Challenges Associated with the Safety Assessment of Extractables/Leachables in Large Volume Parenterals (LVPs) and Potential Chemistry Approaches

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Situational Assessment

Among the numerous characteristics that differentiate Large Volume Parenterals (LVPs) from other dosage forms, their large dose volume is particularly noteworthy because of the practical implications of dose volume to the safety assessment of packaging system leachables.

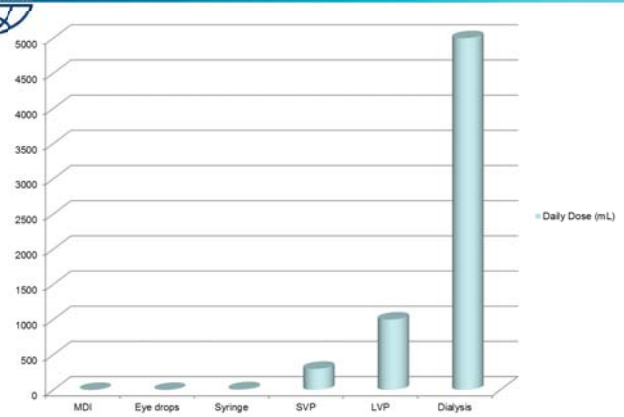
"The Situation" – Relative Dose Volumes

Metered Dose Inhaler
(small volume - large number of doses)

By definition in USP <1>, a large volume parenteral is a single-dose injection that is packaged in containers labeled as containing more than 100 mL. It is noted that large daily dose volumes may also reflect the use of multiple SVPs on a daily basis.

Large Volume Parenteral
(large volume - small number of doses)

Daily Dose Volumes for General Classes of Pharmaceutical Products



While certain dosage forms have relatively small Daily Doses Volumes (MDI, eye drops), other dosage forms have relatively large Daily Dose Volumes (LVP, dialysis).

What is the Big Deal About Daily Dose Volume?

One of the most basic concepts in toxicological assessment is that:

"The dose makes the poison"

Paracelsus, the "Father" of modern toxicology

That is to say that a substance can adversely affect health only if the amount of the substance to which an individual is exposed exceeds a tolerable threshold.

Now the dose of a substance that an individual is exposed to when receiving medication in a liquid form is the product of the concentration of the substance in the liquid medication and the volume of the liquid medication that is administered:

Dose = concentration in medication x volume of medication used

What is the Big Deal About Daily Dose Volume?

Thus an important consideration in establishing the safety of a medication is to establish that it contains no substances that exceed the permissible dose (PD). PD is typically expressed in units of amount per day (for example, mg/day).

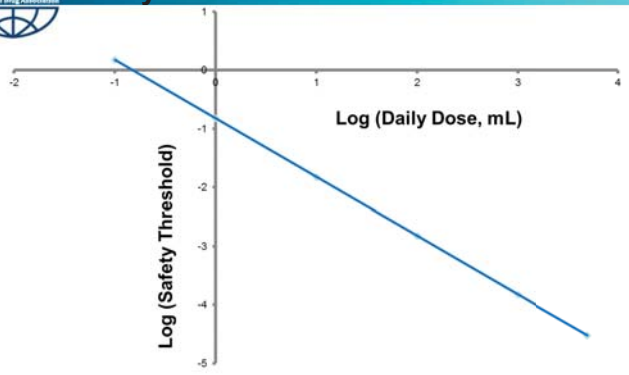
For this reason, medications are tested for their levels of substances that could be potentially unsafe. These test results are expressed as a concentration of the substance in the medication in units of amount per volume (for example, mg/L).

To establish whether the level of the substances exceeds the permissible dose, the permissible dose is "converted" to concentration units by dividing the PD by the daily dose volume V (for example, liters per day)

$$PD_{concentration} = PD_{amount}/V$$

Clearly, as the dose volume V increases, the magnitude of $PD_{concentration}$ decreases.

Effect of Daily Dose Volume on an Analytical Threshold



The value of the Analytical Threshold decreases in direct proportion to the increase in Daily Dose Volume.

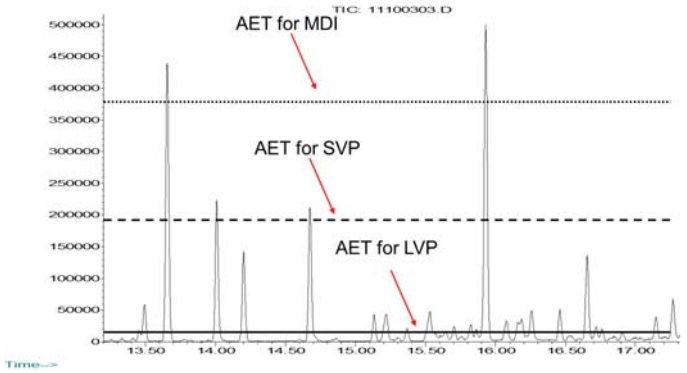
A Numerical Illustration

Case #1: **MDI**, 0.5 mL of drug product in a canister that has 200 labeled actuations with a recommended daily dose of 10 actuations. For an individual organic leachable, the estimated **AET** would be **6.0 μg/mL**. *Easy to accomplish!*

Case #2: **Inhalation Solution**, 3 mL of drug product in a LDPE container with a recommended dose of 3 containers per day. For an individual organic leachable, the estimated **AET** would be **0.017 μg/mL**. *Doable but much more difficult!*

Case #3: **LVP**, 1 L of drug product in an appropriate container with a recommended dose of one container per day. For an individual organic leachable, the estimated **AET** would be **0.00015 μg/mL**. *Practically impossible to accomplish!*

Effect of Daily Dose Volume on the AET



Practical Implication: More peaks to identify at lower concentrations

Problem Statement, Safety Assessment of Leachables in LVPs

AETs for LVPs may be so low that even state of the art, best demonstrated practice analytical methods may not be able to accomplish the functions of discovery and identification for all necessary leachables.

If leachables cannot be detected and identified then obviously they cannot be toxicologically assessed by numerical means and thus their potential safety impact cannot be established by such numerical means.

Potential Analytical Approaches to Address the LVP Situation

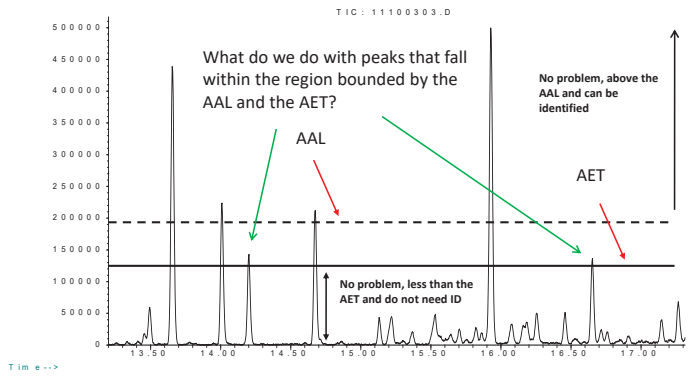
1. The Analytical Action Limit.
2. Use of the "Right" Analytical Threshold
3. The Safety Assessment Triad.
   - Controlled Extraction Study (material characterization and screening).
   - Simulation study (Extractables as worst case leachables, initial safety assessment, target ID).
   - Migration study (target leachables assessment).

The Concept of the Analytical Action Limit

The **Analytical Action Limit (AAL)** is that concentration of an analyte below which the activities of discovery and identification cannot be reliably performed.

If the AAL can be established for a particular analytical method, the AAL can be compared to the AET and the safety risk associated with the difference between the AET and AAL can be established.

The Issue with the Analytical Action Limit



The Concept of the "Right" Threshold

1. Analytical thresholds for leachables are based on certain toxicological characteristics of the leachables (i.e., are they carcinogens?), certain generalizations about product usage (i.e., duration of clinical exposure) and no allowance for "benefit versus risk" analysis.

