Regulatory Perspective on Safety Qualification of Extractables and Leachables

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Risk-based Approach in Evaluating E&L

- **Safety considerations (e.g., toxicity, immunogenicity, etc.)**
- **Efficacy considerations (e.g., L interacting with a product → loss of activity; L may induce development of neutralizing activity via NAb formation)**
- **Quality considerations (e.g., impact on the manufacturing process, product stability, etc.)**

Safety considerations

- **Toxicity (e.g., acute, chronic, synergistic, additive, carcinogenicity, endocrine dysregulation, etc.)**
- **Adjuvant effects:**
  - **Adjuvants are substances that increase the activity of the immune system without having any specific antigenic effect**
  - **In contrast to vaccines where adjuvant effect is a desired effect, this may be a serious safety concern for therapeutic proteins**
  - **May promote development of anti-drug antibodies**
    - **A decrease or loss of efficacy due to development of neutralizing activity**
    - **May be life-threatening if NAbs are developed against a non-redundant endogenous protein (e.g., erythropoietin - anemia/PRCA; thrombopoietin - thrombocytopenia)**
    - **Altering the PK of the drug**
  - **May promote non-specific inflammation**

Safety considerations (cont.)

- **Drug dose, mode and frequency of administration (e.g., SC vs. IV, life-time dosing and chronic exposure)**
- **Prior clinical exposure to leachables may enhance sensitivity in case of re-exposure**
- **Therapeutic necessity of the drug (higher levels may be tolerated if drug is considered a part of essential therapy)**

Manufacturing considerations

- Place in the process stream (e.g., upstream vs. downstream; typically risks are greater as production moves closer to the finished product)
- Type of the processed/stored material (e.g., purification buffer vs. final product)
- Storage temperature (e.g., freezing vs. 2-8 C)
- Surface-to-volume ratio
- Contact time
- Type of polymeric material (e.g., PVC at risk for leaching di(2-ethylhexyl)phthalate, which is linked to various toxicities)
- Formulation/choice of excipients; (e.g., liquid vs. lyophilized; pH; phosphate buffer)
- Risks often assessed on a case-by-case basis

What's done in practice…

- **Extractables studies are performed using exaggerated conditions (organic solvents, accelerated T°, pH, etc.)**
  - **Alternatively, the drug manufacturer may rely on the E studies done by the vendor**
  - **Note: Drug Product vehicle may or may not be used as an extraction medium (role of excipients important)**
- **Analysis of extractables is done in conjunction with stability studies, which monitor changes in product quality over time**
- **Leachables studies are often omitted**

Risk assessment and risk reduction with regard to leachables studies

- **Safety considerations:**
  - Toxicity studies are usually acute studies that do not measure chronic exposure to potential leachables
  - Potential for adjuvant effect and immunogenicity is not addressed
  - Mode of administration (e.g., SC is often more immunogenic than IV)
  - Product that is at the end of its dating period is rarely evaluated in clinic
- **Product Quality considerations:**
  - Stability studies are often not geared to detect leachable impurities (e.g., inorganic leachables such as tungsten, Fe, Al, etc., are never evaluated and organic leachables may be missed)
  - Differences in the levels of leachables at the extremes of the manufacturing, storage and transportation conditions (no worst case scenario risk assessment)
  - Inappropriate sample size that is needed to understand the true variability in the C/C system (e.g., tungsten and PFS syringes)

Leachables studies

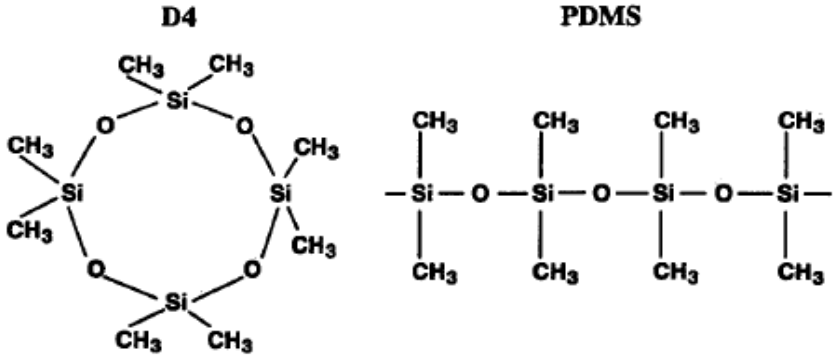
- **Monitor the clinical outcome in conjunction with measuring leachables over the entire shelf-life of the product**
  - **In the presence of product**
  - **Without the product (i.e., in placebo alone)**
- **Ideally, Drug Product material that is at the end of its shelf-life should be tested in clinic**
- **Perform stability studies, which monitor impurities and product physico-chemical and biological properties throughout the expiry period**

