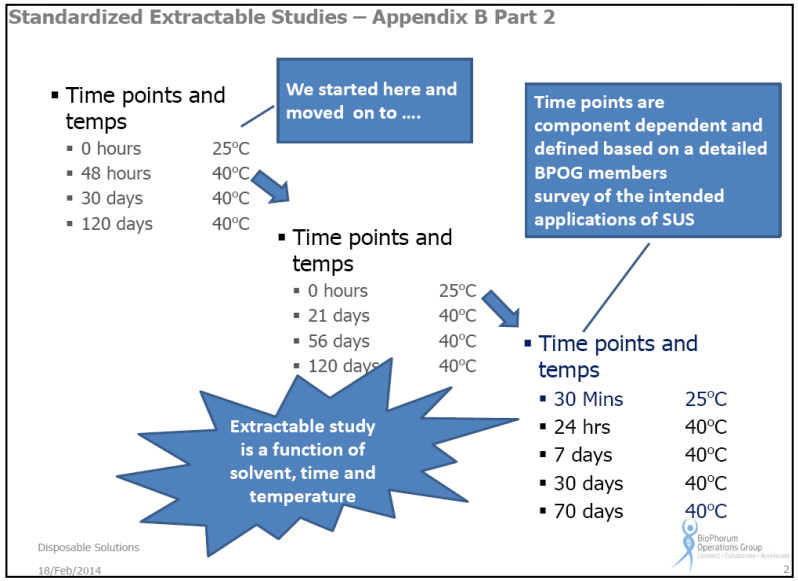
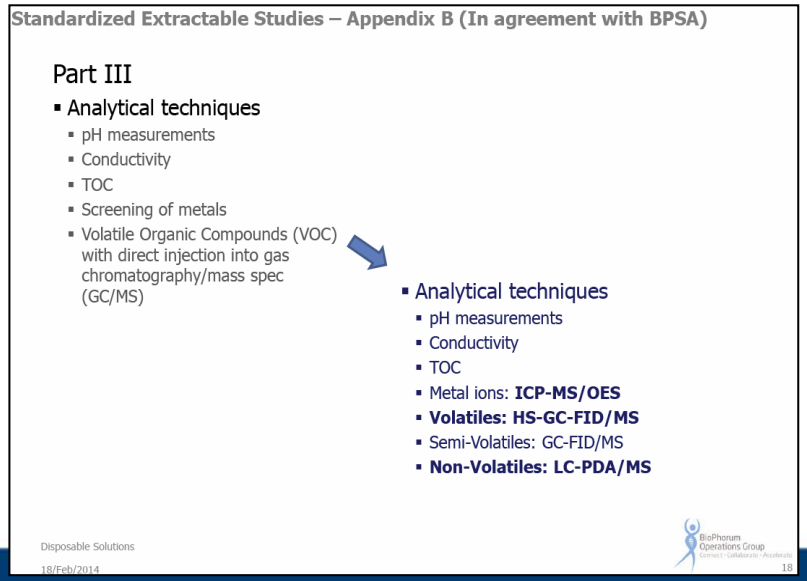
PALL Life Sciences

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)