2. The values for analytical thresholds differ with respect to the aspects noted in point (1) above.

3. Matching the analytical threshold to the specific scenario being addressed insures that the analytical processes are being held to the proper performance expectations.

Using the "Right" Threshold; Is the Leachable Carcinogenic? (I)

The exact and formal definitions of the analytical thresholds such as the AET, SCT and QT bear close scrutiny:

AET = concentration threshold at or above which a chemist should begin to identify a particular leachable and/or extractable and report it for potential toxicological assessment.

SCT = amount threshold below which a leachable would have a dose so low as to present negligible safety concerns from carcinogenic/noncarcinogenic toxic effects.

QT = amount threshold below which a given non-carcinogenic leachable is not considered for safety qualification (toxicological assessments) unless the leachable presents structure-activity relationship (SAR) concerns.

The important points are:

1. The value of the QT will be significantly higher than the SCT.
2. The AET can be based on either the SCT or the QT if the carcinogenicity of the leachable can be established.

Using the "Right" Threshold; Is the Leachable Carcinogenic? (II)

Compound presents an unacceptable safety risk in terms of both potential carcinogenic and non-carcinogenic toxic effects

QT ≈50 µg/day

SAR Assessment

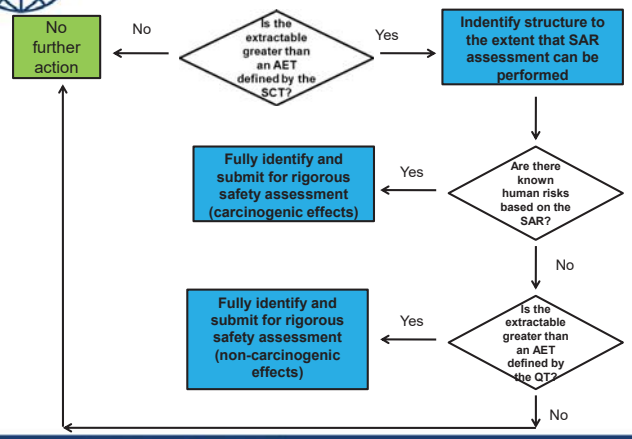
Compound presents an acceptable safety risk in terms of potential non-carcinogenic toxic effects but not in terms of potential carcinogenic toxic effects

SCT 1.5 µg/day

PQRI PODP Recommendation

Compound presents an acceptable safety risk in terms of both carcinogenic and non-carcinogenic toxic effects (no toxic effects)

Using the "Right" Threshold; Is the Leachable Carcinogenic? (III)



Using the "Right" Threshold; Is the Leachable Carcinogenic? (IV)

Compound identity must be confirmed is all cases

QT — **≈ 50 µg/day**

SAR Assessment

Compound's identity can be "approximate" or tentative as long as it supports SAR and as long as SAR comes back with no alerts. Compounds with SAR alerts must be identified.

SCT — **1.5 µg/day**

PQRI PODP Recommendation

Compound does not need to be identified in any case.

Using the "Right" Threshold; Is the Leachable Carcinogenic? (V)

Lesson:

It is very important that one remembers the "SAR endpoint" as a viable identification objective. However, even if the SAR endpoint is applicable, one may still be inclined to pursue full identification. If an identification is "easy", then by all means get the confirmed ID. However if the ID is "hard", then maybe one can stop once a "tentative" or "estimated" ID has been secured to support the SAR.

This is especially important for LVPs as it can be anticipated that LVPs will have lower AETs, regardless of whether the AET is based on the SCT or the QT.

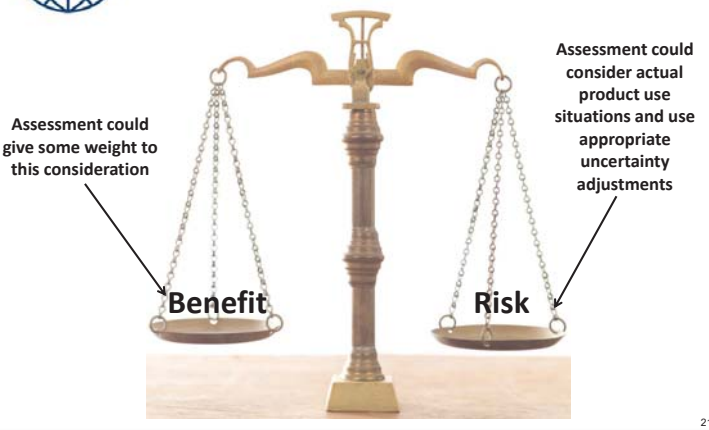
Using the "Right" Threshold; What is the duration of clinical exposure?

The magnitude of the threshold depends on the duration of clinical exposure, with higher thresholds being appropriate for shorter durations.

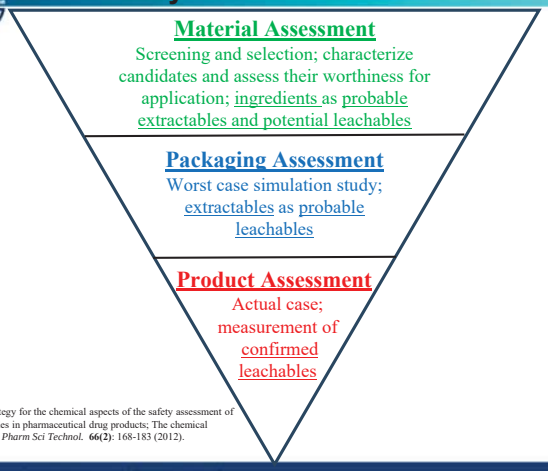
M7 Acceptable Thresholds for Genotoxic and Carcinogenic Impurities						
	Duration of Clinical Exposure					
	< 14 days	14 days – 1 month	1 – 3 months	3 to 6 months	6 to 12 months	> 12 months
Genotoxic and carcinogenic impurity threshold (µg/day)	120	60	20	10	5	1.5

Guidance for Industry. M7 Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk. 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). May, 2015.

Using the "Right" Threshold; Focusing on both sides of the Balance



A Process Answer to the LVP Challenge; The Safety Assessment Triad



D. Jenke. A general strategy for the chemical aspects of the safety assessment of extractables and leachables in pharmaceutical drug products; The chemical assessment triad. *PDA J Pharm Sci Technol.* **66(2)**: 168-183 (2012).

The Safety Assessment Triad: Material Assessment

Purpose:

Chemically characterize candidate materials to establish their composition.

Extraction:

Conditions sufficiently aggressive to establish the composition, little or no consideration given to mimicking the conditions of contact when the materials used in packaging, utilization of standardized extraction and testing protocols

Safety Assessment:

High–level, generally semi-quantitative toxicological assessment looking for "compounds of potential impact". Assessment to be used in screening of packaging candidates.

Outcome:

Approval or rejection of material as a packaging system candidate.

The Safety Assessment Triad: Material Assessment

Value Proposition:

"The best way to ensure that a packaging system does not materially affect the safety or quality of a packaged pharmaceutical product is to construct the packaging systems from raw materials that are well-characterized and appropriately inert."

The Safety Assessment Triad:

System Assessment, Simulation Study

Purpose:

Establish the worst case (highest possible) accumulation of leachables.

Extraction:

Conditions chosen to mimic the worst case conditions of contact between the drug product and packaging; conditions may be adjusted to accelerate (but not greatly exaggerate) attainment of the worst case. Justified simulating solvents used.

