Risk Management Strategies for Safety Qualification of Extractable and Leachable Substances in Therapeutic Biologic Protein Products

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Abstract

Extractables and leachables (E&L) are chemical entities, which can be released into intermediate material or final therapeutic biologic protein product at various times during upstream and/or downstream manufacturing steps, packaging operations and/or storage. These substances may pose a safety risk to the patient by causing toxicity, carcinogenicity, immunogenicity and/or endocrine dysregulation. They may also alter product physico-chemical properties via direct interaction with the active pharmaceutical ingredient or, indirectly, by interacting with the excipients in product vehicle, thereby adversely affecting the product quality. Current paper will address a risk-based approach to conceptualizing, evaluating and executing identification and characterization of E&L along with regulatory considerations regarding the impact of these impurities on product quality, patient safety and clinical efficacy. Selected case studies are presented and discussed.

Introduction

Extractables (E) are defined as chemical entities that can be extracted from components of a material by exertion of an exaggerated force (e.g., organic solvent, extreme temperature, ionic strength, pH, contact time, etc.). Leachables (L) are defined as chemical entities that can migrate from product-contact and/or non-product contact surfaces into a process stream, bulk drug substance, product intermediate and/or final drug product under specified conditions of production, storage and use. While leachables are considered a subset of extractables, they can also be derived by chemical modification of the original extractable component. In addition, not all substances identified as extractables

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will be detected as leachables given that widely opposing extraction conditions are utilized for isolation of each set of compounds. Evaluation of extractables is usually considered an essential step in the accurate prediction of leachables as well as in selection of adequate in-process equipment and/or final container/closure system employed in production of a given biologic product (Figure 1). In general, leaching can occur at any of the multiple steps comprising a manufacturing process. Such include but are not limited to upstream operations (e.g., media preparation, fermentation); downstream operations (e.g., concentration/buffer exchange, purification); formulation/fill; packaging operations and long-term storage of the product throughout its expiry period.

Evaluating Extractables and Leachables

Potential sources of E&L include materials used in the manufacturing, packaging, storage, filtration and transfer systems. These include but are not limited to components which are in direct contact with the process fluid or product such as single use/stainless steel bioreactors, bags for intermediate and long-term storage, containers, filters, transfer tubing, elastomeric closures, ampoules, vials, syringes, bottles, etc. In addition to materials in direct contact with the product, secondary packaging components, which are non-product contact (e.g., cardboard containers, overwraps, overseals, container labels) can also be the source of leaching. For example, ink, epoxy adhesives and organic solvents originating from container labels have been detected in products packaged in prefilled syringes. Regarding their chemical nature, E&L are diverse compounds which may include but are not limited to phthalates (i.e., plasticizers), nitrosamines, vulcanizing agents, accelerators, silicone, organic acids, hydrocarbons, cyclic esters derived from urethane adhesives, anti-oxidants, residual solvents, antistatic agents, cleaning agents, residual metals, inorganic compounds (e.g., metal oxides), etc.

Quality Risk Management Approach

The presence of leachables during any stage of the production process or storage may pose a safety risk due to their potential to cause toxicity, carcinogenicity, immunogenicity and/or endocrine dysregulation [1, 2, 3, 4, 13]. In addition, these substances may adversely impact the physico-chemical characteristics (e.g., via aggregation, oxidation, degradation, formation of particulates, etc.) of the final protein product [5]. Furthermore, there are concerns that leachables may pose a risk to cell viability during storage of live cells or during cell culture fermentation (e.g., in single use bioreactors) likely negatively affecting product yield and product quality characteristics. It is of note that biologic therapeutics may be especially susceptible to impact of chemical leachables due to their (1) large size (e.g., in the KDa range) and complex structure (e.g., secondary, tertiary, quaternary); (2) extensive surface area and high frequency of potential sites of interaction; (3) route of administration (i.e., most are sterile injectables) and dosing/volume (i.e., may be dosed at mg/ml and at relatively high volumes); and (4) because proteins may be more efficient in solubilizing leachables compared to small molecules due to abundance of both hydrophilic and hydrophobic sites.

