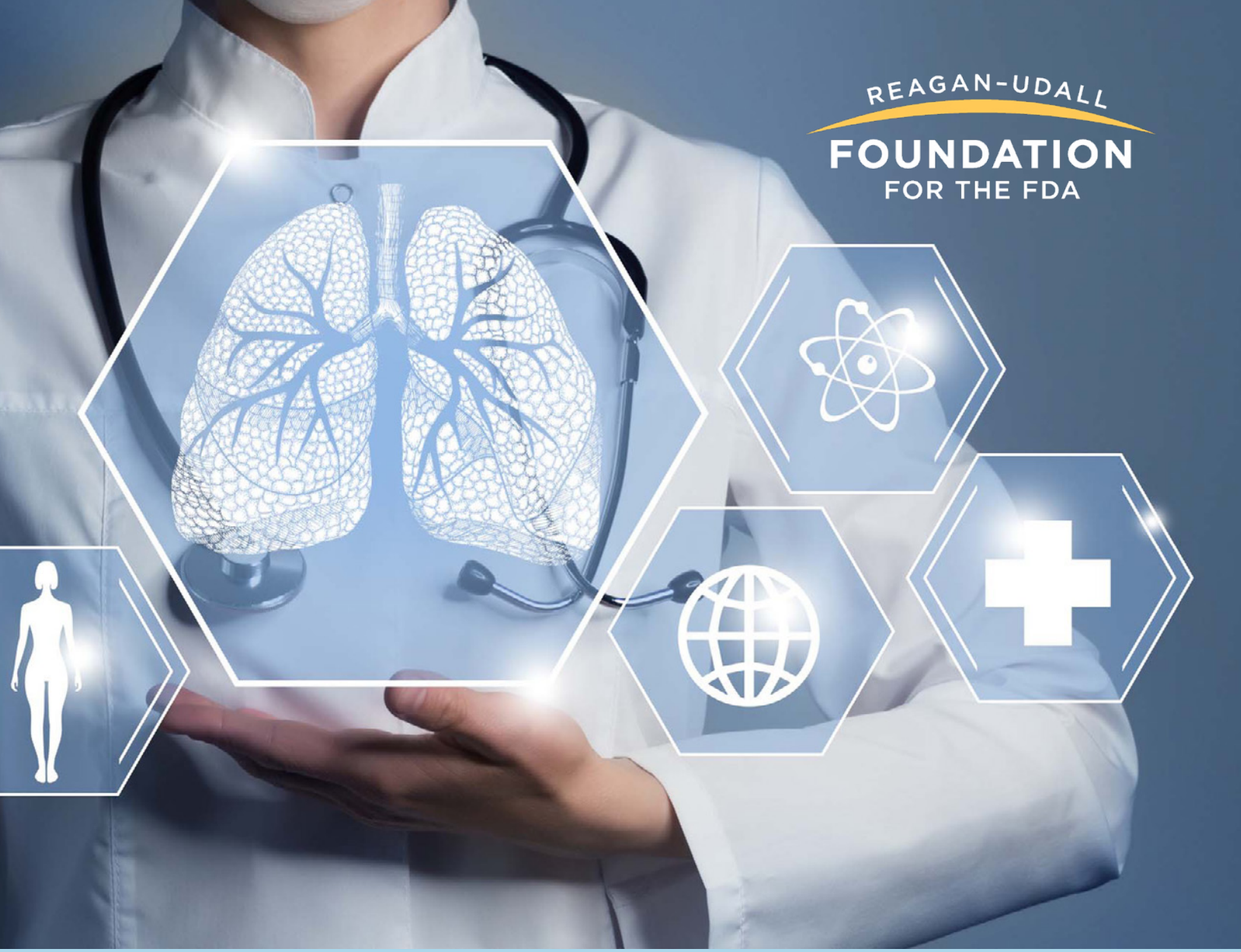


REAGAN-UDALL FOUNDATION FOR THE FDA

Advancing Drug Development by Reducing Reliance on Animal Testing

CASE EXAMPLE: PRECLINICAL ANIMAL MODELS IN LUNG TOXICOLOGY

Summary Report

REAGAN-UDALL FOUNDATION FOR THE FDA

ABOUT THE REAGAN-UDALL FOUNDATION FOR THE FDA

The Reagan-Udall Foundation for the FDA (the Foundation) is an independent 501(c)(3) created by Congress to advance the mission of the FDA to modernize product development, accelerate innovation, and enhance product safety. The Foundation works to advance regulatory science, support development and dissemination of reliable information, and facilitate engagement and information exchange.