Safety Assessment:

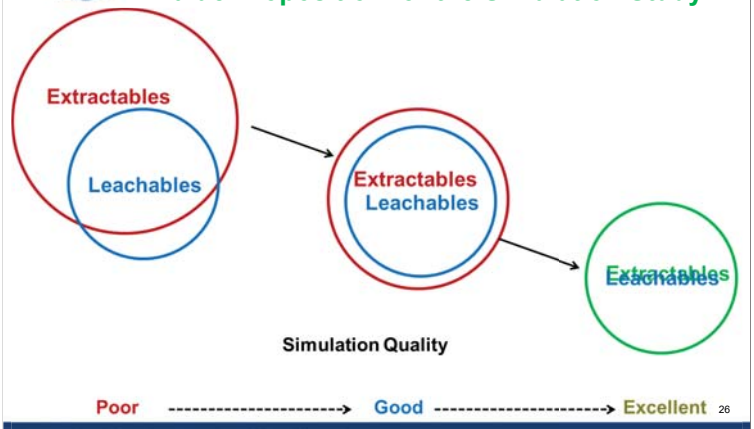
Detailed toxicological assessment of all extractables (as potential leachables) above the AET. Output is a safety risk assessment for all such extractables.

Outcome:

Some extractables will have negligible safety risk (safety assessment completed). Some extractables may have unacceptable safety risk. Either packaging is rejected or such extractables are targeted as leachables in migration studies.

The Safety Assessment Triad:

Value Proposition for the Simulation Study



The Safety Assessment Triad:

Value Proposition for the Simulation Study

1. The simulating solvents are more analytically expedient than are drug products, therefore one can more easily achieve lower AETs.
2. Use of accelerated conditions produces a more realistic profile in less time then exaggerated or real time studies.
3. Use of a small number of simulating conditions can build a design space that is applicable to a larger number for drug products.
4. Helps to focus leachables migration studies on targeted compounds as it establishes the basis of target selection.

The Safety Assessment Triad:

Product Assessment, Targeted Migration Study

Purpose:

Establish the actual accumulation of target leachables.

Leaching:

Actual conditions of use. Drug-containing solution.

Safety Assessment:

Detailed toxicological assessment of all targeted leachables. Output is a safety risk assessment for all such leachables.

Outcome:

Some leachables will have negligible safety risk (safety assessment completed, approve packaging). Some leachables may have unacceptable safety risk. In this case, reject packaging.

The Safety Assessment Triad:

Migration Study, Use of the AET (I)

- At this point in the assessment process the focus is target leachables
- Because these are target leachables, toxicological data is available and has already been assessed (e.g., a Permissible daily exposure, PDE, has been determined).
- The PDE (expressed in µg/day) can be converted to a maximum allowable concentration in the drug product (MAC, expressed in units of µg/mL). The MAC establishes the quantitation target concentration for the analytical method used to measure the target leachables.

MAC = PDE/Daily dose volume (mL)

The Safety Assessment Triad:

Migration Study, Use of the AET (II)

- Analyte concentrations less than the MAC are intrinsically safe and do not need to be numerically determined and reported (for safety assessment purposes) but may be used for trending over time.
- Analyte concentrations greater than the MAC represent an unacceptable safety risk.

Thus the AET is used in the Migration Study to address the possibility of "new" leachables that were not previously discovered as extractables or the possibility that a leachable has insufficient tox data to do a proper assessment.

References:

1. Guidance for Industry. M7 Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk. 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). May, 2015.
2. D. Jenke. A general strategy for the chemical aspects of the safety assessment of extractables and leachables in pharmaceutical drug products; The chemical assessment triad. *PDA J Pharm Sci Technol.* 66(2): 168-183 (2012).
3. Safety Thresholds and Best Demonstrated Practices for Parenteral and Ophthalmic Drug Products. Product Quality Research Institute (PQRI). September, 2017.

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Thank you!

CLOSING THE GAP BETWEEN EXTRACTABLES AND LEACHABLES

PDA TRAINING COURSE
EXTRACTABLES – LEACHABLES
Berlin
28 – 29 September, 2017

Dr. Piet Christiaens

CONTENT

Content

The more we know,
the more we know we don't know!

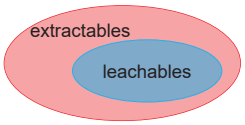
Anonymous
Berlin, 2017

Extractables / Leachables Testing: a Relatively New Science!

- ✓ Regulatory Requirements are becoming more and more Stringent.
- ✓ This leads to more and more Testing.
- ✓ More Testing increases the Understanding of the Interaction of the Materials with the Drug Products
- ✓ In order to have a proper "*Risk Mitigation*" a good Understanding of what can happen is of premordial importance!

2. LEACHABLES: A SUBSET OF EXTRACTABLES?

➔ THEORY:



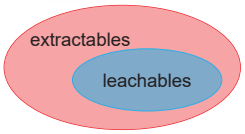
In early stages of E/L research (5 – 10 years ago):
- *Consensus: Leachables are a subset of Extractables*
- *Extractable study should be designed to identify all potential leachables*

FDA and EMA also include this thinking in their Guidelines and Guidances

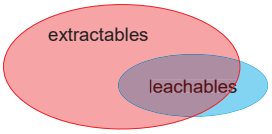
emea

Migration studies may only be omitted if, based on the outcome of the extraction studies, the calculated maximum amount of individual leachable substance that may be present in the active substance/medicinal product leads to levels demonstrated to be toxicologically safe. When a migration study is not considered necessary and thus is not conducted, a justification should be provided.

➔ THEORY:



➔ PRACTICE:



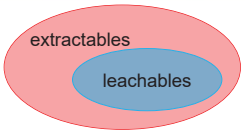
MIND THE GAP!

In the last 6-7 years, there is a growing consensus that – based upon experimental evidence – **Leachables are not always a subset of Extractables**!!

Yet, a lot of pharma companies adhere to the risk assessment of pharmaceutical containers and closures, solely based upon Extractables Data...

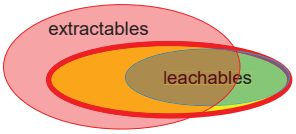
2. LEACHABLES: A SUBSET OF EXTRACTABLES?

➔ THEORY:



CLOSING THE GAP!!

➔ PRACTICE:



Additional Study Design

2. LEACHABLES: A SUBSET OF EXTRACTABLES?

TRADITIONAL STEPS IN THE SAFETY EVALUATION OF A PHARMACEUTICAL CONTAINER/CLOSURE

- A WELL DESIGNED **EXTRACTABLE STUDY** IS THE **FIRST STEP** IN THE SAFETY ASSESSMENT OF A CONTAINER CLOSURE SYSTEM
- **TARGET COMPOUNDS** FOR **LEACHABLE STUDIES** ARE SELECTED **BASED UPON THE RESULTS OF EXTRACTABLE STUDIES** ***(Remark: Pharmacopoeial tests are not equivalent to a well-designed extractable study!!)***
- **LEACHABLES CAN BE CONTROLLED/ASSESSED THROUGH EXTRACTABLES**
- USE **PLACEBO** AS AN EXTRACTION SIMULANT IN EXTRACTABLE STUDIES

3. CONSIDER THE STERILIZATION

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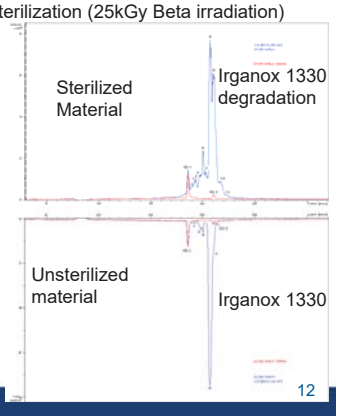
CASE STUDY

