Recommendations for Extractables and Leachables Testing

Part 1: Introduction, Regulatory Issues, and Risk Assessment

the Extractables and Leachables Subcommittee of the Bio-Process Systems Alliance

Determination of extractables and leachables for disposable manufacturing systems must be addressed as part of process validation when single-use technology is used. The idea that compounds leach into pharmaceutical formulations or process fluids (e.g., buffer solutions and bulk storage) from processing and storage materials is not new or even unique to plastics. All materials have extractables and potential leachables. When properly evaluated, these are easily addressed and rarely lead to disqualification of a disposable component. Ideally, processing methods and equipment are chosen early in the development lifecycle of a pharmaceutical product. The choice should be made by a dedicated team of scientists, quality assurance and/or regulatory affairs (QA/RA) representatives, and validation specialists working in partnership with component and system suppliers.

It is important first to understand the distinction between extractables and leachables. In recent years, industry and regulatory agencies have reached a consensus in concept on the following general definitions:

Extractables: Chemical compounds that migrate from any product-contact material (including elastomeric, plastic, glass, stainless steel, or coating components) when exposed to an appropriate solvent under exaggerated conditions of time and temperature.

Leachables: Chemical compounds, typically a subset of extractables, that migrate into a drug formulation from any product contact material (including elastomeric, plastic, glass, stainless steel, or coating components) as a result of direct contact under normal process conditions or accelerated storage conditions. These are likely to be found in the final drug product.

The distinction is important to understand. Extractables are determined by exposing components or systems to conditions that are more severe than normally found in a biopharmaceutical process, typically using a variety of solvents at high temperatures. The goal of an extractable study is to identify as many compounds as possible that have the potential to become leachables. A positive outcome is one where the list of extractables from a material is sizable. Although it is not expected that many of those extractables will actually leach into the drug product at detectable levels, a materials extractables profile provides critical information in pursuit of a comprehensive leachables test.

However, it is important to note that not all leachables may be found during the extractables survey (Figure 1). For instance, drug formulation components or buffers may interact with a polymer or its additives to form a new "leachable" contaminant that was not previously identified during extractables analysis. In addition, leachables that were not identified as extractables also will be found if the drug product formulation and processing conditions are unique and more severe than the conditions at which extractable tests were performed — or when the analytical methodologies used in the two types of studies are different.

Photo 1: Example of a single-use bag system (SARTORIUS STEDIM BIOTECH, WWW.SARTORIUS-STEDIM.COM)

Regulatory Requirements

There are as yet no specific standards or guidances that reference extractables and leachables from single-use (disposable) bioprocessing materials. Many references that do apply were written to address all processing materials and equipment without regard to the materials of construction. But it is clear that they are sufficiently broad to include leachables.

North American Requirements (United States and Canada): The foundation for the requirement to assess extractables and leachables in the United States is introduced in Title 21

Product Focus: All biologics

Process Focus: Manufacturing

Who Should Read: QA/QC, Product and process development, analytical, and manufacturing personnel

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Level: Intermediate

of the *Code of Federal Regulations* (CFR) Part 211.65, which states that "Equipment shall be constructed so that surfaces that contact components, in-process materials, or drug products shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements" (**1**). This regulation applies to all materials including metals, glass, and plastics.

Extractables and leachables generally would be considered "additive," although it is also possible for leachables to interact with a product to yield new contaminants.

The US Food and Drug Administration (FDA) regulatory guidance for final container–closure systems, though not written for process contact materials, gives direction about the type of final product testing that may be provided regarding extractables and leachables from single-use process components and systems (**2**). The May 1999 guidance document from the FDA's Center for Drug Evaluation and Research (CDER) indicates the types of drug products and component dosage form interactions that the FDA considers to be the highest risks for extractables (Table 1). Drugs that will be administered as injectables or inhalants will have higher levels of regulatory concern than oral or topical drugs. Similarly, liquid dosage forms will have higher regulatory concern than tablets because extractables migrate into liquids more easily than into solids.

In addition, pharmaceutical-grade materials are expected to meet or exceed industry and regulatory standards and requirements such as those listed in the *US Pharmacopeia* (USP) chapters <87> and <88> (**3, 4**). The USP procedures test the biological reactivity of mammalian cell cultures following contact with polymeric materials. Those chapters are helpful for testing the suitability of plastics for use in fabricating a system to process parenteral drug formulations. However, they are not considered sufficient regulatory documentation for extractables and leachables because many toxicological indicators are not evaluated, including subacute and chronic toxicity along with evaluation of carcinogenic, reproductive, developmental, neurological, and immunological effects.