Aer Therapeutics, Avalyn Pharma, Inc., Biotechnology Innovation Organization, Charles River Laboratories, Endeavor BioMedicines, Ionis Pharmaceuticals, and VIDA provided funding for this meeting.

Contents

1. Introduction

As the U.S. Food and Drug Administration (FDA) and regulated industry pursue strategic efforts to reduce animal testing, with more efficient use of animal data and the application of scientifically-validated New Approach/Alternative Methodologies (NAMs),[1] the opportunity to collectively explore specific use cases has the potential to accelerate progress. The Reagan-Udall Foundation for the FDA (the Foundation) convened a hybrid public meeting to provide a platform for discussion of recent achievements in reducing animal testing and, with a collective discussion space, to explore reducing reliance on animal testing in lung toxicology.

Using inhalation toxicology as a case study, speakers and panelists at the February 26, 2026, meeting examined limitations of current preclinical lung toxicology approaches and discussed human-relevant alternatives to traditional animal testing, including 1) the FDA-supported NAMs; 2) new/enhanced monitoring strategies; and 3) weight of evidence (WoE) approach to risk assessment to support human safety without hindering respiratory drug development. Researchers, product developers, regulators, and patient advocates also explored opportunities to better align global regulatory expectations and clinical translation. Particular focus was placed on areas where the current regulatory paradigm, especially reliance on rodent inhalation toxicology and assumptions of nonmonitorability, may limit clinical translation and delay patient access to inhaled therapies.

This report presents a summary of the meeting; full meeting materials including presenter slides, video recording, and a meeting transcript are available at https://reaganudall.org/news-and-events/events/advancing-drug-development-reducing-reliance-animal-testing.

> **"NAMs is something where the acronym has had a long persistent life, but what it spells out has not. New Alternative Methods, New Approach Methodologies. And to me, the roadmap picking New Approach rather than Alternative really suggests it's thinking about NAMs in a completely new way. That it's not just a one-to-one correspondence [of] how do we replace what an animal model does, but how do we really explore the powers of these new technologies?"**
>
> **— Dr. Steven Kozlowski, Chief Scientist, FDA**

1 For purposes of this report, the NAMs abbreviation will be used when referring to both New Approach Methodologies and New Alternative Methodologies.

2. Background

Nearly 90% of drugs that undergo animal testing ultimately fail to gain FDA approval.[2] Approximately 30% of these failures are due to safety issues that were not predicted by animal models.[3] By contrast, animal testing and regulatory challenges can prematurely eliminate potentially beneficial drugs because these models can fail to reflect human biology, leading drug sponsors or regulators to halt development before human effects are fully understood.

> **"We don't need perfection. We just need better. We have high death rates for every disease in the space. We can do better, but we also have to strengthen…collective knowledge and understanding. We have to have journals that are willing to publish on new models and not just the standards. We need flexibility so that studies can be done in the U.S.… Our patients miss out when studies are done outside this country, that we have patients internationally, but we have a lot of U.S. patients. When they can't participate, that's a loss not only for the patients, but for the entire community."**
>
> — Teresa Barnes, PF Warriors

Human Respiratory Tract

Inhalation through the nose and mouth results in air flow into the trachea, then moving through the conducting airways of the respiratory tract, and into the smaller pulmonary-alveolar structures. (Figure 1). Pulmonary disease and toxic effects of inhaled substances can occur anywhere along the respiratory tract. Pulmonary research examines how inhaled substances (e.g., droplets, aerosolized particles) interact with the lungs, focusing on where these particles deposit and the potential beneficial or harmful effects resulting from that deposition. (Figure 2)

2 Sun, D., Gao, W., Hu, H., & Zhou, S. (2022). Why 90% of clinical drug development fails and how to improve it? Acta Pharmaceutica Sinica B, 12(7). https://pmc.ncbi.nlm.nih.gov/articles/PMC9293739/.

3 Van Norman, G. A. (2019). Limitations of animal studies for predicting toxicity in clinical trials. JACC: Basic to Translational Science, 4(7), 845–854. https://doi.org/10.1016/j.jacbts.2019.10.008.