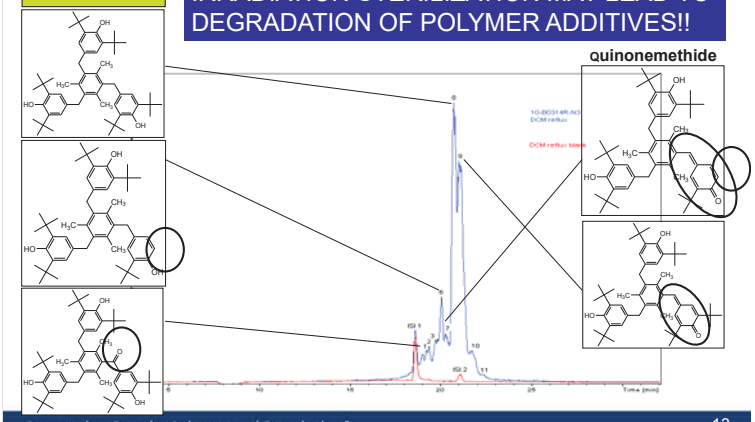
- Polypropylene Containers, Before and after sterilization (25kGy Beta irradiation)
- Extracted with Dichloromethane
- Ratio: 1 g/ 10 mL, reflux for 8h
- Analysis (presented): LC/MS (APCI-)



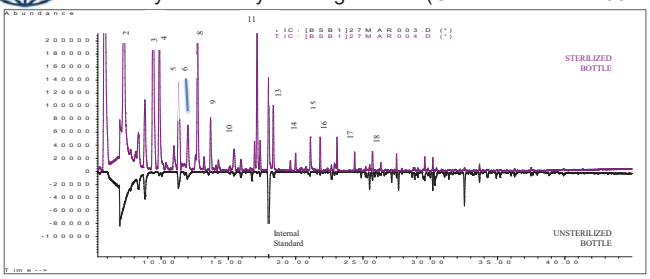
3. CONSIDER THE STERILIZATION

IRGANOX 1330

IRRADIATION STERILIZATION MAY LEAD TO DEGRADATION OF POLYMER ADDITIVES!!



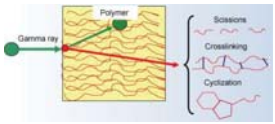
Sterilization of a Polyolefin: Polymer Degradation (Gamma Irratiation 50 kGy)



(1) TIC: n-butane
(2) IC: n-pentane
(3) IC: 3—methylpentane
(4) IC: n-hexane
(5) IC: butanal
(6)TIC: Hydrocarbon
(7) IC: cyclohexane
(8) IC: acetic acid
(9) IC: n-heptane
(10) IC: propanoic acid
(11) IC: 3-methylpentane
(12) TIC: Hydrocarbon
(13) IC: n-octane
(14) IC: 2-Hexanone
(15) IC: Butanoic acid
(16-18): TIC: HC

3. CONSIDER THE STERILIZATION

AGEING - STERILIZATION



POLYMER DEGRADATION (e.g. Scissions, Crosslinking, cyclization)

POLYMER ADDITIVE DEGRADATION (see example for Irganox 1330, but also the case study on biological reactivity (I168ox-diester)!)

CHANGES IN POLYMER CRYSTALLINITY

This will impact the: LEACHABLES SOLUBILITY

LEACHABLES MIGRATION

CONCLUSION: TEST FOR EXTRACTABLES AND LEACHABLES ON STERILIZED C/C SYSTEMS

4. CONSIDER THE WHOLE DEVICE / ADMINISTRATION PROCEDURE

4. CONSIDER THE WHOLE DEVICE

Typical Cases:

- Connectors, Tubing of Administration Set (tubing), Glue, Ports, Filters in I.V. Bag applications (not only film!)
- Silicone Oil, Glue extractables, Extractables from Barrel Manufacture
- Integrated Filter in Sterile Administrations (e.g. Ophthalmic)
- Reconstituting Solution (WFI, 0.9% NaCl), stored in Separate Vial / Syringe
  (Case study: see part E/L for Lyo Products)
- Cross Contamination during Sterilization (e.g. Autoclaving)
- ....

5. CONSIDER THE SECONDARY PACKAGING

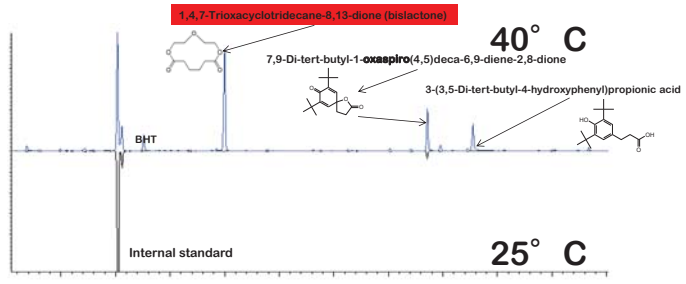
5. CONSIDER THE SECONDARY PACKAGING

- Regulatory requirements
  - FDA guidance document: 'Container Closure systems for Packaging Human Drugs and Biologics', 1999:
    "if the packaging system is relatively permeable, the possibility increases that the dosage form could be contaminated by the migration of an ink or adhesive component…In such case the secondary packaging component should be considered a potential source of contamination and the safety of its materials of construction should be taken into consideration…"
  - EMA: 'Guideline on Plastic Immediate Packaging Materials', 2005:
    "it should be scientifically demonstrated that no components of ink or adhesives, applied to the outer surface of the container closure system, will migrate into the medicinal product."

5. CONSIDER THE SECONDARY PACKAGING

Case study LEA: 100 mL flexible multi-layer bag containing a drug solution
ageing at 25° C and 40° C for 3 months
Results for S-VOC (Semi-Volatile Organic Compounds)

Conclusion:
1. MAIN Leachable: bislactone, from adhesive of ALUMINUM Multilayer overwrap!!
2. T increase leads to increased leaching behaviour of additives / degradation products



CASE STUDY 2

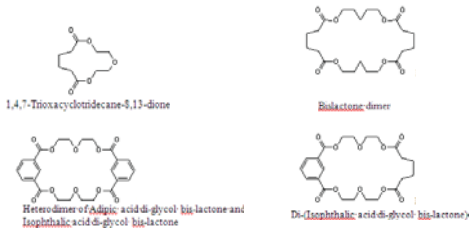
- Label
  - Adhesive
  - paper
  - Ink
  - Varnish
  - Typical extractable compounds:

    curing agents (e.g. Benzophenone, Irgacure 184), solvents (e.g.Toluene, acetone), residual monomers (e.g. Acrylates)

CASE STUDY 2

- Overpouch
  - Multilaminated foils often containing Aluminium layer
  - Typical extractable compounds:

    Bislactone related compounds originating from polyurethane binding layers:



5. CONSIDER THE SECONDARY PACKAGING

Typical Cases:

- Overwrap (I.V.-Bags, Blow-Fill-Seal, ...)
- Label migration (Ophthalmic, I.V.-Bags, Polyolefin Containers)
- Ink Migration (I.V.-Bags, Blow-Fill-Seal)
- Needle Shield (Pre-Filled Syringe)

More delicate for Primary Packaging, made of materials with low barrier properties.