Polymer formulations approved for contact with foods have a list of allowed compositions and additives specifically intended to limit extracted or transferred substances. To the extent that such regulations limit composition, they also allow some prediction of extractables. In the United States, these listings are complex and difficult to use because allowable additives vary by intended use. European Union food-contact requirements are narrower and better defined. However, the predicted extractables profile still should be confirmed. Use of food-contact–listed materials cannot be substituted for determination of extractables.

International Requirements: In the European Union, a related statement to the US 21 CFR 211.65 is found in the rules governing manufacture of medicinal products. The EU good manufacturing practice document states that "Production equipment should not present any hazard to the products. The parts of the production equipment that come into contact with the product must not be reactive, additive or absorptive to such an extent that it will affect the quality of the product and thus present any hazard" (**5**).

The European Medicines Evaluation Agency (EMEA) published a guideline on plastic immediate packaging materials in December 2005 that also addresses container–closure systems and has been used to provide direction for single-use process contact materials (**6**). Extractables and leachables data that must be submitted depends on the nature of an active substance (e.g., solid or liquid) and the route of administration (e.g., inhalation, parenteral, ophthalmic, oral, or topical). If a plastic material is described in the *European Pharmacopoeia* (Ph. Eur.), the level of information necessary for submission is lower. For "nonsolid" active substances, the general requirements include the complete qualitative and quantitative composition of a given plastic material. Data to be included relating to extractables and leachables come from extraction studies ("worst-case leachables"), interaction studies, migration studies (similar to leachable studies), sorption studies (interaction

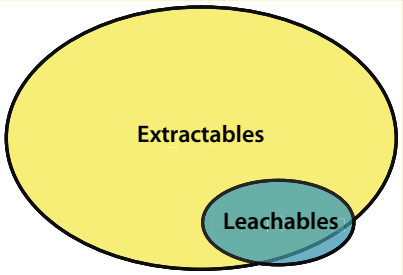
Table 1: Examples of packaging concerns for common classes of drug products

	Likelihood of Packaging Component–Dosage Form Interaction		
Degree of Concern	**High**	**Medium**	**Low**
Highest	Inhalation aerosols and solutions; injections and injectable suspensions	Sterile powders and powders for injection; inhalation powders	None
High	Ophthalmic solutions and suspensions; transdermal ointments and patches; nasal aerosols and sprays	None	None
Low	Topical solutions and suspensions; topical and lingual aerosols; oral solutions and suspensions	Topical powders; oral powders	Oral tablets and oral (hard and soft gelatin) capsules

Part One of Two

The Bio-Process Systems Alliance (BPSA) was created to give greater visibility and a united voice in promoting the benefits of single-use manufacturing. A key focus of BPSA's core activities is to educate and to develop guides that safeguard the quality of drugs produced using this technology. As one of its initiatives, the BPSA Extractables and Leachables Subcommittee is developing a series of documents as a starting point for addressing leachables and extractables in single-use systems. This first of two parts contains information regarding the key definitions and regulatory requirements, and it describes a risk assessment approach with which to initiate an extractables and leachables program. Part 2 will describe a recommended process to evaluate extractables and leachables.

Figure 1: The relationship between extractables and leachables can be illustrated by a Venn diagram. Extractables include known additives, impurities in additives and polymers, and reaction products of materials with extraction solvents. Leachables include known extractables as well as those that are chemically modified by a drug formulation or processing conditions.



between drug formulation and packaging), and toxicological information/documentation.

A GMP guide from the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) considers the impact of equipment on the final product by stating that "Equipment should be constructed so that surfaces that contact raw materials, intermediates, or APIs do not alter the quality of the intermediates and APIs beyond the official or other established specifications" (**7**).

Industry Guides: Extractables and leachables have been addressed specifically for filters, which gives an indication what might be expected for all disposables. The Parenteral Drug Association (PDA) published a technical report to guide users with the selection and validation of liquid-sterilizing–grade filters (**8**). In addressing extractables, it specifies that "It is the user's responsibility to demonstrate that the product does not contain objectionable levels of extractables from the filter. . . . The filter user is responsible for obtaining extractable data for the drug product formulation" (**8**).