FIGURE 1. INHALATION PATHWAY[4]

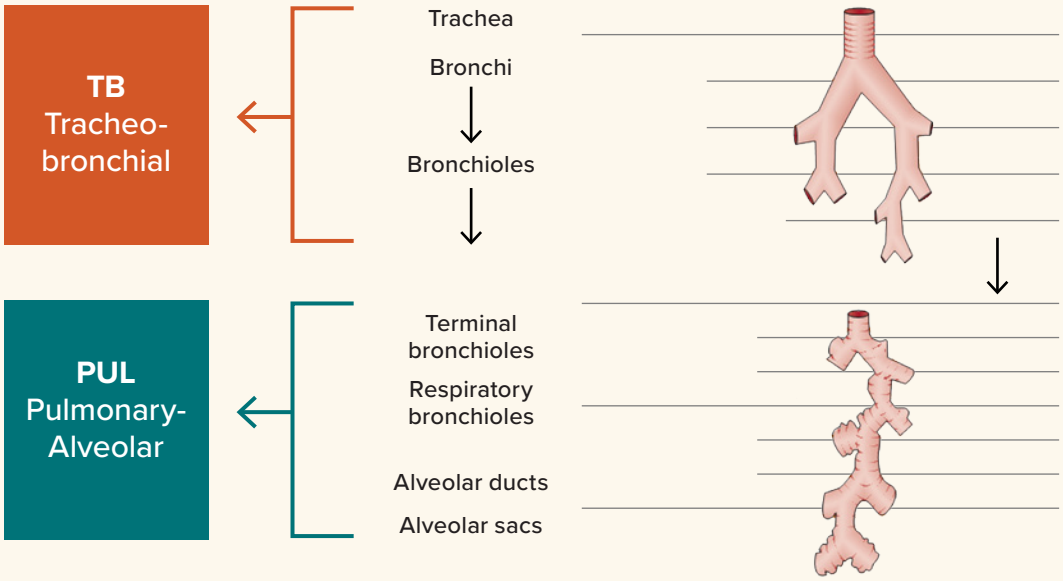
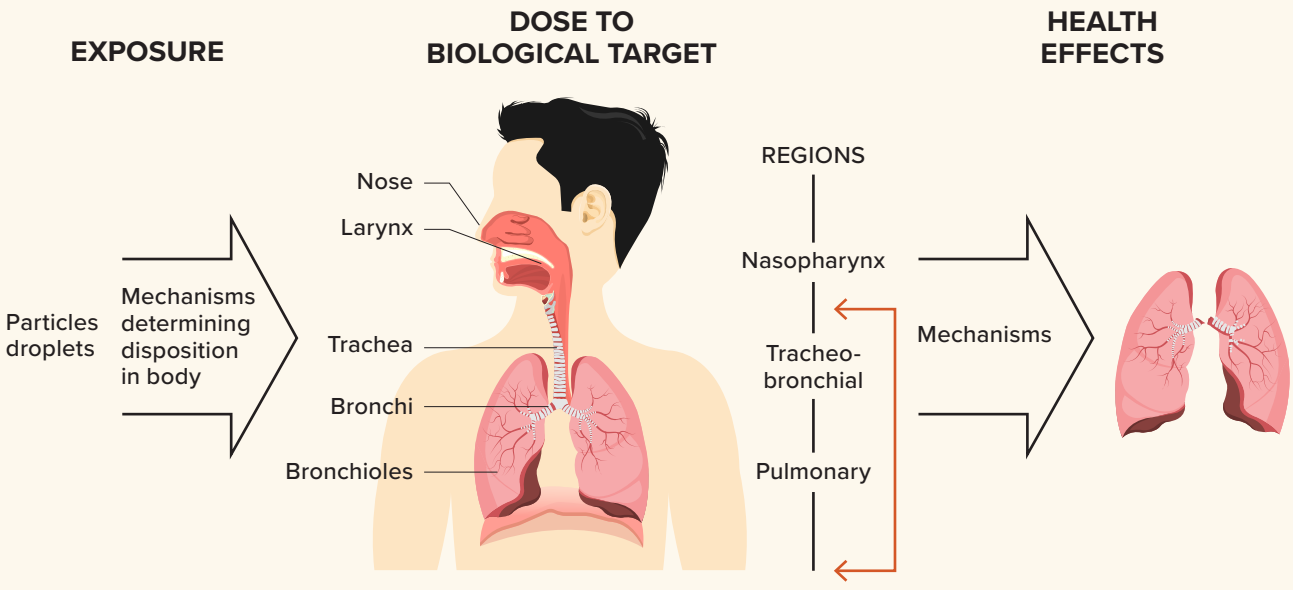


FIGURE 2. PARADIGM OF TRANSLATIONAL DOSE AND HEALTH EFFECT (POSITIVE OR NEGATIVE)[5]



Schematic representation of the major elements of inhalation delivery oriented toward understanding the efficacy or toxicity of pharmaceutical aerosols

4 Reed, M. What are we doing here? Baselining Pulmonary Delivery and Dose Determination in Pulmonary Drug Development; February 26, 2026. https://reaganudall.org/news-and-events/events/advancing-drug-development-reducing-reliance-animal-testing.