Leachables as adjuvant and/or immunomodulatory factors

Some support in the literature

Leachables as adjuvant and/or immunomodulatory factors

- **Silicone oil – polydimethylsiloxane and octamethyl-cyclotetrasiloxane (D4)**
  - **Naim et al., 1995, Immunol Invest, 24:537-547**
  - **Naim et al., 2000, Clin Diagnostic Lab Immunol, 7:366-370**
  - **Locatelli et al., 2004, Nephrol Dial Transplant, 19:288-293**



Leachables as adjuvant and/or immunomodulatory factors (cont.)

- **Di-(2-ethylhexyl) phthalate (DEHP) and Mono-2-ethylhexyl phthalate (MEHP)**
  - **Larsen et al., 2001, Tox Letters, 125:11-18**
  - **Larsen et al., 2001, Toxicology, 169:37-51**
  - **Larsen et al., 2007, Tox Letters, 170:223-228**
- **Polycyclic aromatic hydrocarbons (PAH)**
  - **Lovik et al., 1997, Toxicology, 121:165-78**
  - **Nilsen et al., 1997, Toxicology, 124:225-232**

Leachables as adjuvant and/or immunomodulatory factors (cont.)

- **Soluble iron**
  - **Beck-Speier et al., 2009, Particle and Fibre Toxicology, 6: 34-46**
- **Cadmium**
  - **Marth et al., 2001, Inter J Occupational Med and Environ Health, 14:375-386**
- **Nickel**
  - **Schmidt et al., 2010, Nature Immunology, 11:814-820**
- **Alkyl phenols**
  - **Yano et al., 2003, J Health Sci, 49:195-204**

Establishing threshold levels for leachables in biologics:

Known's and Unknown’s

What we know

- **Toxicological threshold levels have been proposed and/or established (e.g., PQRI, ICH Q3C, published literature, etc.) that can be applied across board**

- **What we don't know: Can the same approach be applied to biologics?**

A feasibility exercise

- **Threshold for the adjuvant effect:**
  - **Adjuvant effects of leachables may be studied in animal models (e.g., in mice)**
  - **Such studies may be useful in looking at relative differences (e.g., after a change in the C/C system) and in identifying potential risks**
  - **However, the threshold levels identified in animal studies are unlikely to be predictive of the clinical outcome (e.g., mice are 1,000x less sensitive to LPS compared to humans)**
- **Threshold for product quality**
  - **Needs to be assessed on a case-by case basis due to diversity of protein products, formulation composition and C/C systems**
  - **E.g., tungsten oxides had a very different effect on two analogous API that had different formulation: in one case tungsten caused unfolding and aggregation, whereas in another case, it had no effect**

Biologics may deserve a special consideration for the following additional reasons…

- **Manufacturing and stability issues:**
  - **Protein conformation (e.g., secondary, tertiary) is sensitive to external environment**
  - **Aggregation and/or degradation**
  - **Deamidation and/or oxidation**
  - **Changes in glycosylation**
- **Routine analytical testing often doesn't detect finite changes in the protein (e.g., release testing is unlikely to detect areas of protein unfolding unless it impacts the function)**
- **Large size (e.g., MAb 150 KD) and extensive surface area ensures → high frequency of potential sites of interaction**
- **Proteins may be more efficient in solubilizing leachables due to abundance of both hydrophilic and hydrophobic sites (the latter are usually buried in the interior of the protein)**
- **Drug dose, mode and frequency of administration (e.g., many biologics are sterile injectables administered frequently at relatively high volumes and doses of mg/ml)**

