Extractables and Leachables

Challenges and Strategies in Biopharmaceutical Development

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The 13th WCBP CMC Strategy Forum on extractables and leachables was held in Bethesda, MD, in January 2008. The purpose of this forum, cosponsored by CASSS (an international separations society) and the FDA, was to discuss questions related to extractables and leachables in the context of biopharmaceutical manufacturing and find consensus on some of those topics. Morning sessions began with "Extractables and Leachables: Challenges and Strategies in Biopharmaceutical Development" with program cochairs Stacey Ma of Genentech, Inc., Ingrid Markovic of FDA CDER, Edwin Moore of Baxter Healthcare Corporation, and Susan Yu of FDA CBER, FDA. "Analytical Tools for Testing Extractables and Leachables" followed, with session chair Edwin Moore. Presentations included

- "Extractables and Leachables: CBER Perspective," by Susan Yu
- "General Concepts in Leachables and Extractables," by Dennis Jenke of Baxter Healthcare Corporation
- "A Strategy for Developing Analytical Methods and a Database to Address the Questions of Extractables," by Jim Castner of Bristol-Myers Squibb Medical Imaging.

Susan Yu defined extractables and leachables. *Extractables* are chemicals generated under exaggerated temperature and time conditions in the presence of an appropriate solvent. *Leachables* are chemicals that migrate spontaneously from a container–closure system, packaging components,

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and/or processing equipment under recommended or routine conditions of use and storage. Leachables are often a subset of extractables.

In his presentation, Dennis Jenke pointed out that every packaging system should be suitable for its intended use over its entire market lifetime. He also defined a contact system as any material or set of materials that contacts a final drug product or its associated precursors during the product lifetime, including manufacturing (processing), storage, and administration (delivery). It has been well established that some extractables from indirect contact materials, components, and systems become leachables in a drug product under actual conditions of use; and it is well established that some extractables from indirect contact materials, components, and systems do not become leachables in a drug product under actual conditions of use. Thus, it is not currently possible to definitively and quantitatively establish without scientific investigation which of those two cases will occur for a specific drug product/system. Therefore, a complete and scientifically rigorous safety assessment must include data-based evaluation of both direct and indirect contact components in both immediate and remote contact situations.

Jim Castner discussed the values and challenges of an industry extractables/leachables database and described materials in published literature as well as software-based applications and analytical tools that are being used (or proposed for use) in such an endeavor. He stated that during the early stages of a drug-development program, the scope of analytical methods for monitoring impurities should not be limited to formulation degradants, but extend also to leachables. Additionally, a database containing the chemical, physical, and application properties of potential leachable compounds can be a valuable predictive and diagnostic tool for developing a new drug under the quality by design (QbD) paradigm.

Why Worry?

Patient safety is the first concern in all biopharmaceutical endeavors. Leachables in biological products can affect patient safety by causing toxicity, carcinogenicity, or (through

interactions with the protein) immunogenicity if administered to patients. Materials leached from a container or other contact surface during manufacturing into a product may cause chemical affects such as oxidation or aggregation. They may affect the look of the formulation: its color, for example. Leachables could affect product stability over time, lead to immunogenicity caused by altering the product, or change its potency. Leachables may also affect medical diagnostic tests and assay results for a drug substance or product.

Control of extractables and leachables is also required by regulations. The US federal Food, Drug, and Cosmetic (FD&C) Act refers to adulterated products. Avoiding product contamination from equipment and contact surfaces is required by 21 *CFR* 211.65 (a), 21 *CFR* 600.11 (b), and ICH Q7 (**1–3**). Extractables and leachables from drug product containers and closures are covered in 21 *CFR* 211.94 (a), 21 *CFR* 600.11 (h), and the FDA guidance on container–closures (**2, 4, 5**). They are also mentioned in 21 *CFR* 600.3 (b) (**6**). An understanding of extractables and control of leachables is expected as part of the filing package for a marketing application. Requirements at earlier points (e.g., at IND filing) are less well-defined but should be risk based. Companies can rely on prior knowledge and vendor supplied data for the necessary information.

Morning presentations were followed by a panel discussion with the presenters talking about their presentations and taking questions from the audience.

