Regulatory Perspective on Extractables & Leachables in Biologics: Quality Considerations

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Ingrid's role at CBER

- Provide technical and scientific support to the center regarding E&L issues
- Assist with developing and implementing E&L policy within the center
- Serve as internal and external point of contact for E&L issues
- Chair of the CBER E&L Subcommittee

Outline

- CFRs
- Special considerations for biologics
- E&L impact on product quality & manufacturing
- CBER's approaches to E&L assessment under discussion
- Case studies focusing E&L impact on product quality
- Lessons learned

CFRs

Title 21 of the CFR

- 600.11(b) Equipment

"All surfaces that come in contact with products shall be clean and free of surface solids, leachable contaminants, and other materials that will hasten the deterioration of the product or otherwise render it less suitable for the intended use."

- 600.11(h) Containers and closures

"All final containers and closures shall be clean and free of surface solids, leachable contaminants, and other materials that will hasten the deterioration of the product or otherwise render it less suitable for the intended use."

Risks Associated with E&L

- Quality considerations (e.g., interaction with the API or excipients may negatively impact stability, etc.)
- Safety considerations (e.g., direct → due to toxicity; indirect → due to effect on the API or excipients)
- Efficacy considerations (e.g., L interacting with a product → loss of activity; L may induce development of neutralizing activity via neutralizing Ab formation)

Biologics Typically Differ from Traditional Small Molecule Drugs for the Following Reasons

- Large MW and complex structure (e.g., Factor VIII is about 300,000 Da; it has 3 different domains and it is also glycosylated)
- Abundance of both hydrophilic and hydrophobic sites may render biologic drugs more efficient extraction media
- Could be heterogeneous mixtures: analytical challenges?
- Produced by a complex manufacturing process employing living organisms

Special Considerations for E&L in Biologics

- A non-toxic leachable can present a high risk to product quality including stability
- An indirect effect as opposed to a direct toxic effect - a change in product quality may occur due to interaction with non-toxic leachables, which may lead to potentially serious safety concern
- Possible outcome: A minor change in the product may induce immune response with a significant clinical impact
- Underlying issue: The relationship between a quality attribute and clinical safety and efficacy for biologics is generally not well understood

The Critical Questions Asked when Assessing the Risks from E&L to the Final Drug Product

- **Product Quality:**
  - Is the product altered and therefore less stable due to leachables interacting with the active pharmaceutical ingredient and/or excipients in the formulation?
- **Safety:**
  - What is the (a) chemical identity and (b) amount (e.g., µg/dose); and (c) how often will the patient be exposed to leachables present in the product.

Quality Considerations: What can go wrong?

Leachables may:

- Appear as in-process and/or final DP impurities
- Interact with the API and/or excipients (affecting structure, function and stability of the API)
- Negatively impact process performance
- Interfere with biological assays
- Interfere with medical diagnostic tests

Quality Considerations (cont.)

- **Leachables may affect protein products in any of the following ways (noted in the course of regulatory review):**
  - Oxidation
  - Unfolding
  - Aggregation
  - Increase in particulates
  - Formation of clipped variants
  - Formation of protein adducts
  - Post translational events during fermentation (e.g., glycosylation)
  - Altered protein translation (i.e., product yield)

Quality Considerations (cont.)

- **May interfere with biological assays**
  - Multiple published reports are available
- **May interfere with medical diagnostic tests**
  - Leachables with surfactant properties caused patients to be falsely identified as positive for a mitochondrial dysfunction disease (Belaiche et al., Clin Chem, 2009, 55:1883-1890)

Manufacturing Considerations

- **May negatively impact process performance:**
  - **May affect cell growth (e.g., detrimental to growth and viability) and therefore product yield**
    - Seen in the regulatory files, also multiple published reports are available
  - **Leachables from the tubing lead the manufacturer to believe that the product was not properly sterilized because the filter integrity test failed** (Meyer and Vargas, PDA J Pharm Sci Tech, 2006, 60:248-253)

Approaches for E&L Assessment Under Discussion at CBER

Not a guidance!