Understanding of the system suitability criteria that are capable of defining and controlling E&L as critical quality attributes built into the design space is of paramount importance to ensure continuous production of high quality therapeutic biologic products with desired efficacy and minimal safety adverse events. For these reasons, it is recommended that drug product manufacturers perform a risk-based analysis as part of E&L evaluation taking into consideration product quality parameters as they relate to product safety and efficacy. The following factors may provide predictive parameters for identifying, evaluating and mitigating risks to critical quality and safety attributes (note that the factors are not ranked in the order of importance):

- Toxic potential of studied E&L including synergistic and/or additive acute affects as well as chronic toxicity
- Drug dose, mode and frequency of administration (e.g., many biologic therapies are presented as sterile injectables likely administered frequently at relatively high volumes and doses whereby higher (i.e., unacceptable) levels of leachable impurities may be delivered)
- Prior clinical exposure to a particular leachable
- Level of risk for adverse impact on product quality (e.g., may need to be assessed on a case-by-case basis as biologic products and respective formulations likely have different susceptibilities to changes in the product due to interaction with leachables)
- Surface area of contact and duration of contact between material component and process fluid, product intermediate or final drug product
- Process fluid storage temperature (i.e., leaching will be exacerbated at elevated temperatures; e.g., 37°C vs. – 196°C)
- Type of the processed/stored material (e.g., purification buffer vs. formulated Drug Product)
- Position in the process stream (e.g., upstream vs. downstream operations; typically the risks are greater as production moves closer to the finished product as opportunity to clear potential impurities is diminished)
- Type of construction material in use (e.g., PVC containers, bags or tubing are at high risk for leaching di(2-ethylhexyl)phthalate, which has been shown to exert various types of toxicities to liver, testis, mammary, nerve, immune system, blood and fat tissue)
- Formulation type whereby a number of factors may be used to predict the risks for leaching. For example, liquid formulations are in continuous drug product-contact with elastomeric closure and/or container material compared to lyophilized ones and therefore at higher risk; formulation excipients due to interaction with leachables can jeopardize product quality [note case study #5]; pH of the formulation buffer may be important where alkaline solutions are thought to exacerbate leaching, etc.).
- Therapeutic necessity of the drug where higher levels may be tolerated if a given drug is considered part of essential therapy [1]

Analytical Characterization of E&L

Detection, identification, characterization and quantitation of leachables could be challenging since these substances represent diverse chemical classes of organic and/or inorganic compounds that co-exist in complex mixtures at trace amounts [6]. Well designed extractables studies are important to provide an analytical roadmap and to identify early warnings signs regarding leachables released during up-stream and/or downstream operations and/or in storage (Figure 1). In situations where extractables are anticipated to adversely impact the physico-chemical/biological properties of a protein, characterization studies spiking the extractables into the product may be of value in assessing risks to product quality.

Product manufacturers may choose to rely on the vendor of particular material component as a starting point for general information on the extractable substances. Such data are generated using model extraction solvents and exaggerated and/or exhaustive conditions of vendor's choice. In general, extraction studies are initiated by selecting appropriate exaggerated and/or exhaustive