5 Adapted from McClellan, R. O., & Henderson, R. F. (1995). *Concepts in Inhalation Toxicology* (2nd ed., p. 44). Taylor & Francis.

Inhalation Toxicology

Toxicology examines where hazard and risk intersect by assessing exposure location (e.g., dose deposition) effects in animal models, and the translation of those doses and effects to humans. To extrapolate animal data to humans, inhalation toxicologists need to account for differences in anatomical structures and cellular processes, including differences in resident and circulating cells. Understanding clinical and nonclinical exposure and dose metrics are essential for inhaled hazard assessment and defining safety margins, given differences in particle deposition, aerosol distribution, and cell-type interactions within the respiratory tract.

> **"We have to understand what dose means so that we are understanding how we impact disease and then how we impact hazard assessment and potentially safety as well."**
>
> **— Dr. Matthew Reed**

Regulatory Science

Regulatory science focuses on developing tools and standards to support the safe and effective development of FDA-regulated products.

Nonclinical safety assessments for inhaled drugs generally follow M3(R2) guidance for small molecules[6] and ICH S6 guidance for biotechnology products.[7] However, because of the unique nature of inhaled delivery and dose determination in inhalation toxicology, peer-reviewed publications serve as de facto guidance for how inhaled doses are calculated in nonclinical studies, how No Observed Adverse Effect Level (NOAEL) doses are established, how human equivalent doses are derived, and what safety margins are typically expected by FDA/Center for Drug Evaluation and Research's (CDER) Division of Pulmonary, Allergy, and Critical Care Products (DPACC) for clinical doses.[8]

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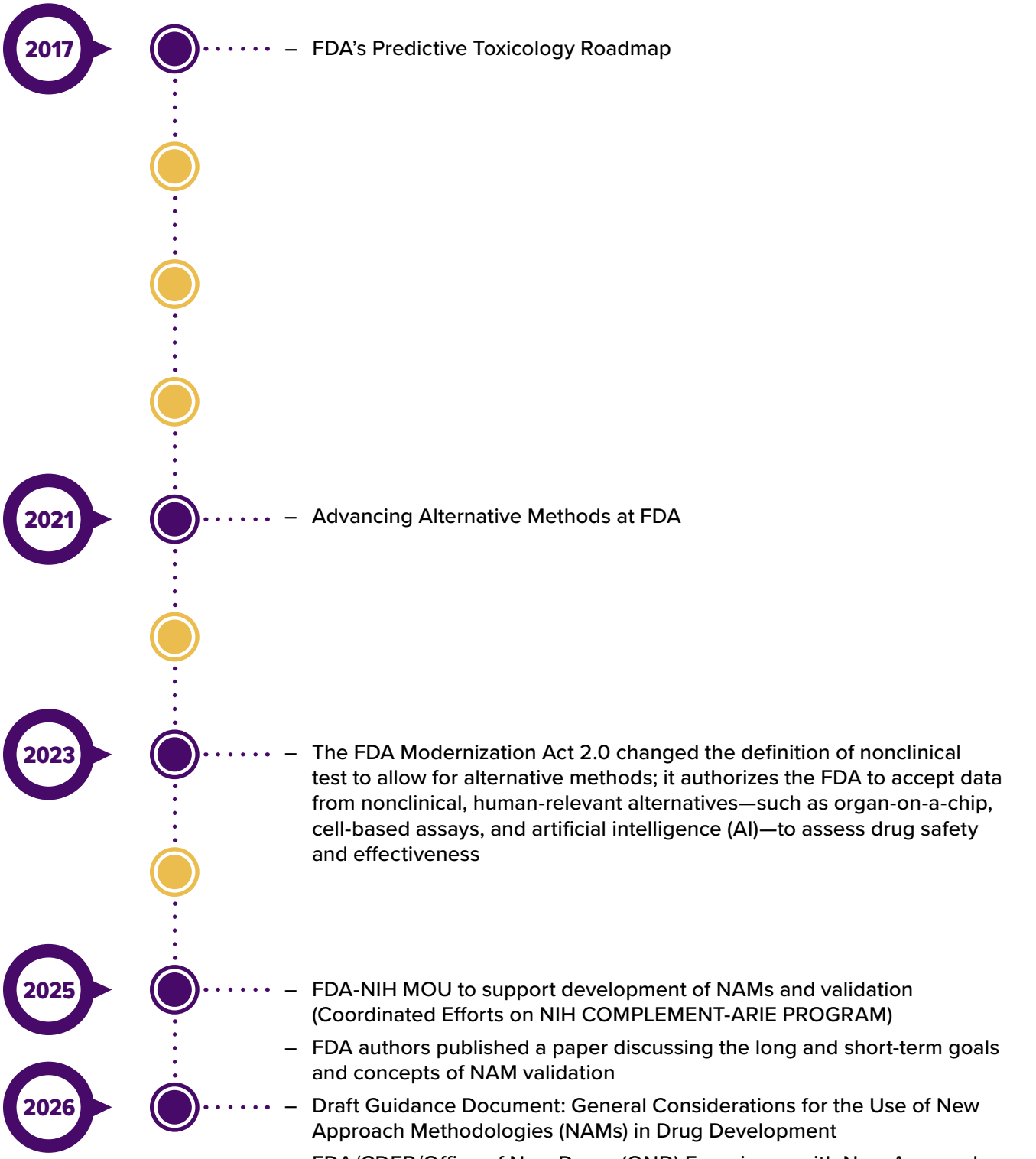
6 U.S. Food and Drug Administration. (January 2010). Guidance for Industry: M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals. www.fda.gov. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/m3r2-nonclinical-safety-studies-conduct-human-clinical-trials-and-marketing-authorization.