Challenges

- CBER regulates a wide diversity of biologic products including:
  - Vaccines (recombinant and non-recombinant)
  - Allergenics
  - Whole blood
  - Blood components
  - Blood derivatives (e.g., recombinant and natural fractionated blood derived clotting factors)
  - Tissue and tissue products
  - Cellular and gene-therapy products
- The nature of biologics (e.g., large MW, complexity) and leachable effects that are unique to biologics add to the regulatory review challenges

Approaches for E&L Assessment Under Discussion at CBER

- We support a risk-based approach to assess an impact from E&L to product quality and patient safety/efficacy
- For INDs:
  - E&L and other impurities are considered to be controlled in the IND phase because the clinical outcomes are closely monitored; therefore, E&L studies are generally not required

Approaches for E&L Assessment Under Discussion at CBER (cont.)

For BLAs:

- Leachables studies conducted under real-time conditions is generally expected to support a BLA
- Leachables studies should be reflective of product expiry with multiple stability time-points evaluated
- Ideally, incorporated into the stability program and considered when establishing product expiry
- E&L for each component used in the process is not necessary for a BLA
- Leachables need only be qualified once, if no changes in the process, Drug Product formulation, container closure system and/or storage conditions are made
- Use validated methods

Approaches for E&L Assessment Under Discussion at CBER (cont.)

- For BLA supplements with various manufacturing changes - a combination of controlled extraction, accelerated, and/or real-time leachables, and stability studies, may be justified depending on the extent of the change and the risks to product quality and safety.
- The following risk factors should be considered in an E&L assessment for post-approval changes:
  - Proximity of the process step(s) undergoing a change to the final product (consider leachables clearance, dilution, etc.)
  - Duration of contact
  - Surface area of contact
  - Storage/process conditions (e.g., temperature, pressure, pH extremes, buffer extraction propensity, etc.)

Recommended Analytical Testing for E&L

- Organic compounds:
  - Non-volatile organic compounds
  - Semi-volatile organic compounds
  - Volatile organic compounds
- Inorganics:
  - **Metals** (of special interest for biologics)

Case studies

Focus is on product quality!

Leachables from Uncoated Stoppers Linked to Immunogenicity

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

- **Change**: HSA was replaced with polysorbate in the DP formulation buffer
- **Outcome**: Vulcanizing agents leached into the product during storage from the uncoated rubber stoppers in PFS
- **Impact**: incidence of immune reaction (PRCA) potentially via an adjuvant effect was correlated to the presence of leachables
  - There were no warning signals at release or on stability
- **Resolution**:
  - Switch to coated stoppers with the stricter control of the cold-chain
  - S.c. route of administration was contraindicated in CRF patients, which was subsequently reversed