Case studies

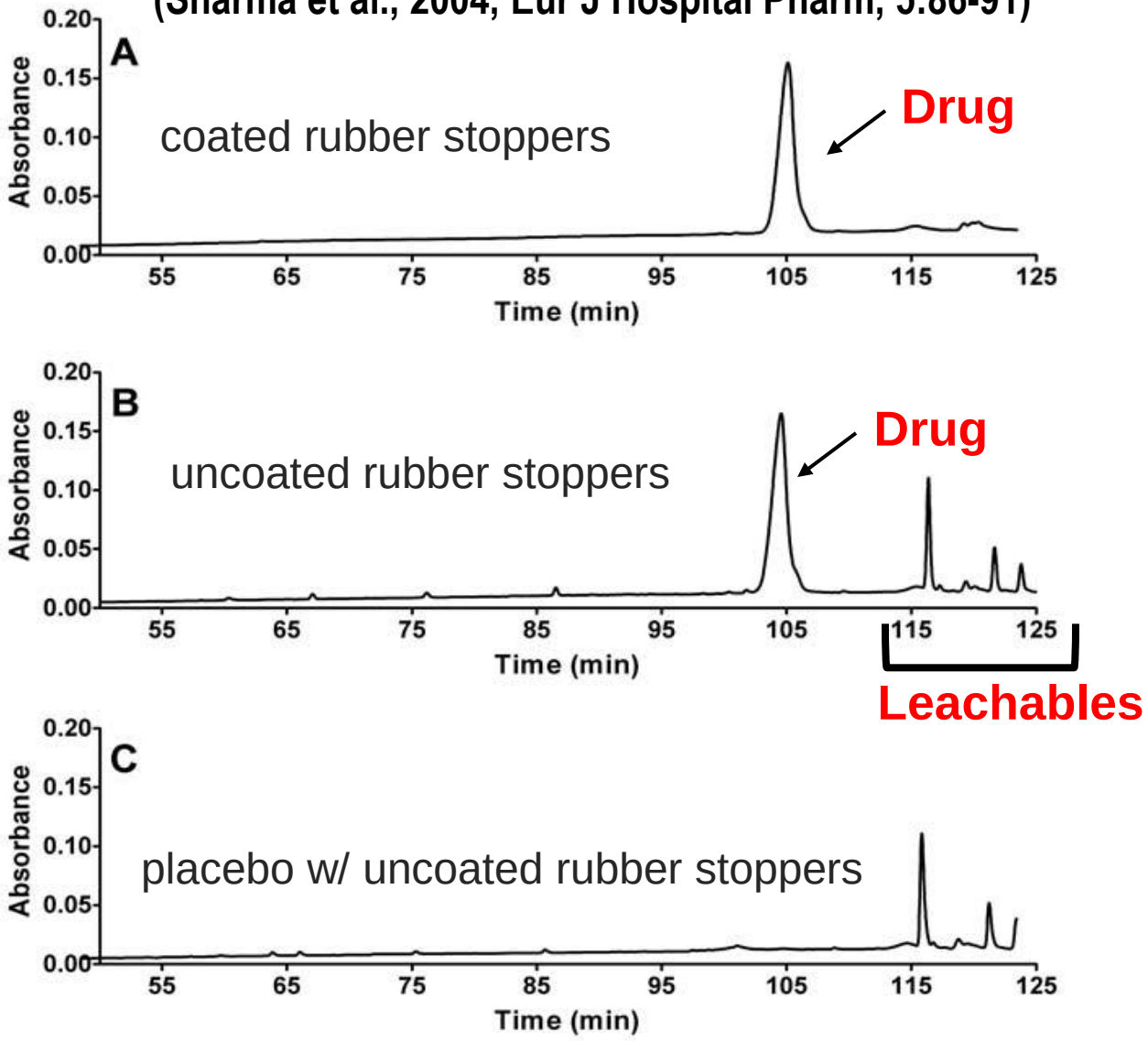
Leachables from the uncoated stoppers

(Casadevall et al., 2002, N Engl J Med, 346:469-475; Sharma et al., 2004, Eur J Hospital Pharm, 5:86-91)

- **Change from HSA to polysorbate formulation**
- **C/c system: pre-filled syringes with uncoated rubber stoppers**
- **Source: Vulcanizing agents leached from the rubber stopper during storage (e.g., Vultac 2 di, tri, tetra, penta, hexasulfide, etc.)**
- **Impact:**
  - **no notable changes in protein physico-chemical properties**
  - **safety: serious adverse event (pure red cell aplasia, PRCA)**
- **Hypothetical MoA: leachables acted as adjuvants leading to formation of neutralizing Abs to endogenous protein**
- **Resolution:**
  - **Switch to teflon-coated stoppers**
  - **Stricter control of the cold-chain from manufacture to administration**
  - **S.c. route of administration was contraindicated in CRF patients, which was subsequently reversed**

RP-HPLC profile of the drug + leachables

(Sharma et al., 2004, Eur J Hospital Pharm, 5:86-91)