7 European Medicines Agency. (2018, September 17). ICH S6 (R1) preclinical safety evaluation of biotechnology-derived pharmaceuticals — scientific guideline European Medicines Agency. https://www.ema.europa.eu. https://www.ema.europa.eu/en/ich-s6-r1-preclinical-safety-evaluation-biotechnology-derived-pharmaceuticals-scientific-guideline.

8 Tepper, J. S., Kuehl, P. J., Cracknell, S., Nikula, K. J., Pei, L., & Blanchard, J. D. (2016). Symposium Summary. International Journal of Toxicology, 35(4), 376–392. https://doi.org/10.1177/1091581815624080.

Figure 3 summarizes agency documents, guidance, and actions related to NAMs, (these are broad documents that encompass but are not specifically directed to inhalation toxicology).



3. Current Practices and Challenges: Implications for Pulmonary Drug Development and Patient Access

Speakers and panelists examined the historical value, limitations, and translatability of animal models in predicting human safety for pulmonary drugs. Presentations included the current science in nonclinical safety assessment, including the rationale for animal model use and where limitations are most evident. Participants shared their experiences to help us understand how current regulatory and scientific considerations are shaping drug development decisions.

The Current State of Determining Clinical Dose from Nonclinical Safety Studies

For inhaled drugs, "dose" is inconsistently defined, leading to confusion and misunderstanding.[9] Across animal species, nonclinical inhalation dose relies on the same principles that control human drug dose and exposure, including administration systems, characterization of aerosol concentration, particle size, deposition, lung anatomy, and airflow. This framework has remained largely unchanged for over 50 years.

The NOAEL is ''the highest dose level that does not produce a significant increase in adverse effects in comparison to the control group''[10] and is the primary outcome of good laboratory practices (GLP) toxicology studies. In most nonclinical studies, the NOAEL is driven by histopathological observations.

NOAEL vs. NOEL

- NOAEL indicates the highest dose *without harmful (adverse) effects*, allowing for minor, nonharmful changes
- NOEL (No Observed Effect Level) indicates the highest dose with *no detectable changes* at all

There is no globally accepted definition of adversity, although the European Society of Toxicological Pathology defines it as the impairment of function to maintain homeostasis[11] while the U.S. Society of Toxicological Pathology defines adversity as indicating harm.[12] Importantly, the NOAEL should only refer to the specific studies being evaluated and should not include theoretical human patient extrapolations and issues related to clinical nonmonitorability. Theoretical considerations, such as these, can be addressed by increasing the safety

9 Ibid.

10 U.S. Food and Drug Administration. (July 2005). Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. www.fda.gov. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/estimating-maximum-safe-starting-dose-initial-clinical-trials-therapeutics-adult-healthy-volunteers.