What are the primary considerations in analyzing extractables for disposable/in-process systems and final container–closure systems? It is important to ensure that the materials you will be working with during product manufacturing and storage cannot pose a leachables problem. Information provided by equipment suppliers may be valuable if used with caution. A supplier of containers or product-contact components should be audited during routine vendor audits. Also, consider the types of extracting solutions your supplier uses and how relevant they are for your product. A contact component that does not pose a problem in one type of product (e.g., a chemical drug) may react differently with a biological.

Drug manufacturers also need to take into account how they are using/processing materials they purchase from suppliers. If something is cleaned or prewashed/pretreated, then extractables data provided by its vendor may no longer be applicable. A vendor also might be unaware of the composition of components it supplies if they come from other raw-materials manufacturers and thus could miss some extractables that might be present.

It is critical to analyze data available for the composition of material yourself. Analyze their manufacturing process and be aware of what goes into each vessel or type of piping in question, and determine whether it will come into contact with your product. For example, you can check existing databases for materials information and use risk assessment to determine what to analyze — whether in the materials themselves or in your product.

A number of things should be taken into account when developing an extractables program. For example, what extractable tests may be required? The *United States Pharmacopeia* lists compendial tests in Chapters <1>, <87>, <88>, <381>, and <661>. Factor in your own worst-case scenarios and specific buffer programs.

When designing an extractables program, be sure you have appropriate methods available to assess possible extractables. Take a thorough but realistic approach. Consider testing at pH extremes, with organic and water-based solvents, and at appropriate temperatures. However, be careful not to overdo extraction conditions that may themselves affect the extractables, particularly thermally labile or volatile compounds. Different conditions are appropriate for different extractables, such as volatile and nonvolatile compounds or thermally labile and stable compounds.

Do not use solvents that can't be analyzed readily using available technologies or those that can affect equipment (e.g., extremely acidic or highly volatile compounds affecting column resins). The length of exposure to an extracting solution also should be considered. Too short a duration might not extract everything that is relevant; too long may induce chemical reactions with materials that would not occur in real-life use and therefore produce irrelevant results.

There is no standard set of techniques for making these decisions. You need to understand the chemistry of the compounds with which you are working to define their analysis. Keep in mind that analytical methods for extractables should be comprehensive rather than for organic compounds only. Some available analytical tools include

- For nonspecific tests, total organic carbon (TOC) analysis, UV absorbance, pH and conductivity measurement, and visual inspection
- For specific testing, gas chromatographic methods (GC/FID, GC/MS, GC/IR), liquid chromatographies (LC/PDA, LC/MS, LC/NMR), Fourier-transform infrared analysis (FTIR) and metals analysis (ICP/MS).

The CMC Strategy Forum Series

The purpose of the CMC Strategy Forum series is to provide a venue for biotechnology/biological product discussion. These meetings focus on relevant chemistry, manufacturing, and controls (CMC) issues throughout the lifecycle of such products and thereby foster collaborative technical and regulatory interaction. The forum committee strives to share information with regulatory agencies to assist them in merging good scientific and regulatory practices. Outcomes of the forum meetings are published in this peer-reviewed journal with the hope that they will help assure that biopharmaceutical products manufactured in a regulated environment will continue to be safe and efficacious. The CMC Strategy Forum is organized by CASSS, an International Separation Science Society (formerly the California Separation Science Society), and is cosponsored by the US Food and Drug Administration (FDA).

Quality Concerns

The afternoon session was titled "Impact of Extractables and Leachables on Product Quality and Clinical Performance (Safety and Efficacy)," with session chair Edwin Moore. Presentations included

- "Relevant Case Studies Illustrating the Safety and Quality Considerations for Extractables and Leachables in Therapeutic Biologics: A CMC Perspective," by Ingrid Markovic
- "Case Examples of Qualification of Extractables and Leachables in Therapeutic Biologic Products: A Toxicological Perspective," by Timothy Robison of FDA CDER
- "Role of Elastomeric Closures in the Degradation of a Lyophilized Product: A Case Study," by Munir Nassar of Bristol-Myers Squibb
- "A Strategy for Developing Analytical Methods and a Database to Address the Question of Extractables," by Jim Castner
- "Extractables and Leachables from Single-Use Systems: BPSA Perspective," by Jerold Martin of Pall Life Sciences.