The Product Quality Research Institute (PQRI) is a collaborative process that includes members of the US FDA, industry, and academia. In 2006 it undertook a substantial effort to define the appropriate testing procedures and safety evaluations to qualify final inhalation containers for orally inhaled nasal drug products (OINDPs) with regard to extractables and leachables (**9**). OINDPs are considered high-risk products (Table 1), so the PQRI recommendations are not meant to be applied to container–closures for other products such as parenterals — or for biopharmaceutical processing materials. However, the PQRI tested custom formulations of polypropylene resin, sulfur-cured, and peroxide-cured elastomers. Extraction techniques and analytical methods were investigated that advanced the science and can form a basis for successful extractables and leachables programs.

Initiating an Extractables and Leachables Program

Know Your Process: The first step of initiating an extractables and leachables program is to understand your manufacturing process completely from start to finish. A detailed process description is usually derived from research reports, batch records and associated standard operating procedures (SOP), technical reports, and batch analyses, all tools to use in preparation for mapping the process. Discrepancies between those documents should be resolved so that an accurate representation of the process is made. For example, the actual time it takes to fill a bag through a filter may be different from what was originally depicted in an SOP.

Product Contact is defined as contact with any liquid that has the potential to be included in a final product — not only the finished product. This absolutely includes all liquid contact for starting materials and process intermediates. It also may include materials that contact gases or solids, depending on the chemical interactions involved. Materials that may have product contact include but are not limited to

- Tubing and connectors used to transfer starting buffers into a process stream
- Syringes and needles used to transfer liquids
- Sterile and nonsterile filters used to filter the starting buffers and intermediate process formulations
- Bags and tubing used to store and transfer intermediate process formulations
- Tangential-flow (cross-flow) filters used for concentration and diafiltration of process intermediates
- Column chromatography sorbents and chromatography membranes used for concentration and purification of process intermediates
- O-rings used to seal connectors and sanitary fittings.
- Final bulk containers (including closure materials of construction) and filters used before final filling.

If there is no product contact as defined above, it is unlikely that further analysis needs to be performed for a given item. For instance, a connector may have components that are not exposed to an actual product stream, and they may not have to be addressed in an extractables/leachables assessment. However, it is important to note that not all materials need to be in direct contact with a drug product to be of concern. Some extractables and leachables may migrate through a direct-contact layer. An example would be a bag with multiple layers that contain differing materials. Extractables and leachables from the outer layers may migrate into a pharmaceutical formulation. Similarly, adhesives and inks from labels may migrate through the layers of a bag.

Single-use systems often combine individual components into a unique, customized package. For example, the bag system illustrated in Photo 1 could have a bag from Vendor A, tubing from Vendor B, connectors from Vendor C, and a filter from Vendor D. The challenge of an extractables and leachables program is to collect and organize all the available extractables and leachables information for those components, identify what additional information or testing is required, and then set and execute a plan to fill in the gaps.

Risk Assessment

Once all materials that have product contact have been defined, an extractables and leachables risk assessment can be performed. People evaluating the importance of detailed extractables and leachables data should consider certain risk factors. They include materials compatibility,

proximity of components to a final product, composition of the product stream and formulation, surface area of the product-contact components, contact time and temperature, and pretreatment steps.

Compatibility of Materials: Most biopharmaceutical formulations are aqueous-based and therefore compatible with the materials used in most disposable processing components. Still, a check to make sure that the process stream and/or formulation does not violate any of the manufacturer's recommendations for chemical compatibility, pH, and operating pressure/temperature is warranted before proceeding. A full analysis of data generated by the vendor should be completed up front as a preparatory step.

Proximity of a Component to the Final Product: Product contact immediately before the final fill increases the risk of leachables in a final product. Alternatively, tubing or connectors used to transfer starting buffers probably present a lower risk because of their upstream location. Processing steps such as diafiltration or lyophilization that could remove leachables from a process should also be considered because they may reduce associated risk. However, it cannot be assumed that a step that can potentially remove some leachables will remove all leachables. In such cases, supporting data should be obtained.

Product Composition: In general, a product stream or formulation that has higher levels of organics, particularly high or low pH, or solubilizing agents such as surfactants (detergents), will increase the regulatory and safety concern for potential leachables. Neutral buffers lower concern about potential leachables.

Surface Area: The surface area exposed to a product stream varies widely. It is relatively high for filters, in which the internal surface area is 1,000× the filtration area. Conversely, surface area is relatively small for O-ring seals.

Time and Temperature: Longer contact times allow for more potential leachables to be removed from a material until equilibrium is reached. Higher temperatures lead to more rapid migration of leachables from materials into a process stream or formulation.