11 Palazzi X, Burkhardt JE, Caplain H, et al. (2016) Characterizing "Adversity" of Pathology Findings in Nonclinical Toxicity Studies: Results from the 4th ESTP International Expert Workshop. Toxicol Pathol,44(6):810–24. doi: 10.1177/0192623316642527.

12 Pandiri AR, Kerlin RL, Mann PC, et al. (2017) Is It Adverse, Nonadverse, Adaptive, or Artifact? Toxicol Pathol,45(1):238–247. doi: 10.1177/0192623316672352.

factor; however, the current regulatory algorithm used by DPACC does not specify where to incorporate these factors, which can make it more difficult to distinguish the NOAEL from other considerations that may influence clinical dose selection.

Dosing of inhaled drugs is guided by conservative safety margins. DPACC recommends minimum safety margins based on drug burden when normalized for body or lung weight. Default margin values of 10 for mice and rats, 6 for dogs, and 5 for monkeys are used when determining the maximum clinical dose. These margins reflect:[13]

- **High risk:** The FDA classifies oral inhalation, along with injections, as having the highest risk among drug administration methods leading to challenges with clinical monitorability
- **Unique Toxicity Concerns (nonmonitorable effects):** Inhaled drugs may cause lung inflammation or tissue damage that is difficult to monitor in human clinical trials, necessitating conservative dose calculations distinct from preclinical starting doses.

Historically, regulators have considered many histologic lung findings nonmonitorable; however, advances in clinical imaging, particularly high-resolution CT, challenge this assumption and demonstrate that a number of these effects can be detected and longitudinally monitored in humans.

Current Challenges: Scientific and Technical Limitations of Nonclinical Animal Lung Toxicology

TERMINOLOGY

Current terminology is not standardized and causes confusion. Terminology used in Figures 4 and 5, for example, is used and interpreted differently by different stakeholders. Outcomes terminology also has changed over time. For example, publications in the 2010s refer to “respiratory sensitization” but earlier studies utilize the term “extrinsic allergic alveolitis” making it unclear whether authors were discussing irritation or respiratory sensitization.[14] These inconsistencies limit clinical translatability, monitorability, and assessment of progression or reversibility across species, underscoring the need to curate and harmonize legacy data.

DOSE DETERMINATION

I) Translating inhaled (or lung-deposited) dose from animal models to humans remains challenging. Current DPACC practices emphasize rigid determination of maximum clinical dose based on “nominal” device loads and animal NOAELs, with limited consideration of the drug delivery factors or translatability in humans.

II) Figures 4 and 5 illustrate the divergence between the FDA guidance on maximum recommended safe *starting* dose vs. current DPACC practices of setting a maximum *clinical* dose, respectively.

Reliance on predefined deposition fractions has notable limitations. Deposition fractions in animals from aerosolized drugs are derived from research published in the 1980s and 1990s. Certain assumptions may no longer be valid given advances in particle engineering, delivery device optimization, and *in silico* modeling, which could better inform realistic deposition factors and subsequent safety margin calculations. As an

13 Fahy, JV. Monitoring drug-induced lung toxicity in patients; February 26, 2026. https://reaganudall.org/news-and-events/events/advancing-drug-development-reducing-reliance-animal-testing.

14 Maertans, A. From Environmental Health to Precision Medicine; February 26, 2026. https://reaganudall.org/news-and-events/events/advancing-drug-development-reducing-reliance-animal-testing.

example, the Multi-Path Particle Dosimetry (MPPD) model can compute deposition factors for humans and five laboratory species. The U.S. Environmental Protection Agency (EPA) has endorsed this as the primary tool to determine deposition in different species. Additionally, inhaled dose estimations do not always adequately account for the compromised health status of patients receiving inhaled therapies where deposition assumptions for healthy lungs may not accurately translate to deposition in diseased lungs.