6. CONSIDER THE RIGHT EXTRACTION SOLVENT

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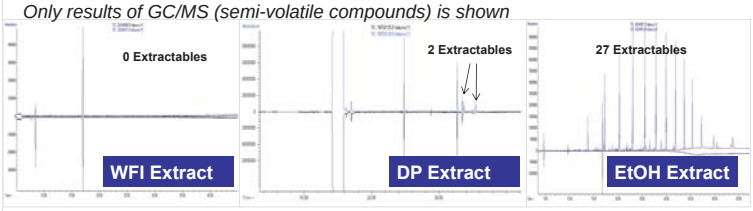
CASE STUDY: impact of contact solution on migration / extraction behavior

Extractable study of a POLYOLEFIN CONTAINER, using 3 solvents:

1. *Water for Injection (WFI)*
2. *Drug Product (containing 3% organic material)*
3. *Ethanol (96%)*

Identical extraction conditions for 3 experiments: refluxing for 8 h at 1 bottle/30mL ratio
Only results of GC/MS (semi-volatile compounds) is shown



Solubility of targets in WFI < Solubility of targets in DP << Solubility targets in EtOH

Interaction polymer-WFI < Interaction polymer-DP << Interaction polymer-EtOH

6. CONSIDER THE RIGHT EXTRACTION SOLVENT

CASE STUDY: PROVE OF EQUIVALENCY OF OLD VS NEW MATERIAL

SITUATION 1

PROOF OF EQUIVALENCY WITH **WFI**

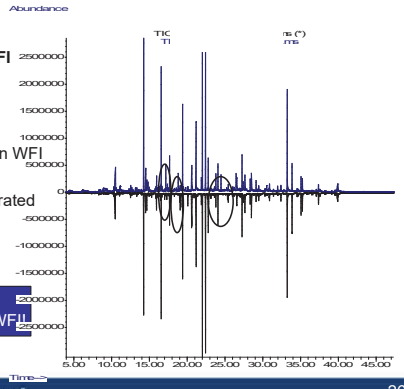
WFI as extraction solvent

2 materials were refluxed for 8 hours in WFI

Extracted with DCM, subseq. concentrated

Analyzed with GC/MS (semi-volatiles)

Conclusion
almost the same extraction profile in WFI!!



6. CONSIDER THE RIGHT EXTRACTION SOLVENT

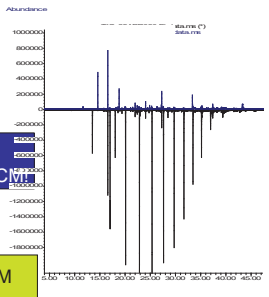
SITUATION 2

DCM as extraction solvent

2 materials were refluxed for 8 hours in DCM

Analyzed with GC/MS (semi-volatiles)

Conclusion:
COMPLETELY DIFFERENT extraction profile in DCM!!



MECHANISTIC CONSIDERATIONS

Solubility of targets in WFI << Solubility targets in DCM

Interaction polymer-WFI << Interaction polymer-DCM

ADVISE : Consider relevancy of adding additional solvent!

6. CONSIDER THE RIGHT EXTRACTION SOLVENT

THE CRITICALITY OF USING THE DRUG PRODUCT (VEHICLE) (DP(V)) AS A SOLVENT

Perform E-study in Drug Product (Vehicle), suggested in:

FDA-Container/Closure Guidance (1999), (eg parenteral/Ophthalmic)

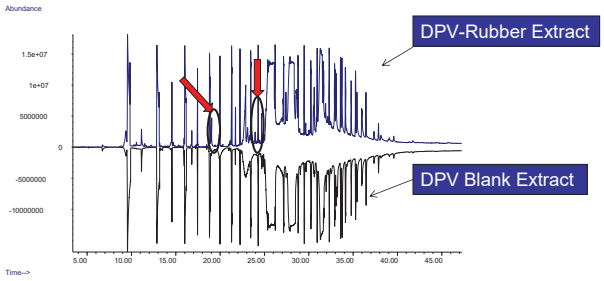
- If the extraction properties of the drug product vehicle may reasonably be expected to differ from that of water (e.g., due to high or low pH or due to a solubilizing excipient), then drug product should be used as the extracting medium.

EMEA-Guideline - immediate packaging (2005)

stress conditions to increase the rate of extraction. The solvent used for extraction should have the same propensity to extract substances as the active substance/dosage form as appropriate. In the case of medicinal products the preferred solvent would be the medicinal product or placebo vehicle. The

THE CRITICALITY OF USING THE **DP(V)** AS A SOLVENT

- Complex DPV: COMPLEX INTERPRETATION OF E-STUDIES!!



THE CRITICALITY OF SELECTING **DP(V)** AS SOLVENT

Similar advantages/disadvantages as for WFI:

ADVANTAGE: simulation of extractables behaviour in DP(V): same extraction propensity!

DISADVANTAGE: Risk of missing the presence of compounds

- *Matrix interference of DP(V) (see previous slide)*

Risk of misinterpretation of analytical data

- *DP(V) Matrix degradant may be misinterpreted as extractable!*

Risk of underestimating the concentration of compounds

- *Extraction conditions – may potentially be to mild*
  - *Difficult to select the right set of extraction conditions (e.g. extraction time, temperature!)*

EXAMPLE for DP(V) – does 8 hour reflux mimic a 3 year shelf life?

THE CRITICALITY OF SELECTING **DP(V)** AS SOLVENT

ADVICE when selecting DP(V) as extraction solution:

1. Combine it with organic model solvent (e.g. IPA, DCM, Hexane)
   - *Minimize the risk of missing the presence of extractables*
2. If necessary: Use validated methods, developed for extraction study with DP(V) as solvent
   - *Eliminate matrix interference from DP(V) matrix*
   - *Assess DP(V) matrix degradation during extractable study*
3. Consider the right set of extraction conditions, relevant for the DP(V) contact
   - *Extraction time*
   - *Temperature*

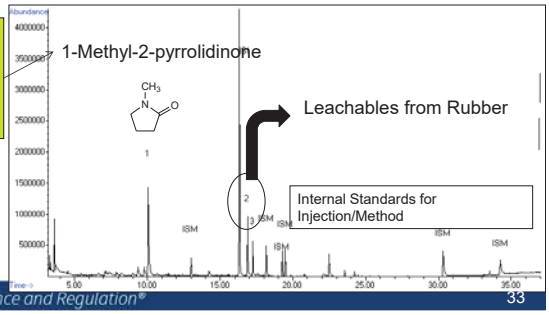
7. CONSIDER THE PROCESSING STEPS

7. CONSIDER THE PROCESSING STEPS

CASE STUDY: Leachable Study on a **vial system** (vial + rubber)
Using **Validated Methods** for Target Compounds, defined after Extractable Study + **Screening Method (**unexpected compounds)

RESULTS: 3 leachables were detected: 2 target compounds, 1 non-target compound (no increase in concentration over time)

Origin of non-target Compound: Sterile Filtration prior to filling in the PFS!



7. CONSIDER THE PROCESSING STEPS

Typical Cases:

- Filtration
- Tubing for Filling
- Storage Containers of Excipients
- Intermediate Storage of API
- Lyophilization Equipment
- Cross Contamination during Sterilization (e.g. autoclaving)
- Inner/Outer layer cross contamination of Films.
- Diptubes in Storage Containers
- ....

8. EVEN THEN, THINGS CAN GO WONG!!

8. EVEN THEN, THINGS CAN GO WRONG

The more we know,
the more we know we don't know!