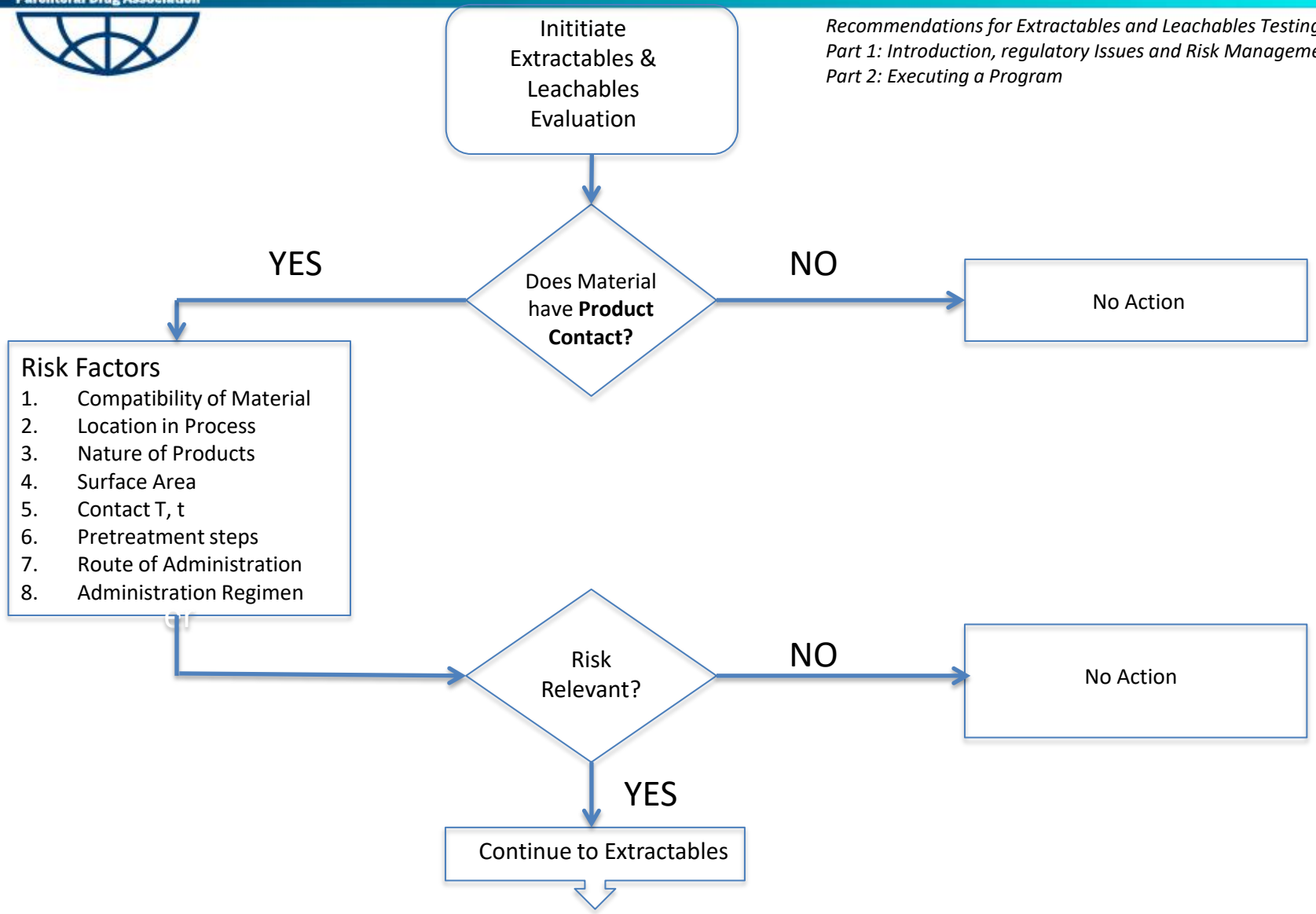
BIOPRODUCTION PROCESS

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
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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)
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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)
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RISK FACTOR 3: Solution Composition

- Extreme pH
- High organic or alcohol content
- Surfactants

Recommendations for Extractables and Leachables Testing (2008)
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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)
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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)
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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

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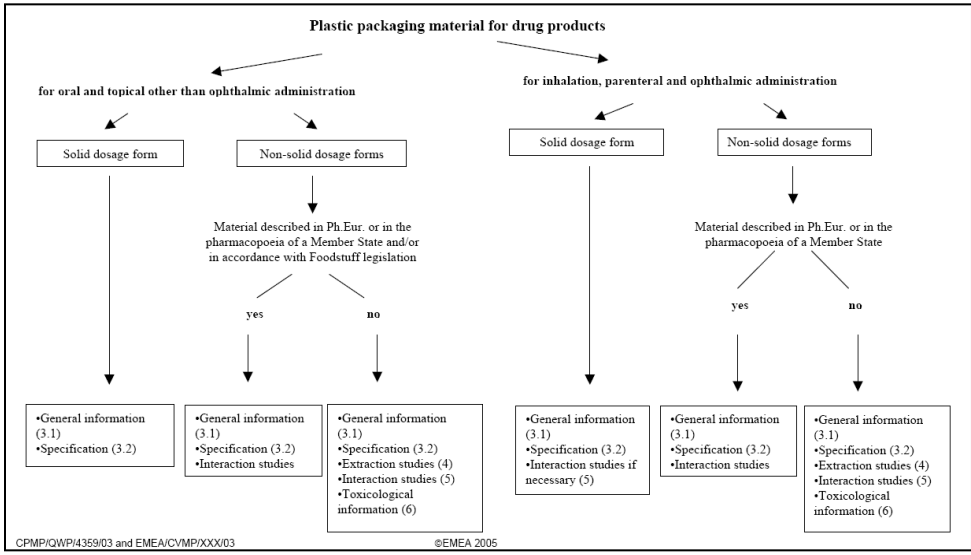
RISK FACTOR 7: Route of Administration