Figure 1

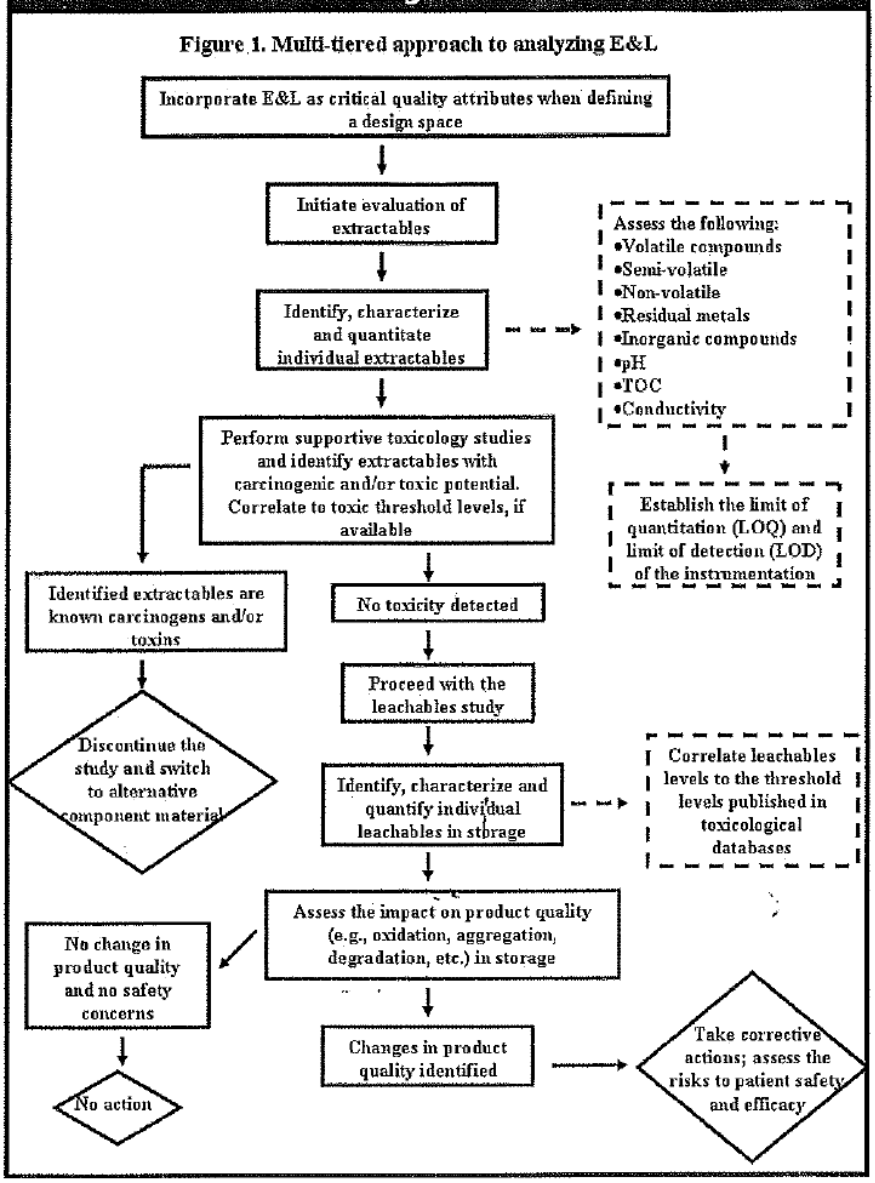

Figure 1. Multi-tiered approach to analyzing E&L

conditions not stipulated for manufacture, storage and/or use in order to isolate chemicals from relevant material components. It is recommended that the extraction studies employ worst-case conditions with regards to pH, ionic strength, contact time, temperature, surface area-to-volume ratio and, if applicable, with organic solvents of varying polarity (i.e., from highly polar to non-polar) as extraction media. In cases where material component under examination is not compatible with organic solvents, aqueous media may be used. Use of detergent (i.e., polysorbate 20)-containing extraction media is also recommended.

Process fluid representative of the actual process (e.g., cell culture media, Drug Product formulation buffer, etc.) should be used as a representative extraction solution in addition to others listed above for accurate prediction of extractables. In the event that representative process fluid is not applicable (e.g., due to interference with the analytical method), a well justified surrogate solution closely resembling the original is considered an acceptable alternative. Extraction study may be performed using a soaking or a recycling method for a given contact time (e.g., 180 days), under accelerated temperature conditions (e.g., 40°C) and preselected surface area-to-volume ratio, while exhaustive conditions may involve more stringent temperatures (e.g., autoclaving a material component for a given time period, [7]). It is of note that small volume components (e.g., bags, containers, etc.) have a greater solution-contacting surface compared to large volume components. In general, they can be expected to generate higher level of extractables and therefore represent the worst-case scenario with regards to surface area-to-volume ratio. Consideration should also be given to selecting the appropriate sample size (i.e., number of bags, filters, containers, elastomeric closures, etc.), which is greater than one in order to achieve accurate representation of the sample population.

Testing of extractable and leachable substances typically includes the analysis of non-volatile, semi-volatile and highly volatile organic compounds as well as analysis of trace light and heavy metals. In addition, some less specific tests such as pH, conductivity and analysis of total organic carbon (TOC) may be performed. It is of note that TOC analysis can not be used on extract solutions that contain carbon. Highly selective analytical techniques should be employed for detection, characterization and quantification of these chemicals. Such include but are not limited to High Performance Liquid Chromatography coupled with Mass Spectroscopy (HPLC-MS), Gas Chromatography coupled with Mass Spectroscopy (GC-MS), Inductively Coupled Plasma with Mass Spectroscopy (ICP-MS), Proton Nuclear Magnetic Resonance (1H-NMR), Fourier Transform Infrared (FTIR) Spectroscopy and Atomic Spectroscopy (e.g., atomic absorption, atomic emission spectroscopy). For example, non-volatile compounds can be analyzed with HPLC-MS; whereas, highly volatile and semi-volatile organic compounds can be resolved using GC-MS. Alternative methods, such as ICP-MS, can be used for detection of residual metals. Emerging analytical technologies with appropriate sensitivity and specificity should be considered in addition to the currently available methods in design of E&L characterization studies.

Evaluation of extractables should be complemented with assessment of leachables. In addition to monitoring the substances that are leaching under recommended conditions of use and storage, leachables studies may be designed to identify interactions and the resulting effect of such interactions on the in-process material and/or on the product under accelerated conditions. In many cases, Drug Substance and Drug Product stability studies should be used to support conclusions regarding the impact of these substances on product quality over time. This is particularly important in situations where there is no downstream purification step that could eliminate the impurities such as in the final formulated Drug Product.