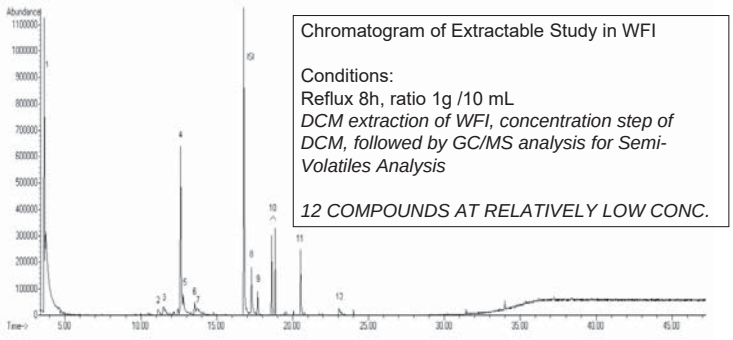
Anonymous
Berlin, 2017

8. EVEN THEN, THINGS CAN GO WRONG

- Prefilled Glass Syringe
- Filled with WFI
- Stored for 3y at 25° C/60% R.H.
- Initial Extractables Study on Plunger (WFI, IPA)
- Leachables (Screening) Analyses after 3 years
  - Headspace GC/MS: Volatiles
  - DCM extraction + GC/MS: Semi-Volatiles
  - DCM extraction + LC/MS (APCI+/-): Non-Volatiles
- 6 different Combinations (Syringe/Plunger/Needle Shield) were tested.
- Results: for Semi-Volatiles, indicative for other groups of compounds

8. EVEN THEN, THINGS CAN GO WRONG

RESULT OF **WFI EXTRACTABLE STUDY** OF THE PLUNGER



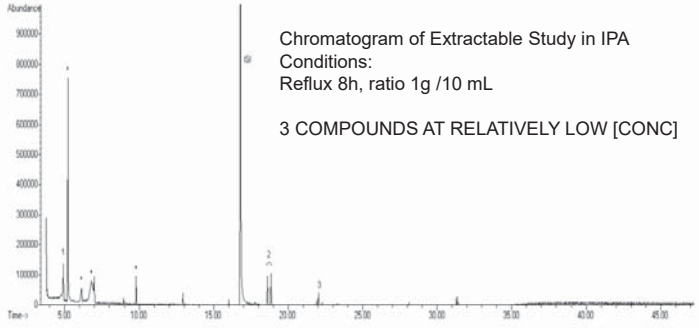
Chromatogram of Extractable Study in WFI

Conditions:
Reflux 8h, ratio 1g /10 mL
DCM extraction of WFI, concentration step of DCM, followed by GC/MS analysis for Semi-Volatiles Analysis

12 COMPOUNDS AT RELATIVELY LOW CONC.

8. EVEN THEN, THINGS CAN GO WRONG

RESULT OF **IPA EXTRACTABLE STUDY** OF THE PLUNGER

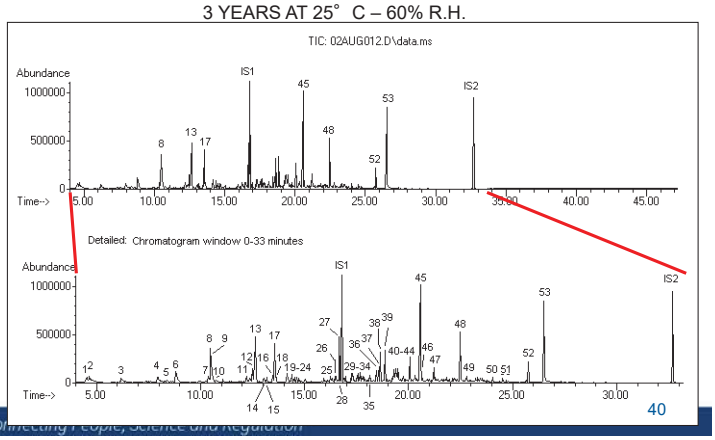


Chromatogram of Extractable Study in IPA
Conditions:
Reflux 8h, ratio 1g /10 mL

3 COMPOUNDS AT RELATIVELY LOW [CONC]

8. EVEN THEN, THINGS CAN GO WRONG

RESULT OF THE **LEACHABLE STUDY** OF THE **WFI- PREFILLED SYRINGE**
3 YEARS AT 25° C – 60% R.H.



8. EVEN THEN, THINGS CAN GO WRONG

LEACHABLES: compounds originating from:

1. **Rubber Plunger**
2. **Hydrolyzed** Compounds from Rubber Plunger
3. Compounds from **Needle Shield**
4. **Hydrolyzed/Oxidized** Compounds from Needle Shield
5. A lot of **"Unknown" Compounds**, both identity and origin is not clear
6. Results are **independent of Type of Rubber** / **Rubber Manufacturer** of the Rubber Plunger!!

Concentration range: from 10 µg/L to > 10 mg/L!

8. EVEN THEN, THINGS CAN GO WRONG

Observations when comparing the results of the Extractable Studies on the Rubber Plunger with the Leachable studies on the PFS system

- **Concentrations of Leachables was Higher** than the Extractables found with WFI as an Extraction Solvent
- Also for more **Aggressive solvents** (e.g. IPA), **not a good match** between Extractables and Leachables
- The observation was **independent of the type of rubber**

How can we **try** to explain these results?

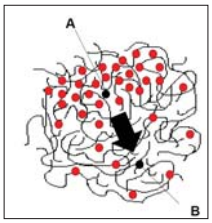
Extractable Studies: Temperature Dependence of Diffusion

By Heating up the material (boiling conditions), diffusion of extractables is increased

$$\frac{dC}{dt} = D \frac{d^2C}{dx^2}$$

With D = Diffusion coefficient

$D = D_0 \exp(-E/RT)$

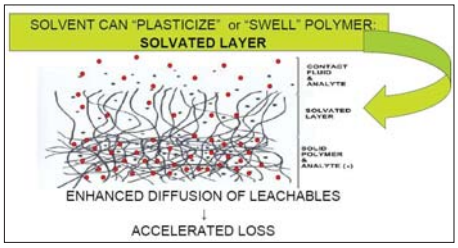


This means that a temperature increase from Room Temperature to solvent boiling point will lead to an increase of D of approx. 2 orders of magnitude *(reference for typical D values: H. Zweifel, « Plastic Additives »)*

Or Reflux extraction of 8h will mimic approx. 800h (=33d of R.T. contact)

8. EVEN THEN, THINGS CAN GO WRONG

Extractable Studies: Interaction between Solvent - Material



For Rubbers: Hexane, DCM and IPA will show enhanced diffusion because of the solvent-material interaction

Completeness of extraction can be checked via Asymptotic Extraction Behaviour

Not to the same extent for WFI!

8. EVEN THEN, THINGS CAN GO WRONG

What is not investigated (sufficiently) in an extractable study?

8.1 MATERIAL DEGRADATION (ageing)

8.2 The **REACTION** (WFI: hydrolysis / O_2: oxidation) **of the leachables with the Drug Product** (solution)

8.1 MATERIAL DEGRADATION

What is not investigated (sufficiently) in an extractable study?

1. MATERIAL DEGRADATION – ASTM 1980 – 02:

Material Degradation: In general ASTM 1980 can be a "general" guidance

$$AAF = Q_{10}^{[(T_{AA} - T_{RT})/10]}$$

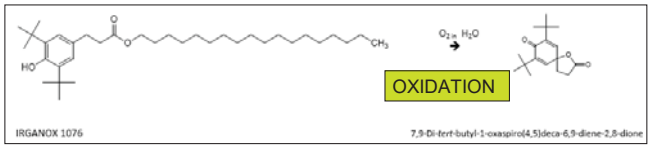
AAF: Accelerated Aging Factor
Q_{10}: Aging factor (10° C increase in T)
T_{AA}: Accelerated Aging Temperature
T_{RT}: Room temperature

8h at 100° C (eg. Refluxing in WFI) represents 1440h (60 days) of RT ageing
8h at 80° C (eg. Refluxing in IPA) represents 15 days of RT ageing

REMARK: Ageing of material is not always representative (Aqueous Environment versus Air (Oxygen!))

8.2 REACTIVITY OF LEACHABLES - DRUG PRODUCTS

EXAMPLE N° 1 (Oxidation):

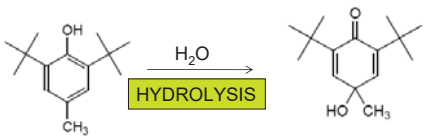


Dissolved Oxygen in WFI /DP(V) will Oxidize Irganox 1076 over time!

Occurrence of "oxaspiro" as a leachable is much more frequent than as an extractable!

8.2 REACTIVITY OF LEACHABLES - DRUG PRODUCTS

EXAMPLE N° 2 (Hydrolysis):



BHT BHT-OH

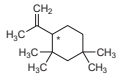
BHT-OH is seldom seen as an extractable, but it is regularly seen as a leachable!