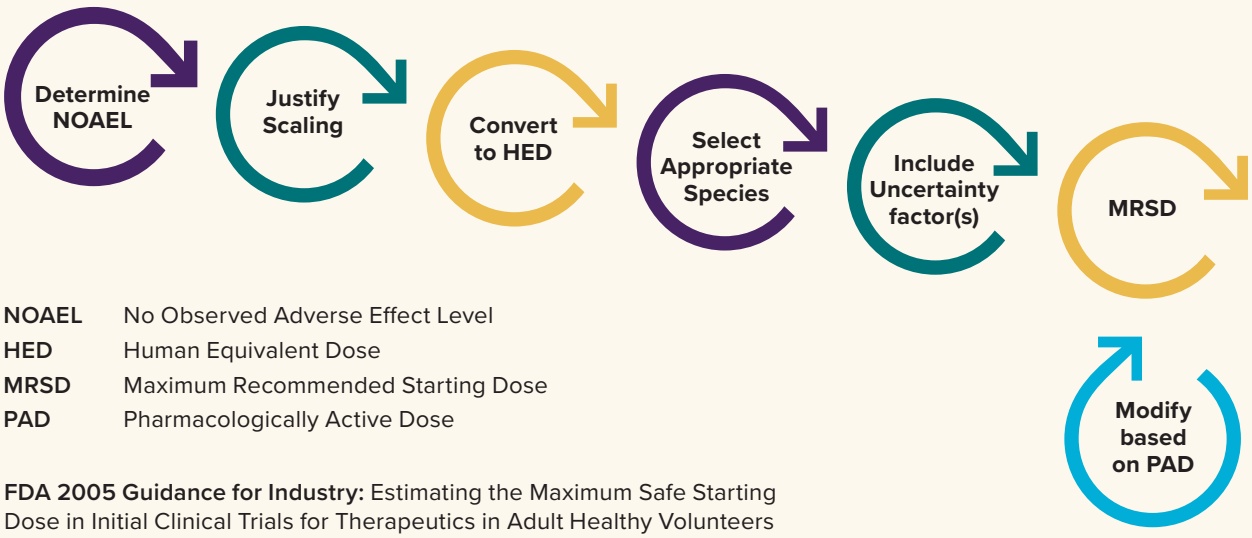
CONSERVATIVE SAFETY MARGINS

DPACC's highly conservative interpretation of nonclinical findings and clinical dose "capping" based on predetermined safety margins continue to limit clinical dosing for inhaled drugs. This approach is a significant concern for developers and has contributed to their decisions to initiate and conduct significant development outside the U.S.

As monitoring science has advanced, ongoing efforts aim to demonstrate that some potential lung effects can, in fact, be clinically monitored. This also raises the question of whether minimal, nonprogressive or reversible changes observed in nonclinical pathology from animal studies represent meaningful safety concerns to humans.

> "So, we would argue for the regulators to really think through accepting weight of evidence arguments, where we might have one species that is an outlier for a particular reason, maybe it's related to the actual drug itself, but to really consider that and allow some of these promising therapies to go forward."
>
> — Dr. William Thelin, Aer Therapeutics

FIGURE 4. 2005 FDA GUIDANCE — MAXIMUM RECOMMENDED SAFE STARTING DOSE (MRSD) FOR FIRST-IN-HUMAN (FiH) TRIALS[15]

