Millipore®
Preparation, Separation,
Filtration & Testing Products

White Paper

Moving Forward Together: Industry Alignment to Eliminate USP <88> *In Vivo* Animal Bioreactivity Testing for Polymer Characterization in Pharma Manufacturing

The time for a shift to well-studied, proven *in vitro* cytotoxicity testing is now

Monica Cardona, Global Senior Program Manager, Single Use & Integrated Systems
Janmeet Anant, PhD., Senior Regulatory Consultant

Introduction

In recent years, there has been growing awareness about the environmental and ethical implications of plastics used in bioprocessing manufacturing. While plastics in pharmaceutical processing are common, the animal (*in vivo*) testing involved has been criticized as unnecessary and unethical. This paper shows how MilliporeSigma is implementing *in vitro* cytotoxicity testing for plastic material qualification. These plastic materials are intended for use in biopharmaceutical manufacturing, including bioprocessing systems and filtration.

A recent 2020 United States Pharmacopoeia (USP) communication highlighted a strategy to eliminate animal testing of plastics. This strategy included the removal of the USP <88> Biological Reactivity Test, *In Vivo* classification system for animal testing and re-emphasizes a cell-based alternative approach provided in USP <87> Biological Reactivity Test, *In Vitro*. This communication from USP allowed the industry to justify the shift away from animal testing for plastics used in pharmaceutical and biopharmaceutical manufacturing.

MilliporeSigma is the U.S. and Canada Life Science business of Merck KGaA, Darmstadt, Germany.

MilliporeSigma

How *in vivo* testing came to be

The pharmaceutical industry has used plastics in manufacturing since 1965 and, shortly after, the scientific community realized that the use of such plastics could impact patient health. That year, the United States Pharmacopoeia (USP) published USP XVIII. It assigned plastics to six classes according to the application use of the plastic and the potential patient risk. In 1975, USP XVIII was renamed *Biological Reactivity Tests, In Vivo <88>*, commonly referred to as USP <88>.

In 1990, USP added *Biological Reactivity Tests, In Vitro <87>*, or USP <87>, and the informational chapter *The Biocompatibility of Materials Used in Drug Containers, Medical Devices and Implants USP <1031>*. USP <87> described *in vitro* tests that could be deployed as decision points to determine whether specific plastics required *in vivo* animal testing. The companion chapter provided guidance via a decision tree for testing based on route of administration and duration of exposure. The international version of USP <88> is called ISO 10993-05.

USP <88> Class VI is the most stringent among the *in vivo* testing classes and is only required for implantable medical devices. But, due to the lack of industry standards for material qualification of plastics and polymeric materials used in bioprocess manufacturing, the biopharmaceutical industry adopted the most stringent medical device standard for biological reactivity testing for these polymers. They adopted the USP <88> Class VI standard even though these products are used in the manufacturing of biopharmaceutical drugs, not implanted in the human body, and thus carry a lower risk of potential health effects.

The lack of industry standards for material qualification of plastics used in bioprocess manufacturing led the biopharmaceutical industry to adopt the most stringent medical device standard for biological reactivity testing for these polymers. The time is now to shift to proven in vitro cytotoxicity testing.

Reconsideration on the horizon

This industry practice has led to unnecessary, excessive animal testing. But recognition of this has led to reconsideration of the tests in USP <87> and <88>.

As awareness of the importance of sustainability and ethical standards has increased, USP has proactively initiated the current revisions of USP <87> and <88> to better meet the animal welfare "three Rs" guiding principles of refine, replace, and reduce the use of animals in product testing and scientific research. Based on the current USP <88> revision draft, the classification numbers I through VI will be removed and replaced with Pharma Grade.[1]

The latest scientific feedback occurred on May 31, 2023 and the implementation date for the USP <88> revision is still to be determined. However, in 2021, the first USP standard specific to plastic components, *USP <665> Plastic Components and Systems Used to Manufacture Pharmaceutical Drug Products and Biopharmaceutical Drug Substances and Products*, was developed. USP <665> will come into effect in May 2026. While earlier versions of <USP 665> included biological reactivity testing, the final version does not include any reference to biological reactivity and rather concentrates on chemical characterization. In addition, the recently finalized *USP <661.1> Plastic Materials of Construction* and *<661.2> Plastic Packaging Systems for Pharmaceutical Use* general chapters have been updated to only reference USP <87> for bioreactivity testing.