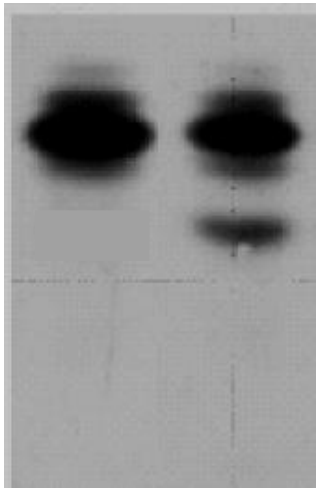


Fe leachables cause formation of protein-preservative adducts

- Change: extension of the expiry period from 15 to 18 months
- Source: uncoated rubber stoppers released iron at levels <1 ppm
- Critical excipients: preservative and other components
- Impact:
  - Fe catalyzed oxidation of the preservative + additional excipient triggering formation of the protein-preservative adducts
  - Several sites on the protein were modified primarily at the N-terminus (primary targets were peptides with – OH, – NH_3 and –SH groups)
  - OOS result for protein content (e.g., >50% of the product was modified)
  - moderate decrease in potency
- Action: >10 DP lots were recalled due to OOS results
- Resolution:
  - Return to the original dating period of 15 months
  - Implement Teflon coated stoppers
  - Conduct additional studies to determine the impact on product Q and S
  - Eventually, the problematic presentation was removed from the market

Metal leachables cause product truncation via metalloprotease activation

- Change: from lyophilized to a liquid formulation
- Source: rubber stopper released divalent metal cations
- Uncovered during stability study under inverted conditions
- Mechanism: activation of a metalloprotease (process-related impurity co-eluted with API)
- Impact: product truncation at the N-terminus
- Resolution: chelator (EDTA) added to DP formulation buffer
- Adverse outcome: new formulation led to cardiovascular adverse events and a change in PK values; it was withdrawn from the market and replaced with the original one; Teflon coated stoppers implemented



Tungsten leachables from PFS barrels #1

- **Container closure system: prefilled syringes**
  - **Tungsten filaments are used to perforate syringe barrel onto which a needle is attached**
- **Source: tungsten salts and tungsten oxides are deposited on the glass and into the product when contacted with liquid**
- **Impact: tungsten caused unfolding and aggregation of the protein**
- **Clinical outcome: Patients developed neutralizing Abs to the endogenous protein**
- **Resolution: Continue product development in vials and discontinue PFS**

Tungsten leachables from PFS barrels #2

- Container closure system: prefilled syringes
- Impact: tungsten salts caused protein oxidation followed by aggregation
  - Up to 60% of aggregated product found in some syringes
  - Up to 1% PFS tested positive for aggregates
- Resolution (different approaches were used by different Sponsors):
  - Optimal - switch to platinum instead of tungsten filaments
  - Alternative - establish tungsten specifications, nitrogen overlay process, special washing procedure, etc.

Alkali oxide extractables cause delamination of glass vials

- **Container closure system: Type I borosilicate glass vials**
- **Glass pitting and surface delamination is initiated by ion exchange at the glass-solution interface causing breaking of Si-O bonds and weakening of the surface layer**
- **Risk factors promoting delamination (not listed in the order of importance):**
  - **Glass vials with high surface alkalinity have higher propensity for delmination**
  - **Specific vial manufacturing process**
  - **Drug solutions formulated at alkaline pH**
  - **Certain buffers**
  - **High ionic strength**
  - **Contact time (delamination is a time-dependent process) and temperature**
- **Risk mitigation strategies include the following:**
  - **Switch to highly resistant glass (high coefficient of thermal expansion)**
  - **Switch to a different vial manufacturing process with more stringent temperature control (leads to lower surface alkalinity)**
  - **Switch to lower risk formulation (e.g., pH, buffers, etc.)**
  - **Reduce product dating period**

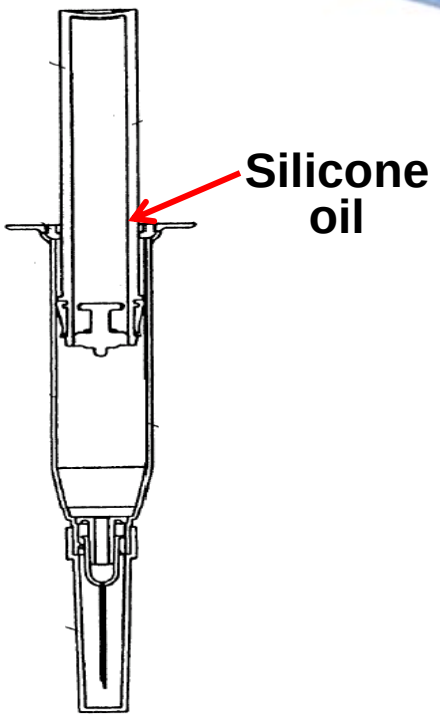
Aluminum leachables from glass form particulates