EXAMPLE N° 3: Halogenated Rubber Oligomers – PART 1

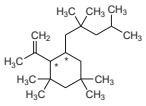
FORMATION OF THE HALOBUTYL ELASTOMERS
(for more details: see presentation "INJECTABLES")

$C_{13}H_{24}$ and $C_{21}H_{40}$ Oligomers

- Considered as
  - Cyclic aliphatic hydrobarbon compounds
  - One double bond
- No experimental data / Literature data is known about toxicity of these compounds
- Structure Activity Relationship Assessment (SAR): compound of low tox. risk.

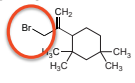


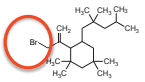
C13 oligomer



C21 oligomer

$C_{13}H_{23}Br$/ $C_{13}H_{23}Cl$ and $C_{21}H_{39}Br$/ $C_{21}H_{39}Cl$ Oligomers





- Considered as
  - **HALOGENATED** Cyclic Aliphatic Hydrobarbon compounds (Allyl Halide)
  - **Alkylating Agents**
  - One double bond
- Structure Activity Relationship (SAR) Assessment:

CARCINOGENICITY IN HUMANS IS PLAUSIBLE

- As no experimental data / Literature data is known about the toxicity of these compounds, a lot of Pharma companies:
  - Rely on the result of a SAR assessment to perform a tox evaluation
  - Conclude that these compounds are of High Concern

For potential Mutagenic/Carcinogenic compounds:

SCT: 0.15 µg/day (PQRI OINDP)

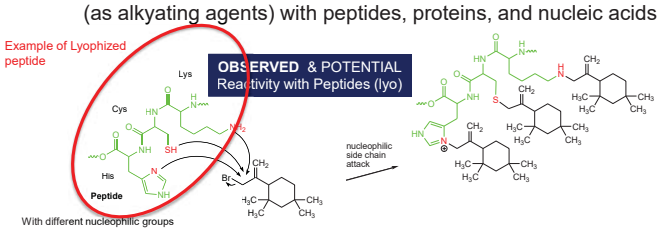
SCT/TTC: 1.5 µg/day (PQRI-PODP; EMA guideline on Genotoxic Impurities)

The low SCT/TTC levels for the Halogenated Oligomers mean:

- Low associated AET levels
- High level of method optimization to obtain these levels (certainly with LVP)
- e.g. SIM mode for GC/MS
- Can only be performed with appropriate analytical standards with known purity
  - *Method Selectivity*
  - *Accuracy*
  - *Sensitivity*
  - *Precision*
  - ...

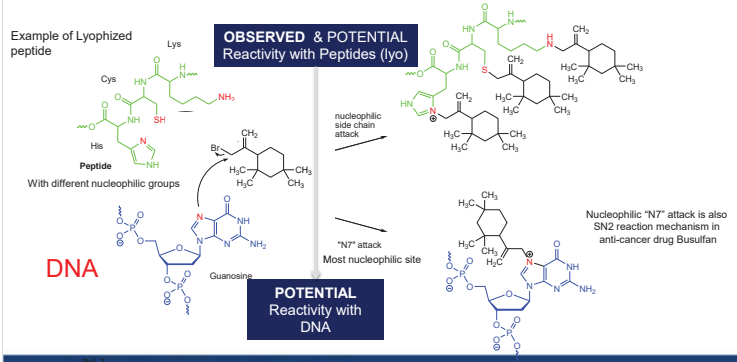
Observed Reactivity of $C_{13}H_{23}Br$ and $C_{21}H_{39}Br$

(as alkyating agents) with peptides, proteins, and nucleic acids



Observed Reactivity of $C_{13}H_{23}Br$ and $C_{21}H_{39}Br$

(as alkyating agents) with peptides, proteins, and nucleic acids

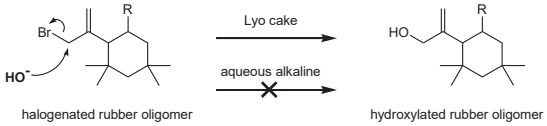


EXAMPLE N° 4: Halogenated Rubber Oligomers – PART 2

Cresol containing drug products, Bromocresol may be formed in the presence of Bromobutyl Stoppers (Mechanism is unknown)

EXAMPLE N° 5: Halogenated Rubber Oligomers – PART 3

Formation of $C_{13}H_{23}OH$ out of $C_{13}H_{23}Br$ in Lyo Products



halogenated rubber oligomer

hydroxylated rubber oligomer

EXAMPLE N° 6: Acrylic Acid reaction with Proteins/Peptide

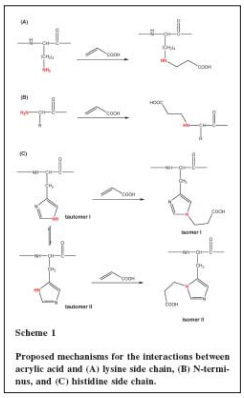
PDA Journal of Pharmaceutical Science and Technology

Interactions between Therapeutic Proteins and Acrylic Acid Leachable

Dengfeng Liu, Yasser Nashed-Samuel, Pavel V. Bondarenko, et al.

PDA J Pharm Sci and Tech **2012**, *66* 12-19
Access the most recent version at doi: 10.5731/pdajpst.2012.00803

EXAMPLE N° 6: Acrylic Acid reaction with



Scheme 1

Proposed mechanisms for the interactions between acrylic acid and (A) lysine side chain, (B) N-terminus, and (C) histidine side chain.

Acrylic acid is a component of the acrylic adhesive used to attach the needle to the barrel of pre-filled glass syringes. Even though acrylic acid was not detected in the syringes currently used by Amgen, it was identified as a leachable (~5 µg/mL) in syringes from one of the potential vendors (X syringes). To investigate the potential interaction between the acrylic acid leachable and our protein drug products, a model IgG 2 antibody was filled into sterilized X syringes. After incubation, the antibody was digested and analyzed using an improved trypsin peptide mapping method (6).

Ten peptides were observed to be modified by acrylic acid (beside four peptides observed in prefilled syringes, another six new peptides were modified). Five peptides were modified through side chain of lysine, one peptide through N-terminus, and four peptides through side chain of histidine. The modification percentage was varied from 0.2% to 5.0%.

Those four modified peptides observed in prefilled syringes were confirmed by the spiking experiments. The selected ion chromatograph (SIC) for unmodified peptides (top) and the corresponding modified peptides (bottom) via spiking experiment is shown in Figure 1. Figure 2 shows MS/MS spectra of unmodified peptides (top) and the corresponding modified peptides (bottom) via spiking experiment.

Acrylic Acid:

- ✓ May be a leachable from the Needle Glue
- ✓ Potential Interaction between Acrylic Acid and Protein Drugs was investigated, with a IgG 2 antibody was used as model
- ✓ 10 peptides were observed to be modified
- ✓ 5 peptides were modified through side chain of Lysine
- ✓ 1 Peptide was modified through N-terminus
- ✓ 4 Peptides were modified through side chain of Histidine
- ✓ Confirmed via spiking experiments

EXAMPLE N° 7: Biological Reactivity of I168ox-diester

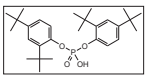
PDA Journal of Pharmaceutical Science and Technology

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.2013.00906

EXAMPLE N° 7: Biological Reactivity of I168ox-diester

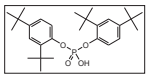


bDtBPP
or
I168ox-diester

Out of a large array of extracted compounds from polymer films used in presterilized, disposable biomanufacturing systems (e.g., BPCs), one compound, bDtBPP, can be shown to be highly detrimental to growth of a range of CHO cell lines, even at concentrations as low as 0.1 mg/L. The effect of bDtBPP on cells is rapid, quickly leading to a decrease in mitochondrial membrane potential. Studies of a film that contains significant quantities of extractable bDtBPP showed an exponential dependence of extracted bDtBPP on extraction temperature, and extracted bDtBPP also increased as a function of incubation time, with significant amounts of bDtBPP continuing to be extracted even after weeks of incubation. Experiments performed to understand the mechanism by which bDtBPP is generated suggest that exposure of oxidized Irgafos 168 (compound 8) to ionizing radiation is the primary pathway to bDtBPP formation, suggesting that manufacturers of single-use biomanufacturing components may have a variety of options to pursue in order to minimize the amount of bDtBPP that could leach from their products and adversely affect cell culture processes.