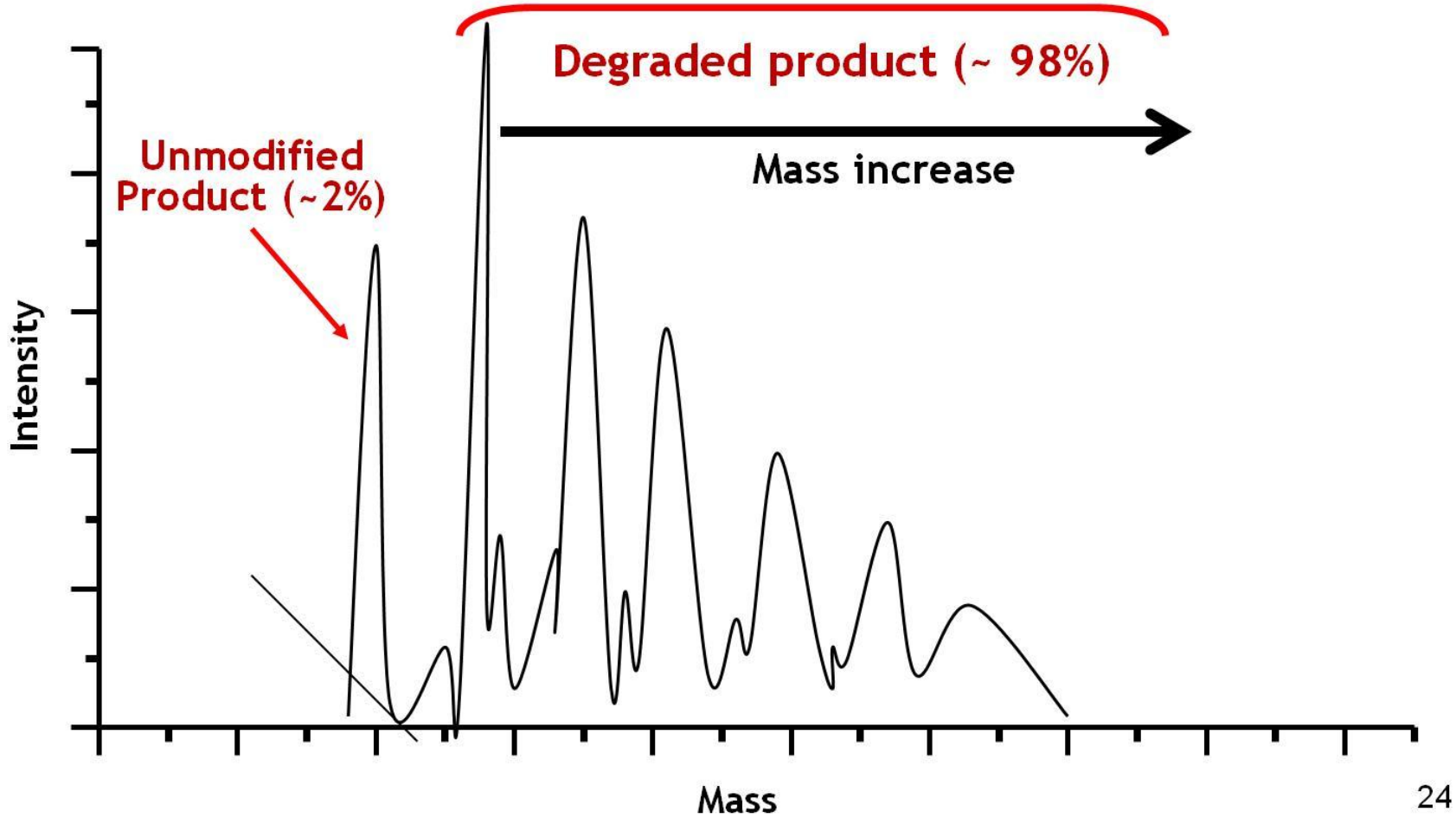
Tungsten Leachables Induced Product Denaturation and Aggregation Eliciting Immunogenicity

- **Change**: Investigation of the s.c. route of administration
- **Outcome**: tungsten oxide and tungsten salts leached from the tip of prefilled syringe barrels and diffused into the product during storage
- **Impact**:
  - To product quality - tungsten leachables induced product denaturation, oxidation and formation of covalent dimers
    - ✓ There were no warning signals at release or on stability
  - To patient safety - Immunogenic reaction was observed in patients administrated via SC route, which was correlated to the presence of covalent dimers
- **Resolution**:
  - Use tungsten free container closure system (i.e., glass vial)
  - Establish acceptance criteria for covalent dimers not to exceed the limit of method detection as an additional control measure

Fe Leachables Caused Protein-Preservative Adducts

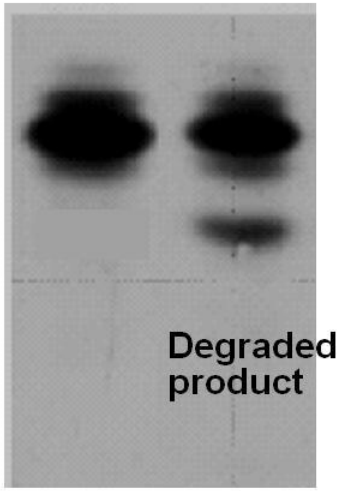
- **Change**: extension of product expiry from 15 to 18 months
- **Outcome**: uncoated rubber stoppers released iron at levels ~ 500 ppb in the presence of a preservative and other components in the formulation
- **Impact**:
  - Fe catalyzed preservative oxidation, which was assisted by another excipient
  - As a result, protein-preservative adducts were formed
  - Several sites on the protein were modified at - OH, - NH_3 and -SH groups
    - OOS result for protein content (e.g., >50% of the product was modified) with a moderate decrease in potency
- **Action**: >10 DP lots were recalled due to OOS results
- **Resolution**:
  - Not to exceed the 15 months expiry period
  - Implement coated stoppers
  - Conduct additional studies to determine the impact on product Q and S