Pretreatment Steps: Sterilization by steam autoclave and/or gamma irradiation may cause higher levels of extractables and leachables depending on the polymer formulation involved in a single-use component. On the other hand, rinsing may lower the concern for extractables and leachables (e.g., when filters are flushed before use).

One risk-assessment approach was published by the ad hoc Biopharmaceutical Process Extractables Core Team after summit meetings held in 2002 (**10**). Priority is established by assigning numerical risks to each category in ranking materials according to their degree of concern. Numerical risks should have supporting data to justify their numerical assignments. If such data are not available, it would be prudent to assign qualitative risks such as "high" or "low" to allow for more general categorization of risk.

Risk assessment is probably the most important part of extractables and leachables evaluation. Each team must evaluate for itself the level of data collection and testing required to address the regulatory and safety risks for its product-contact materials. Some users are comfortable focusing only on the process steps immediately upstream of the final fill. Others will want to perform extractables and/or leachables testing for product contact surfaces throughout the process. It may be that one risk category (e.g., a product formulation with high organic solvent content) is considered of sufficient risk that a more in-depth evaluation of extractables and leachables for it is appropriate without regard to the other risk categories.

Frequently Asked Questions

Extractables and leachables evaluations are part of a validation program for processes using disposable biopharmaceutical systems and components. All materials have extractables and leachables. There is minimal regulatory guidance that directly addresses extractables and leachables in bioprocessing. Therefore, regulatory and safety risks are minimized by addressing extractables and leachables early in a validation program. The suppliers of single-use systems represent a good place to start to obtain information on extractables. However, it is always the responsibility of a pharmaceutical product sponsor to ensure that leachables and extractables are appropriately addressed.

Who Is Responsible for Conducting an Extractables/Leachables Investigation? Current regulatory responsibility for overall assessment and understanding of a finished product and process components involved in its production remains with the product sponsor. This includes evaluations of extractables and leachables.

Do All Elastomer and Plastic Materials Contain Extractables? Yes, all elastomeric and plastic-based materials contain extractables specific to the formulated and cured material(s) from which they are constructed.

Are Extractable Contaminants Detected from Stainless Steel Systems? Yes, such contaminants can be in the form of residues left after cleaning or traces of metals such as iron, nickel, and chromium salts from the stainless steel itself.

Why Are Additives Used If They Can Become Extractables or Leachables? Can I Get a Polymer That Does Not Contain These Additives? Most commercial polymers would be nonfunctional without the use of certain additives. These stabilize polymers during processing, lubricate them during extrusion, and prevent oxidation and ultraviolet degradation throughout the shelf life of a polymer. They are also used as antistatic agents, impact modifiers, catalysts, release agents, colorants, brighteners, bactericides, and blowing agents. One exception to this rule is found with fluoropolymers, which are typically processed without additives, stabilizers, or processing aids.

Does a Vendor Need a Drug Master File (DMF) or Biologicals Master File (BMF) to Support My Product? A DMF or BMF for process-contact equipment is not explicitly required by US regulatory authorities. However, it represents a way for vendors to share proprietary information about a component or raw material with the FDA and to ensure that such information remains up to date.

How Often Is DMF or BMF Information Added or Deleted? Once the file has been submitted to the FDA,

a vendor is required to update that document annually. Information typical to these files includes a physical description, intended use, chemical composition (including extractables), manufacturing process and locations, and raw material types and grades.

Does the Polymerization Process Used in Manufacturing Affect the Level or Types of Extractable Analytes? Yes, it is now well understood that the choice of polymer or method of polymerization (by heat or chemical means) directly affects the levels and types of compounds found as extractables.

Does the Sterilization Process Used After Manufacturing Affect the Level or Types of Extractable Analytes? Yes, it is now well understood that the levels and types of compounds found as extractable analytes are directly affected by the type and degree of sterilization performed (e.g., gamma irradiation, ethylene oxide gas, or autoclaving).

Can a Supplier Guarantee That No Leachable Will Come Out of a Product That Will Be Deleterious to My Process? Absolutely not. The leachable analyte and concentration that may be of issue to one particular drug formulation may have no impact on another. It is the responsibility of product sponsors to qualify and demonstrate applicability of process components within their manufacturing systems. Leachables are final-product–specific.

What Are the Most Common Ways That Product Sponsors Have Trouble with Extractables and Leachables? It is the responsibility of each product sponsor to fully understand its process, including the potential and possible origins for product adulteration. When a sponsor does not understand (or care to look for) the presence of extractable analytes, it runs the risk of less-than-optimum quality product development, potential safety risks, and probable regulatory delays.

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