Ingrid Markovic pointed out the many sources of extractables and leachables. In-process single-use systems include bioreactors, containers and storage bags for product intermediates, intravenous (IV) bags, carboys, filters, tubing, gaskets, valves, rings, purification resins, and so on. Even stainless steel bioreactors and storage tanks can contribute. Primary packaging components that come into direct contact with a drug include vials, syringes (prefilled or not), ampules, and bottles. Closures include screw caps and rubber stoppers. Container liners are also of concern. Secondary packaging components (not necessarily in direct contact with the product) include cardboard containers, overwraps/overseals, and container labels.

Extractables and leachables can be grouped into several groups of compounds. Metals include zinc (Zn), iron (Fe), barium (Ba), cadmium (Ca), aluminum (Al), nickel (Ni), and so on. Fatty acids include stearic acid, palmitic acid, and myristic acid, just to name a few. Cyclic esters come from polyurethane adhesives. Silicone oil is polydimethylsiloxane, for example. Organic solvents include acetone, isopropanol, and others. Accelerators include thiuram, sulfenamide, guanidine, and dithiocarbamate. Antioxidants include butylated hydroxytoluene (BHT), Irganox, Irgafos, and so on. Phthalates such as di(2-ethylhexyl) phthalate (DEHP) are a separate group unto themselves, as are nitrosamines (e.g., diphenylnitrosamines and vulcanizing agents (e.g., Vultac 2). Other groups include polycyclic aromatic hydrocarbons, antistatic agents, and residual cleaning agents. Timothy Robison highlighted the scope of this problem when he pointed out that available scientific literature indicates many hundreds, if not thousands, of individual chemical entities that can appear as extractables/leachables and could be detected, identified, and quantified at similar levels.

Munir Nassar presented a case study: A degradant in a lyophilized product was attributed to a leachable from the vial stopper that was not part of the rubber formulation itself, but likely part of the stopper's reinforcing structure. The chemical that was not initially identified as a leachable migrated out of the stopper as a volatile compound and interacted with the product to form adducts. This incident showed that a company should work with its suppliers to understand the full composition of their materials because not all compounds used during material manufacture may be identified easily as potential leachables.

Jerold Martin introduced the Bio-Process Systems Alliance (BPSA), an organization representing suppliers of disposable process components, single-use systems, and related services to the biopharmaceutical industry. The organization's primary objectives are to encourage and facilitate adoption of single-use systems in biopharmaceutical manufacturing, establish industry consensus guides for the manufacture and use of disposable process components and systems, and communicate industry best practices to biopharmaceutical manufacturers, regulatory bodies, and nongovernment organizations.

Next, the presenters made up a panel to discuss their presentations and questions along with the audience.

What are the primary considerations for analyzing leachables — analytical and stability studies — and assessing their impact on product quality? Before initiating a leachables program, companies can use toxicologists to help define which extractables identified may be important to consider during leachables studies. Based on risk assessment, leachables studies may be performed at any stage of manufacturing (e.g., upstream production, downstream processing, intermediate and/or final storage, and so on). Contaminants are evaluated in process raw material(s), product intermediate(s), drug substance, and/or drug products.

In designing leachable studies, companies should consider using the actual drug product vehicle whenever possible. In addition, because a protein can mask and/or interfere with leachables detection, analysis of leachables also should include a placebo as an additional extraction medium. Specifically for final dosage forms, consider exposing the container–closure system with placebo alone as well as with the protein product throughout the dating period, both inverted and upright. It is becoming an expectation that several stability time points (including end-of-expiry material) be analyzed for leachables and the results provided as part of the stability section in a marketing application.

Some other materials have limited contact periods with a component material (e.g., tubing, filters, and storage bags). The length of contact time with the product, intermediate(s), and/or process raw material(s) should be accounted for in design of leachables studies. For all component materials, take into consideration the appropriate sample size (>1) in light of intralot and lot-to-lot variability. For leachables from component materials, specifically, evaluate leachables in material reuse studies to ensure that your program monitors whether

cleaning affects the results. Cleaning under harsh conditions (e.g., with detergents or organic solvents) may over time alter a material's surface and make it more or less prone to leaching.