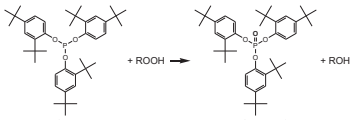
- ✓ A Range of Extracted Compounds were Investigated on their Impact on Cell Growth
- ✓ bDtBPP showed to be highly DETRIMENTAL to Cell Growth
- ✓ Even at < 0.1 mg/L!
- ✓ The effect is rapid, leading to a decrease in mitochondrial potential
- ✓ The Mechanism of Formation: see next slide

EXAMPLE N° 7: Biological Reactivity of I168ox-diester



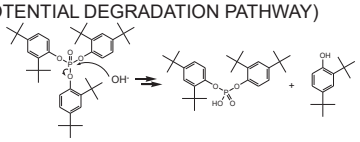
bDtBPP formation:

Step 1: Anti-oxidant I168 is oxidized to I168ox



Irgafos 168 — Irgafos 168 Ox

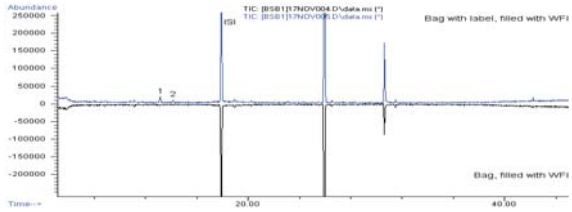
Step 2: During γ-Irradiation: I168ox degrades to I168ox-diester (POTENTIAL DEGRADATION PATHWAY)



Irgafos 168 Ox — bDtBPP

EXAMPLE N° 8: Benzene formation/migration – Label/Ink

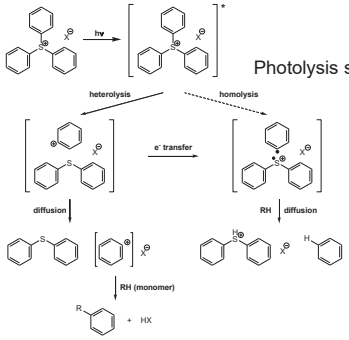
STUDY : Check the Migration of the Adhesive/Ink of the Label through the PVC layer of the Bag (results shown for *Headspace GC/MS*)



- **LABELED BAG vs. UNLABELED BAG – HS GC/MS**
  - **(1) IC: Benzene (5-10 µg/L)**
  - (2) IC: 1-butanol

EXAMPLE N° 8: Benzene Formation/Migration – Label/Ink

Triaryl sulfonium salts are photoinitiators for printing Inks

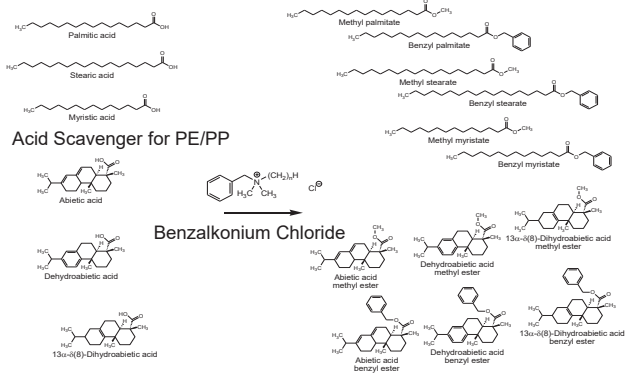


Photolysis step

BENZENE FORMATION

Source: Presentation of A. Hauk at EEA/EL Seminar, 2011

EXAMPLE N° 9: Benzalkonium Chloride Reactivity



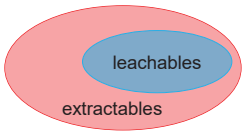
Acid Scavenger for PE/PP

Benzalkonium Chloride

Label Migration Impurities

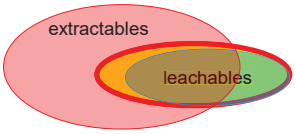
8.2 **REACTIVITY** OF LEACHABLES - DRUG PRODUCTS

➔ THEORY:



CLOSING THE GAP!!

➔ PRACTICE:



Additional Study Design

8. EVEN THEN, THINGS CAN GO WRONG

KINETICS OF	Extraction	Extraction	Accelerated Leachable St.	Real time/temp Leachable St.
	H_2O e.g. 8h reflux	DCM or IPA e.g. 8h reflux	e.g. 6 Mo, 40° C	e.g. 3 y at 25° C
EXTRACTION	SLOW – Incomplete no swelling/enhanced diffusion	FAST – complete Enhanced Diffusion Almost Asymptotic	Enhanced Diffusion controlled leaching is T-dependent $D = D_0 \exp(-E/RT)$	SLOW, but long term contact!
MATERIAL DEGRADATION	Slightly enhanced ASTM 1980: reflux at 100° C/8h: 60d at RT Even if they will be formed, will they come out?	Very Slightly enhanced ASTM 1980: (IPA) reflux at 80° C/8h: 15d at RT	Enhanced ASTM 1980: 6 Mo ageing at 40° C ≡ 17 Mo at 25° C	SLOW, but evaluated over LONG period! (e.g. 3y)
REACTION KINETICS • Dissolved O_2 in H_2O • Hydrolysis (H_2O) • Reaction with DP and leachates/materials • …	Slightly enhanced Low $[extr]_{init}$ will limit the formation of reaction comp. (i.e. for slow reactions)	Not relevant!	Enhanced, $k = k_0 \exp(-E_a/RT)$ E_a: Activation Energy, reaction dependent (Pseudo) first order kinetics	SLOW, but evaluated over LONG period! (e.g. 3y)

9. LESSONS LEARNED

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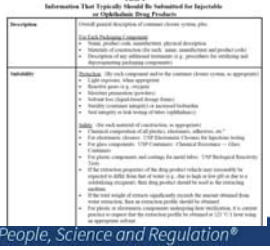
1. Consider All Components of the **Pre-Filled Syringe**
2. Consider the Secondary Packaging (Needle Shield), the Processing Conditions, the right set of Conditions to perform the Extractable Study
3. Do not rely solely on Extractable Studies to perform a risk assessment of your Containers/Closures
   Even if the Guidelines themselves suggest that this could be sufficient

FDA



EMEA

9. LESSONS LEARNED

3. If Safety Assessment is made on Extractables Results: check off with Leachable Studies!
   This will account for "unaccounted" leachables, such as polymer degradation, polymer additive degradants, process leachables, secondary packaging, or other extractables missed because of an ill designed study set-up

4. Consider – if possible – an additional **Accelerated Leachable study** (e.g. with screening methods) to verify the presence of "unexpected leachables"
   (as a step in between extractable studies and full leachable studies)

5. If the above is not possible: add a **screening step in the full leachable study**

Consider – if possible – an additional accelerated Leachable study (e.g. with screening methods) to verify the presence of "unexpected leachables"

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ANY QUESTIONS?

For further questions, please contact:
piet.christiaens@toxikon.be
http://www.toxikon.be/extractables-leachables-parenteral-injectables.html