In addition to determining the chemical identity, quantity and composition of E&L and the impact on process fluid/product quality, E&L should be assessed for their cytotoxicity (e.g., USP chapter <87> [8]), acute toxicity in animals (e.g., USP chapter <88> [9]) as well as chronic toxicity. The chronic toxicity data may be especially helpful in ensuring that safety and clinical efficacy are not adversely affected in patient population which is treated for extensive time-periods (e.g., lifetime treatment) and thereby subjected to chronic exposure of impurities. For acute threshold levels, publically available literature sources such as ICH Q3C(R3) [10] or Product Quality Research Institute Leachables and Extractables Working Group [11], which stipulate the safety threshold levels for such impurities may prove especially useful.

Case studies

Case study #1

Please note that this case study was previously published in [12] with the aim of the current paper to provide an update on the corrective actions taken. A therapeutic protein product was changed from a lyophilized to a liquid presentation. Due to this change, a divalent metal cation migrated from the rubber stopper into the Drug Product vehicle. The released metal cation activated a metalloprotease (a process-related impurity that co-eluted with the active pharmaceutical ingredient) causing N-terminal degradation of the product. The problem was uncovered during stability studies under inverted conditions and was resolved by adding a chelator (i.e., ethylenediaminetetraacetic acid, EDTA) to the Drug Product formulation. Unfortunately, the new formulation was associated with adverse safety outcomes recognized by an increase in cardiovascular events as well as changes in the pharmacokinetic properties of the drug. This formulation was consequently withdrawn from the market and replaced with the original one. The leaching of divalent metal cations was mitigated by implementing a modification in the elastomeric closure, which is now coated with Teflon.

Case study #2

For another protein product, human serum albumin (HSA) was replaced with polysorbate as a critical excipient while keeping the same container/closure system (i.e., pre-filled syringe). Associated with this change, bromine from the coated bromobutyl plunger stopper and tungsten from the syringe needle were found by ICP-MS. Both impurities can be powerful oxidants but the impact on product oxidation and aggregation were inadequately monitored. It is of note that methionine is present as an excipient in the formulation, which may be critical in mitigating the possible damage due to bromine and tungsten. In order to identify the risks to product quality the sponsor was asked to, evaluate the effects of bromine and tungsten on Drug Product quality both individually and in combination using robust analytical methods that included orthogonal methods for monitoring protein aggregation over the shelf life.

Case study #3

This case study pertains to a change in the material of construction of the closure system from latex to chlorobutyl rubber stopper for a lyophilized product. As a result, butylated hydroxytoluene (BHT), a common antioxidant and food additive, leached from the stopper and was uncovered at the 12-month stability time point using Reversed Phase High Performance Liquid Chromatography (RP-HPLC). The leachable (i.e., BHT) was quantified and measured levels proved to be extremely low and moreover significantly below the LD50 values established for BHT in animal models. This alleviated concerns associated with adverse effect on patient safety. In order to assess the impact to product quality in storage, the Sponsor performed additional stability-indicating assays and found no other anomalies in product physico-chemical parameters. Furthermore, additional studies evaluating E&L are being performed with results currently underway. Finally, in order to control and monitor the level of the leachable, it was recommended that an acceptance criterion for BHT be established.

Case study #4

This example involves a change from vials to staked needle prefilled syringe. Due to this change, organic solvent from partially dried epoxy glue used for needle attachment to syringe barrel leached into the product and caused an increase in protein oxidation followed by aggregation via disulfide switching. The problem was resolved by allowing syringe barrels to dry for 6 months prior to use.

Case study #5

In this case study there was a change from molded to tubing glass vials, which resulted in the leaching of aluminum oxide produced as a by-product of the new glass vial manufacturing process. Due to this change, phosphate in the formulation buffer interacted with aluminum forming aluminum phosphate crystals. The problem was observed as an out of specification (OOS) result for visible particulates of up to 150 µm diameter in size in samples that were allowed to age for more than 12 months with no changes in other physico-chemical parameters. A variety of analytical methods was used to characterize the particulates including Scanning Electron Microscopy, Fourier Transform Infrared Spectroscopy and X-Ray Diffraction. The OOS result led to a recall of the lots that failed the acceptance criterion. The issue with leaching was resolved by coating the glass vials with silicone using a baked-on siliconization process.

Summary

Biologic protein products can be very sensitive to seemingly minor impurities and changes in the container/closure system and/or formulation composition. Undetected differences in product impurity profile may have a significant impact on clinical safety and efficacy parameters such has been reported in the case of leachables acting as adjuvants triggering immune response [13]. Presented case studies illustrate that corrective actions should employ a simplest approach to resolve a problem with a least potential to alter product quality as it relates to safety and efficacy.

Acknowledgements

The author gratefully acknowledges Drs. Barry Cherney and Steven Kozlowski from OBP, CDER, FDA for critical review of the manuscript. The author also thanks members of the Division of Therapeutic Proteins, OBP, CDER, FDA for helpful discussions and for providing the case studies.

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