You also need to justify which methods you will use for extractables and leachables studies. For example, to select appropriate analytical methods, you require an understanding from extractables studies to ensure that chosen methods are capable to assess leachables in your product. Those studies should include an assessment of sensitivity (limit of detection, LOD and quantitation, LOQ) and potential interference, as well as selection of specific tests for leachables. We recommend qualifying the methods selected for extractables and leachables testing to ensure that they are fit for their intended purpose. The panel of methods as a whole should be able to detect leachables in both drug substance and drug product. Some leachables may be masked by the protein; thus, as noted above, we recommend testing both in its presence and absence.

During the meeting, some questioned why material before and after product expiry should be analyzed for leachables — if product is tested during clinical studies whether the risk:benefit ratio would account for the presence of leachables. However, others argued that it is rare to test material at the end of expiry in a clinical trial, whereby leachables might account for some adverse events and thus should be studied. Also, the tests used to analyze product on stability may not be such that can identify leachables (especially inorganics) or product interactions with them. And it is highly likely that product interaction with contact surfaces over time can influence leachables profiles, increasing the number of leached chemicals and their quantities as a product ages.

Would a public database of common extractables and leachables be useful, and what information should it contain? A public database would allow for early risk assessments during product development and improved selection of process materials, as well as help companies design appropriate clearance processes, cleaning protocols, and length-of-use studies. It could also prevent reinventing the wheel and reduce extractable/leachable study costs. A database could also assist in development of assays that can cover both product and leachables (or a range thereof), ultimately saving time and costs. It would need to include chemical structure, Chemical Abstract Service (CAS) number, partitioning, materials that chemicals can come from, toxicology data, and spectral/analytical properties.

The forum discussed whether the industry should rely on vendors or collaborate as an industry and create a database, indicating that the latter might be more realistic. Further, there was discussion about where such a database would reside and who would fund it. The Forum agreed that a consortium was most likely. The database would need to have some sort of standardized conditions before it could be really useful, but current information might be a good start. Leachables that migrate into identified formulations would need consideration, as well as extractables extracted under specific, exaggerated conditions.

What are the primary considerations for setting acceptance limits? Defining a true "safety threshold" depends on many variables. It often depends on patient population and the amount of safety data available for humans. It is affected by your ability to translate animal data for your particular product if no human-exposure data exists. It also must consider dose regimen and method sensitivity. The LOD may not be suitable for certain toxic compounds.

Consideration of a toxicological safety threshold rarely takes into account nontoxic and/or weakly toxic leachables (e.g., certain inorganics), which can negatively affect product quality. Such leachables can exert their activity through different pathways including protein aggregation, adduct formation, and generation of clipped variants (e.g., by metalloprotease activation), all of which are likely to adversely affect drug safety and efficacy. Leachables threshold levels that trigger changes in product quality may not correspond to the toxic thresholds of such compounds, which emphasizes the need for well-designed leachables studies.

Assessments of safety are based on systemic toxicity, route-specific toxicity (e.g., inhalation and skin injection), and mutagenic/carcinogenic potential, as well as ICHQ3 classification and limits (**7–9**), existing toxicology databases, structural similarity to known molecules, and actual product/leachable toxicology studies (minimally a 90-day duration for chronic administrations). Safety considerations must also take into account the fact that many databases are based on toxic thresholds that do not include subchronic threshold levels, which can affect patients, as could interaction of subthreshold compounds. Therefore, understanding the range and levels of leachable compounds present in a product at the end of its dating period is critical.

How can a risk assessment of extractables and leachables be developed for different phases of drug development? It is important to develop a risk profile early in product development. Define potential extractables using existing knowledge from material manufacturers, leveraging existing in house data, and taking advantage of chemical toxicology classifications (e.g., benzene is class I, acetic acid is class III). Consider patient factors such as disease severity, treatment impact on disease, dose and frequency of administration, and prior clinical exposure to

leachables. Also take into account your previous manufacturing and clinical experiences with similar materials.

When deciding whether you need to investigate an extractable identified from a particular material, consider where in the process it could be released as a leachable (e.g., upstream or downstream). Also important is the ability of purification to remove chemical contaminants and the composition of buffers that could induce leaching from contact materials (e.g., high or low pH, organic content, and surfactants). Other considerations include contact time and temperature and whether an accelerated stability study can help identify product impact leachables early on. Worse-case safety assessments should be carried out for all extractables unless there is absolute assurance that leaching could not occur.