- **Change: change from molded to tubing glass vials**
- **Source: aluminum oxide leached from the new glass vials**
- **Mechanism: aluminum interacted with sodium phosphate in the formulation forming aluminum phosphate crystals**
  - **Aluminum phosphate (i.e., alum) is widely used as adjuvant in vaccines, although at concentration leached, it is unlikely to exert such an effect**
- **Impact: visible particles (up to 150 µm diam) observed in stability samples over 12 month of age with no other OOS results**
- **Resolution:**
  - **Recall of lots that failed the particulate spec**
  - **New glass vials are coated using a baked-on siliconization process**

Barium leachables from glass form particulates

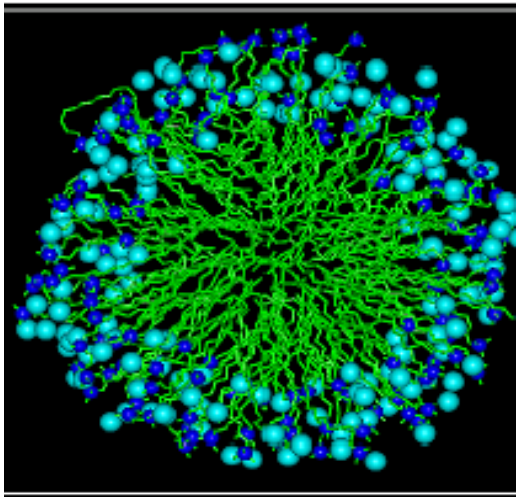
- **Change: vendor for glass vials**
- **Source: barium leached from new glass vials**
- **Mechanism: barium interacted with sodium sulfate in the formulation forming barium sulfate crystals**
- **Impact: visible particles observed in 18 month stability samples with no other OOS results**
- **Resolution: acceptance limit for barium established with commitment to generate stability data for 10 new vial lots**

Silicone oil leachables cause product aggregation



- Source: silicone oil spray was used as a lubricating agent to coat prefilled syringes
- Observation: silicone oil was shed (break-loose effect) from the syringe barrel into the product
- Silicone oil forms micelles in solution, which can interact with proteins and cause protein denaturation and aggregation

  (J Pharm Sci, 2005, 94:918-927)
- Outcome: formation of amorphous polymers visible by the naked eye
- Resolution: product now packaged in non-siliconized syringes



Points to consider…

- **Greater emphasis should be placed on the leachables testing, in conjunction with clinical data mining, in order to reduce clinical uncertainty and minimize patients' exposure to unnecessary risks**
- **Consideration for the improvement and standardization of E&L testing**
- **Consideration of E&L for disposable (i.e., single use) systems**

Summary

- **Biologic products can be sensitive to minor impurities and changes in the C/C system and/or formulation**
- **Undetected differences in product impurities may have a significant impact on clinical safety and efficacy (e.g., leachables acting as adjuvants triggering NAb response)**
- **It's important to monitor leachables over time (e.g., extended time points reflective of product dating period should be included)**
- **Corrective actions should employ a simplest approach to resolve a problem while minimizing changes to product quality as it relates to safety and efficacy**
- **Lack of transparency between a product quality attribute and its safety & efficacy doesn't reduce risk, it's simply, uncontrolled risk (Barry Cherney)**

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Questions? Comments?

Extractables analysis

- **Appropriate extraction procedure is relevant**
  - **Determines chemical profile and maximal levels of extractables (depends on the specific extraction conditions)**
  - **Highlights immediate safety concerns (if any)**
  - **Ensures that methods with appropriate specificity and sensitivity are used**
- **Justification of selected extraction conditions is generally poorly described in submissions**

Examples of more common E&L

- Phthalates (e.g., Di(2-ethylhexyl) phthalate - DEHP)
- Metals (e.g., Zn, Fe, Ba, Ca, Al, Ni, etc.)
- Fatty acids (e.g., stearic, plamitic myristic, etc.)
- Cyclic esters (from polyurethane adhesives)
- Silicone oil (e.g., polydimethylsiloxane)
- Organic solvents (e.g., acetone, isopropanol, etc.)
- Nitrosamines (e.g., diphenylnitrosamines, etc.)
- Vulcanizing agents (e.g., Vultac 2, etc.)
- Accelerators (e.g., thiuram, sulfenamide, guanidine, dithiocarbamate, etc.)
- Antioxidants (e.g., BHT, Irganox, Irgafos, etc.)
- Polycyclic aromatic hydrocarbons
- Antistatic agents
- Cleaning agents