Simulated Chromatogram of a Severely Degraded DP Lot



Metal Leachables Caused Protein Clipping via Activation of a Metalloprotease

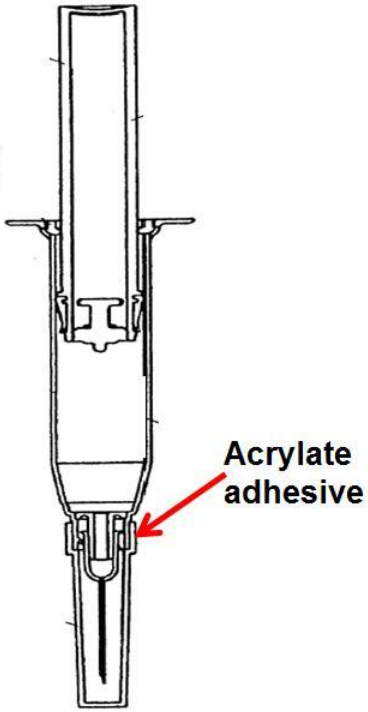
- **Change**: from lyophilized to a liquid formulation
- **Outcome**: rubber stopper released divalent metal ions during storage under inverted conditions
- **Impact**: product was truncated at the N-terminus by a metalloprotease, which was a process-related impurity co-eluted with API that was activated by metal ions
- **Resolution**: chelator (EDTA) added to DP buffer
- **Unexpected event**:
  - New formulation led to cardiovascular adverse event and change in the PK value
  - Modified product was withdrawn from the market and replaced with the original formulation
  - Coated stoppers were implemented to prevent leaching



Acrylate Leachables from PFS Adhesive Caused Protein Adducts

(Liu et al., PDA J Pharm Sci Technol, 2012, 66:12-19)

- **Source**: staked needle prefilled syringe
- **Outcome**: acrylic acid (~ 5 ppm) from acrylate-based adhesive used for needle attachment to the syringe barrel leached into the product
- **Impact**: acrylic acid covalently modified protein at three different sites via interaction with the lysine side chains, N-terminus, and histidine side chains changing the overall protein charge into (-)
- **Resolution**: An alternative PFS was used in the filling platform



Barium Leachables from Glass Caused an OOS for Particulates

- **Change:** there was a switch in the vendor of glass vials
- **Outcome:** barium leached from the new glass vials and interacted with sodium sulfate in the formulation buffer creating barium sulfate crystals
- **Impact to quality:** visible particulates were formed in storage (at 18 month) with no other OOS results
- **Safety concerns:** Similar to other large particulates
- **Resolution:** acceptance limit for barium established with a commitment to generate stability data for 10 new vial lots

Aluminum Leachables from Glass Caused an OOS for Particulates

- Change: there was a switch from molded to tubing glass vials
- **Outcome**: aluminum leached from the new glass vials and interacted with sodium phosphate in the formulation buffer creating aluminum phosphate crystals
- **Impact to quality**: visible particles were formed (up to 150 µm diameter) in storage at 12 months with no other OOS results
- **Safety concerns**: Similar to other large particulates
- **Resolution**:
  - Lots that failed the particulate acceptance criteria were recalled
  - The existing vial was replaced with a new one that is coated with a baked-on siliconization process

Lessons Learned

- Biologics could be very sensitive to changes in the manufacturing process, C/C system or formulation (supported by the published examples and regulatory review files)
- A toxicological threshold level and threshold that impacts product quality could be different
  - I.e., minor or non-toxic leachables may induce considerable changes in product quality, which can in turn become a safety issue (e.g., tungsten causing product aggregation, glass leachables inducing formation of large size particulates, divalent metals clipping the product, etc.)

Lessons Learned (cont.)

- Undetected differences in product impurities may have a significant impact on clinical safety, and possibly efficacy
  - Recall: Leachables acting as adjuvants and tungsten leachables were both correlated to a neutralizing immune response, which is a serious adverse event
- Therefore, a greater emphasis on leachables testing seems warranted in order to reduce clinical uncertainty and minimize patients' exposure to unnecessary risks
  - Recall: The relationship between a quality attribute and clinical safety and efficacy is generally not well understood for biologics (e.g., structure/function/activity relationship may not be fully known)

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Thank you!

Questions/Comments?