- 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
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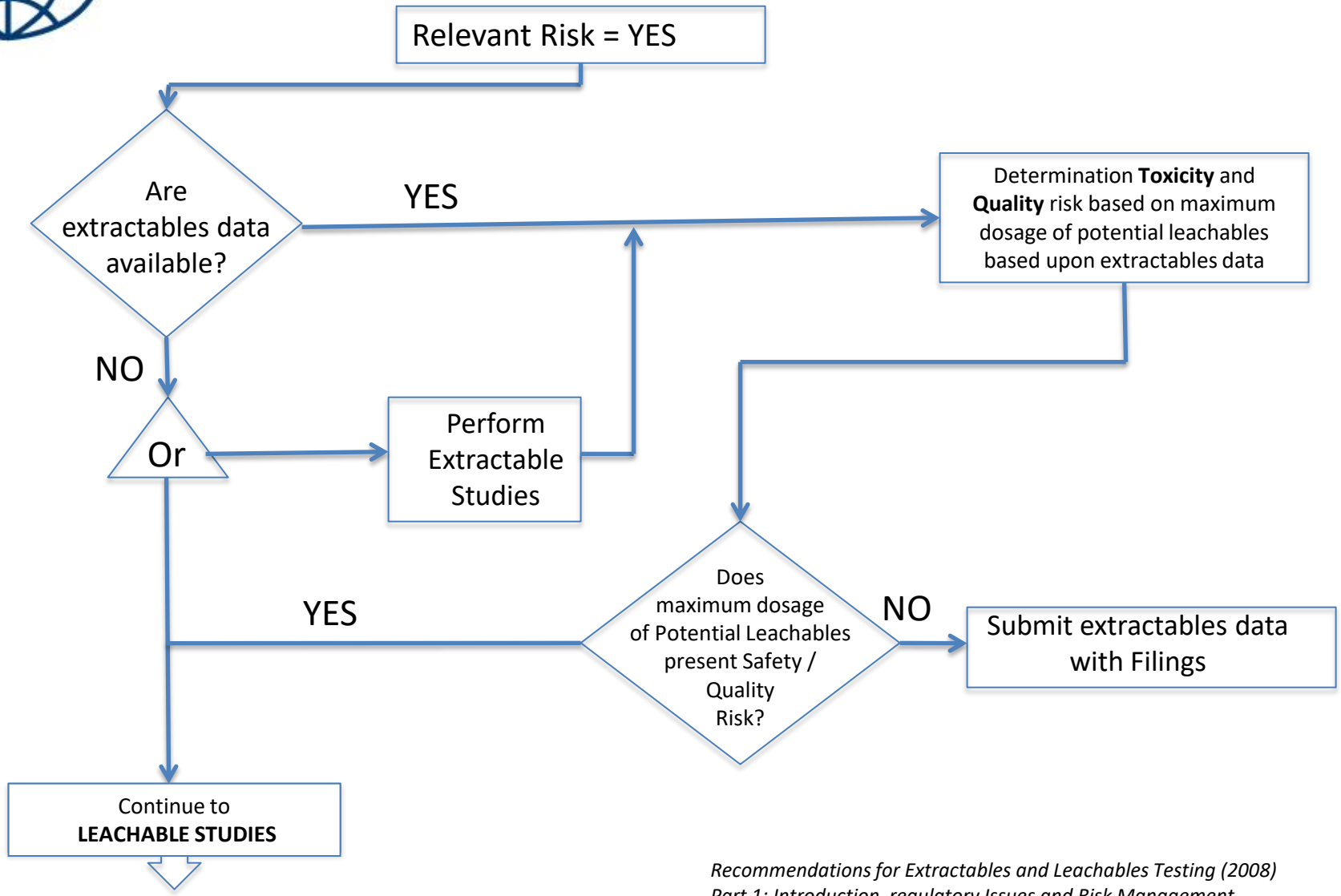
What to do with RISK FACTORS?