For regulatory submissions, the bioreactivity testing for the filters and single-use manufacturing systems are typically not included in the electronic common technical document (eCTD) based on ICH M4Q requirements for the control of materials. Bioreactivity testing is only required to be submitted for drug product container closure systems. For a recent 2022 approval of a lentiviral gene-modified hematopoietic stem cell drug product, ZYNTEGLO (betibeglogene autotemcel), manufactured by Bluebird Bio, Inc., the FDA CBER review team accepted cytotoxicity testing for the final bag containing the cryopreserved cell therapy drug product (ZYNTEGLO | FDA). This highlights that even for higher risk applications, the FDA is accepting *in vitro* bioreactivity testing.

Compared to animal testing, chemical characterization of polymers provides far more useful quantitative data to assess patient risk from plastic components used in biomanufacturing. Therefore, chemical characterization should be the first line of testing, making biological reactivity testing superfluous.

It is time to replace animal testing with alternative methods

Biopharmaceutical companies, as well as regulatory authorities, have been focusing on reducing animal testing and improving animal welfare. If no regulatory burden exists, it is considered inhumane to use animals for tests.

In Europe, it is already more difficult to find companies willing to perform animal testing for material qualification of single-use bioprocessing assemblies, compared to the US. Test facilities in Europe have begun to require documentation (501K or MDR) to confirm that the materials to be tested are indeed medical devices and that a true regulatory requirement for the testing exists.

Stopping animal testing improves sustainability as well as improving animal welfare. The three Rs—reduce, refine, replace—have been a focus for many companies and global regulatory agencies, including MilliporeSigma. When companies find and implement opportunities to eliminate

unneeded testing, we are better able to meet our carbon targets and create a more sustainable supply chain.

Cytotoxicity testing with USP 87/ISO 10993-05 is a proven alternative to animal testing. *In vitro* testing is more appropriate for assessing the risk of polymers used in bioprocessing manufacturing. Testing is done in mammalian cell lines. Its results are more objective and quantitative than animal testing, which involves a subjective rating of animal response to plastic. *In vitro* testing is also far more application relevant to bioprocessing manufacturing.

Pioneering approach to biocompatibility testing

MilliporeSigma's goal is to replace all animal utilization with non-animal alternatives. To achieve this, we began with reducing animal testing wherever possible. MilliporeSigma proposed and began advocating to the industry, via the Bio-Process Systems Alliance and BioPhorum, for industry acceptance of the alternate *in vitro* bioreactivity testing method. This proposal is in alignment with the key principles of the USP 3R initiative: replacing animal experiments with alternatives wherever possible, reducing the number of animals used, and refining experiments to minimize impact on animals.

MilliporeSigma has been advocating for this industry change since 2021 and we have implemented the change to USP <87> for all our plastic polymers used in bioprocessing equipment. We expect this change to be complete for all products used in bioprocessing manufacturing by end of this year or early 2024. Here's how we approached this change.

- Even though there is no regulatory requirement, we did a thorough analysis of the risk of the change.
- We embraced a risk-based approach for polymer characterization of materials used in bioprocessing equipment. See Figure 1 below.
- As cytotoxicity characterization by USP <87> can provide more relevant results for cell-based work, we performed USP <87> testing across a wide range of our single-use and filtration products to ensure that our materials that had already passed USP <88> Class VI also passed the *in vitro* test.

We then created this implementation strategy for the change:

- According to our SOP, we only perform biological reactivity testing as part of material characterization when we add new components into our product library and when there is a raw material change where our internal risk assessment deems it necessary.
- This change will thus be applicable to all new products and those undergoing a raw material change significant enough to require bioreactivity testing based on a risk assessment.

Cytotoxicity testing with USP <87> and/or ISO 10993-05 will be used instead of USP <88> and USP <87> *in vitro* testing has been incorporated into MilliporeSigma's Material Qualification SOP. Components that have already passed USP <88> testing do not need to be retested as it is the most stringent test. Data has shown that components that pass USP <87> would also pass USP <88>, proving that this is sufficient testing.

Libraries of components are being transitioned to ISO 10993-05 testing for any new or changed components. Certifications will now specify that components were tested for biological reactivity under one or a combination of USP <87>, <88>, or ISO 10993-05.