Developing a risk-based profile later in product development involves defining actual extractables that can be extracted from contact components, depending on the level of prior knowledge and data as well as its applicability to actual manufacturing conditions. Extraction procedures should be carried out and those extractables above safety/risk levels focused on for investigation as part of a leachables study. However, as mentioned above, all potential leachables should be investigated on stability, not just those indicated by the extractable studies. It is still unclear how extractable studies can be translated into actual leaching that can occur over time in the presence of a product, especially at the boundaries of recommended conditions for storage and use.

To reduce risk, you can use extractables data to create preextraction procedures (e.g., rinsing) in manufacturing processes to lessen the possibility of leaching. You can develop alternatives to treatments known to induce leachables (e.g., irradiation and ethylene oxide) and reduce risk by developing clearance mechanisms within processes to lower amounts of leachable (e.g., protein A and other resins). Also reduce risk with a quality system focused on vendors of product-contact materials with appropriate specifications to control levels of leachables.

Are biologic therapeutics at a greater risk regarding the impact of chemical leaching? Some factors can both decrease the potential for leachables to occur and increase their impact on a protein product. In general, proteins are more likely to be affected chemically by leachables than are small molecules, but protein manufacturing processes (that is, the chemical nature of buffers and temperatures involved) tend to be milder than those for small molecules.

Several protein attributes increase the potential impact of leachables. Proteins are susceptible to environmental changes due to weak noncovalent interactions that stabilize them in part. They are large and thus present potential surface/attribute exposure to chemicals. Most biologics are injectable products that go directly into a patient's bloodstream or through skin without passing through the gut. Many are dosed in high quantity (mg/mL). Also, liquid drug substances and/or products can be stored for extensive periods, offering more potential for leaching than chemical drugs that are usually dry powders and tablets.

What are the challenges in selecting container–closure and packaging systems, formulation ingredients, and storage conditions to minimize leaching? Biological products pose a number of challenges to leaching from their primary containers. Protein formulations are limited by the nature of delivery — mostly liquid injectables — suitable to keep proteins in solution and stable. Liquid formulations are most able to induce leaching from container–closures; lyophilized injectable products may be more stable and less likely to induce leachables, but they are often seen as a competitive disadvantage. Glass is the most common storage container material for drug products and stainless steel or plastic (e.g., polycarbonate) for drug substances. Each has a distinct leachable potential, which can be surprisingly manufacturer-specific, despite international pharmacopeial standards. Levels of alkalinity and surface components can vary dramatically between glass vendors. Those are particularly an issue for vials because syringe internal surfaces are coated in silicon oil, which protects the inner glass surface from interacting with formulations inside.

Prefilled syringes, however, can contain multiple surfaces with exposure to leachables (e.g., silicon, tungsten, glue, rubber, plastic, or stainless steel). What might be a nontoxic leachable (e.g., silicon, tungsten, and ethylene oxide) in its own right, either directly or by amount, can affect the quality of a biological product. Lot release and stability assays are highly product focused and not usually developed to identify leachables. Manufacturers must consider within-lot and lot-to-lot variability in container–closure leachable levels, especially for trace components that depend highly on process operations and raw materials.

In some ways, biological products have a reduced risk of inducing leaching compared with classical pharmaceuticals. The industry has a lot of clinical experience with liquid formulations. Most modern formulations are at neutral or near neutral pH, which eliminates the levels of salts and buffers known to induce leaching from glass (e.g., citrate). Although therapeutic biologics rarely contain organic solvents, they do often contain detergents (e.g., polysorbate 20 or 80) used as stabilizers, and those may enhance the risk of leaching. Storage of these products is usually either in frozen form (for drug substances) or at 2–8 °C (for drug products). Higher temperatures and light exposure usually should be avoided at all costs.

Start Early for Success

Leachables can negatively affect biological products at various steps during production and/or downstream processing, both at the drug substance and drug product stages. A process altogether devoid of leachables is highly unlikely, so the goal of a well-planned program is to control leachables within predefined limits to ensure that each product has desired quality, safety, and efficacy. Address extractables and

leachables from a very early point in drug development to ensure that all sources and causes are considered throughout the process. This is vital to the success of every biopharmaceutical drug manufacturing process.

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