- 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
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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**

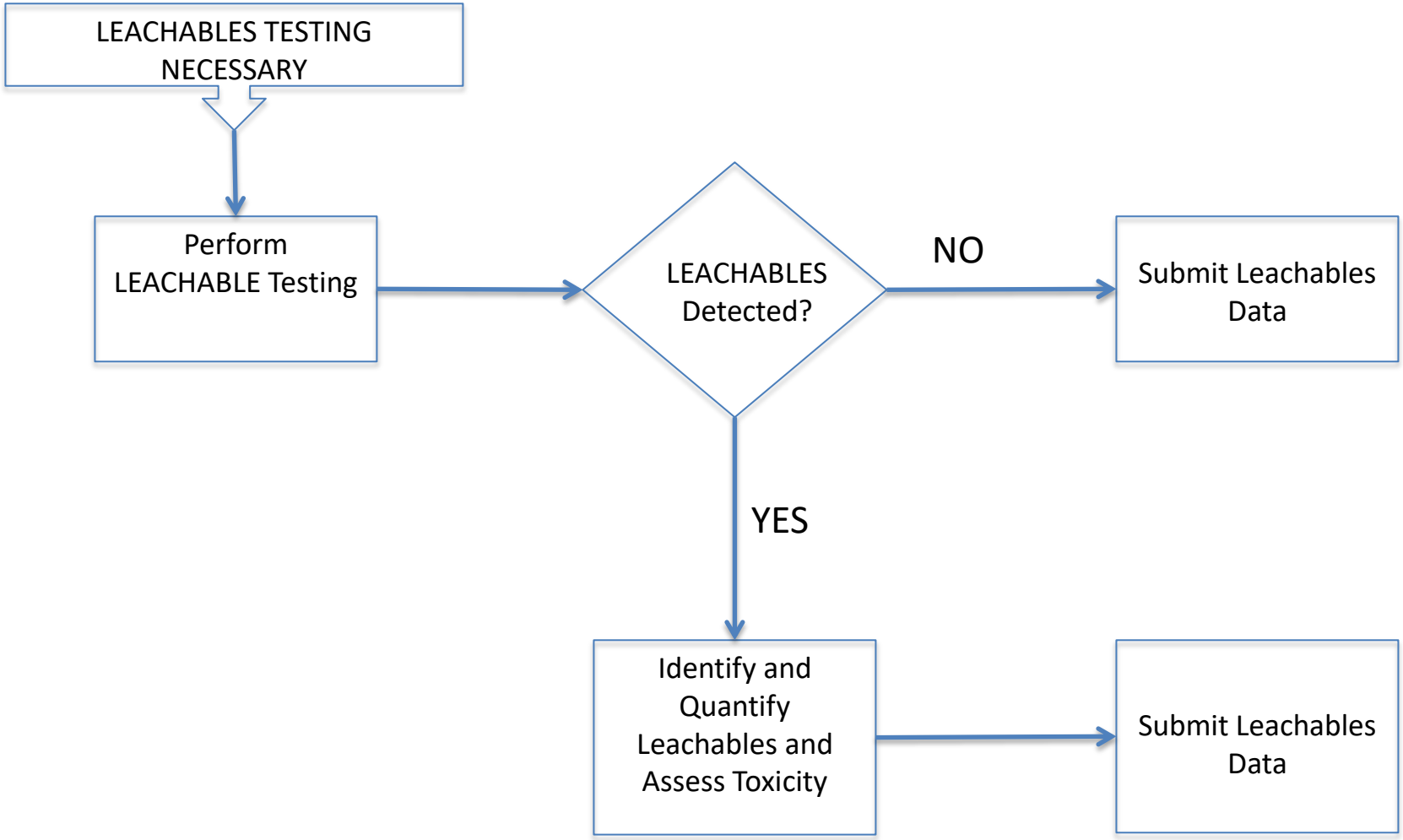
- *Determine if extractables **data** is available **from vendor** or other reference source*

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

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

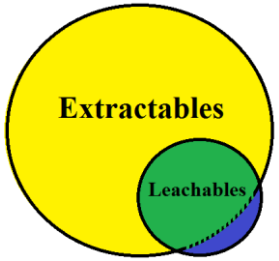
- *A vendor who performs high quality extractables testing and identifies many extractables should be admired and not punished!*

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.

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)