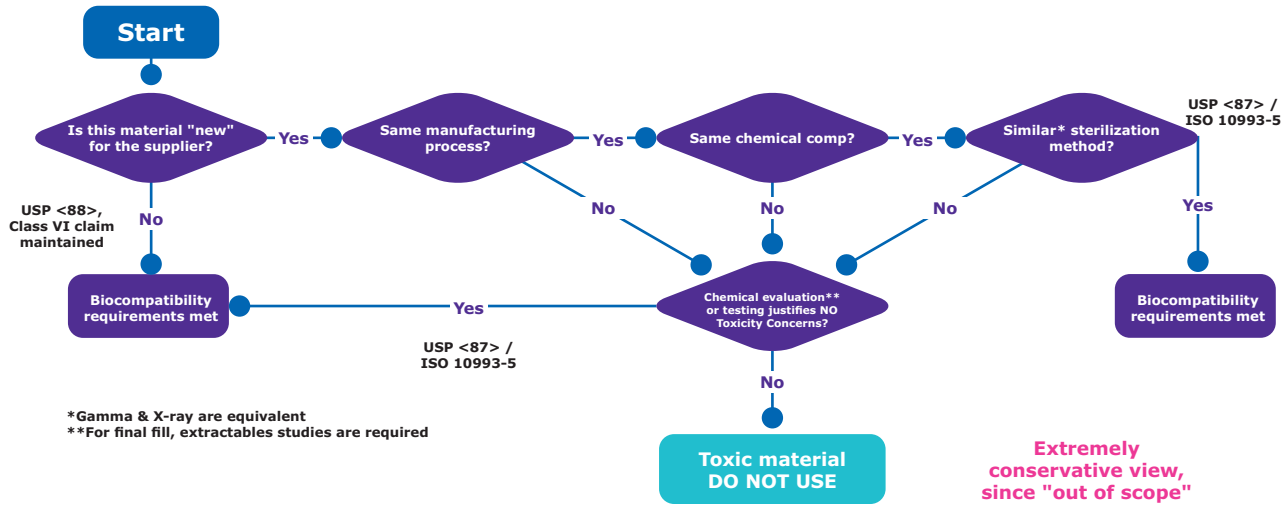

Figure 1. Risk assessment for polymer materials not used before for DS/DP manufacturing. Decision Flow Diagram - based on USP <1031> and ISO 10993-1.

Industry alignment will move us forward together

The first step toward eliminating animal testing is education. It is important to develop communications regarding robust alternative *in vitro* methods to replace antiquated and unreliable animal tests. In addition, updated regulatory and safety standards now make animal testing unnecessary. Building trust among drug manufacturers, suppliers and testing companies will pave the way for change.

The industry tends to be risk averse, and quality systems are slow to change. However, the reality of the impact of animal testing and the availability of better alternatives make the status quo unsustainable. It is no longer acceptable to prioritize "worst case" scenarios over the well-being of animals and the environment.

Even testing companies are questioning whether animal testing of single-use and other plastics makes sense. This is a clear indication that change is necessary, and that the industry should be moving toward more sustainable and ethical practices.

The case for change

All plastics used in pharmaceutical bioprocessing have passed USP <88> testing for over two decades. Plastics used in bioprocessing are already well characterized through a battery of tests. Therefore, the level of animal testing currently expected is unnecessary and out of step with regulatory and safety standards. USP <88> testing is also not aligned with the global governing bodies and regulatory agency initiatives to reduce animal testing as part of sustainability and social responsibility initiatives.

Now is the time for change. Many firms are already following our lead and transitioning away from animal testing in single-use and other plastics. Drug manufacturers are also beginning to request *in vitro* testing in anticipation of this transition. At MilliporeSigma, we are supporting stakeholders transitioning away from USP <88> testing and actively encouraging the industry to employ non-animal testing in determining plastics safety and suitability.

Eliminating animal testing in single-use and other plastics used in manufacturing is an obvious win for all involved. The positive impact across the supply chain for the pharmaceutical industry is significant and will help us reach our sustainability goals. It is time for the industry to align with this important goal and work together to achieve it.

References

1. Slide 6 of https://www.pharma-congress.com/files/userFiles/Presentations/Day1/Compliance-Conference/05_Eakins_Update_on_USPs_Bioreactivity_Extractables_and_Glass_Containers_Packaging.pdf

MilliporeSigma
400 Summit Drive
Burlington, MA 01803

For additional information, please visit
www.Sigmaaldrich.com

To place an order or receive technical assistance, please visit
www.Sigmaaldrich.com/contactPS

Lit. No. MS_WP12673EN
08/2023 Ver 1.0