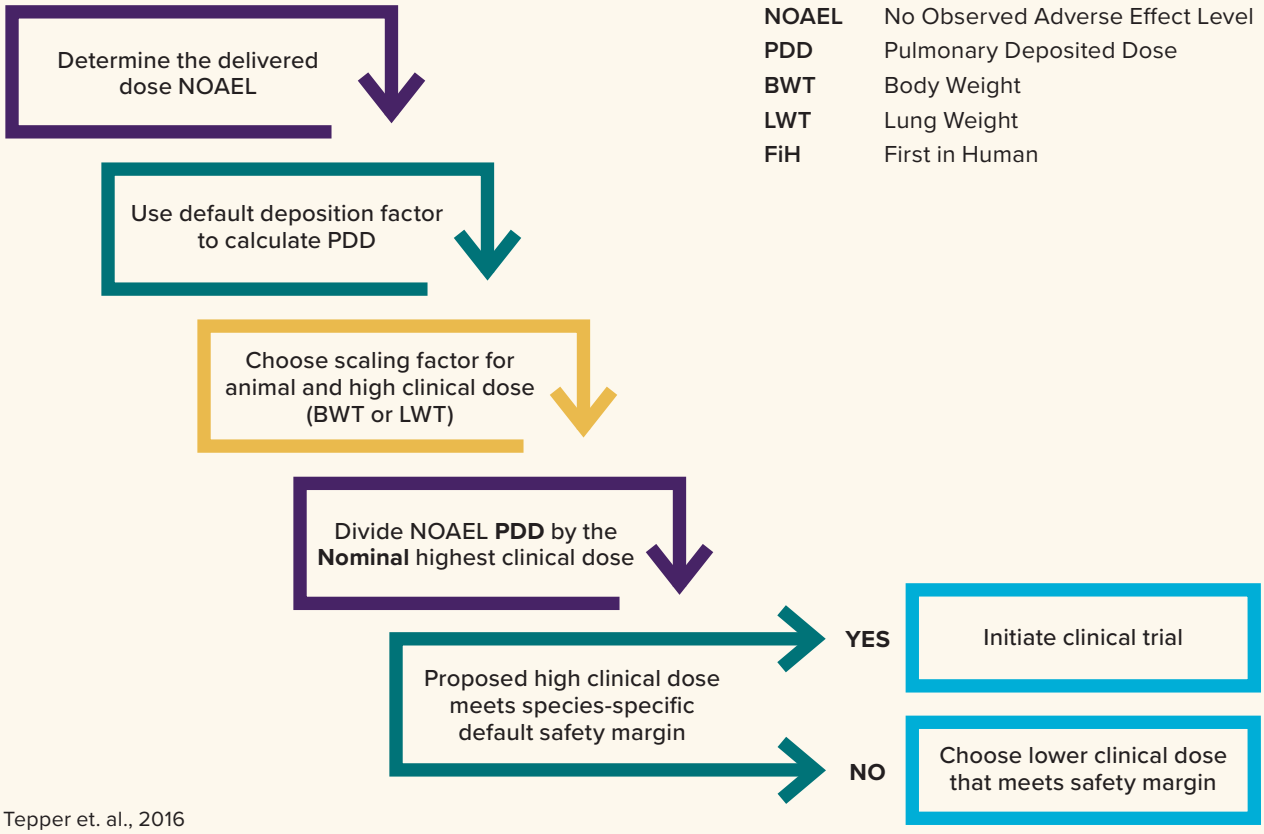


NOAEL	No Observed Adverse Effect Level
HED	Human Equivalent Dose
MRSD	Maximum Recommended Starting Dose
PAD	Pharmacologically Active Dose

FDA 2005 Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers

15 Tepper, J. IRA Demystified; February 26, 2026. https://reaganudall.org/news-and-events/events/advancing-drug-development-reducing-reliance-animal-testing.

FIGURE 5. FDA PULMONARY DIVISION ALGORITHM TO DETERMINE MAXIMUM CLINICAL DOSE FOR FiH TRIALS[16]



Tepper et. al., 2016

Species Difference Challenges

Pulmonary toxicology studies often begin in rodents and progress to larger animals. There are limitations to using rodent models that are minimized in large animal species (e.g., dogs and nonhuman primates) where the latter's airway size, generations, physiology, and/or lung ultrastructure allow for better translation to humans. Table 1 provides considerations for rodent models for studying inhaled drugs which limit the translatability to humans.

16 Ibid.

TABLE 1. CONSIDERATIONS FOR LIMITATIONS IN RODENT MODELS SHARED IN THE MEETING

- The rodent lung is anatomically and physiologically different than a human lung.
- Rodents have a very simple lung branching pattern compared to the dichotomous and more complex human airways.
- Rodents have slower mucociliary clearance from their lungs than humans. This phenomenon can lead to a scenario in which aerosols might deposit at a higher level in the alveolus (due to anatomical differences) and also clear more slowly, thus biasing results towards a higher exposure of rodent lung than human.
- There are instances when rodent lung findings have not accurately predicted human responses, or these models have yielded ambiguous or nonpredictive signals for human risk. A specific example is that of exaggerated macrophage overload response in rats to inhaled poorly soluble particles.[17]
- In this context, reliance on rodent findings without integration of additional species, mechanistic understanding, and/or clinical monitoring strategies may lead to overly conservative decision-making and potential exclusion of therapeutically meaningful doses.
- Some rodent findings have a radiographic correlate in humans that can be used to the advantage of researchers in clinical trials.

Toxicology interpretation is challenging when species exhibit divergent outcomes or when conclusions rely disproportionately on a single species. Determining which species is most informative or predictive becomes especially difficult when results from two-species toxicology studies conflict. Responses in healthy animals may differ markedly from those in disease models with preexisting inflammation (e.g., drugs/doses that are anti-inflammatory in disease models but proinflammatory in normal animals) or the response changes from anti- to proinflammatory at high doses. Overreliance on one species can also drive additional animal studies, undermining efforts to reduce animal testing.

Regulatory Challenges

Challenges extend from the laboratory to the regulatory space, where variable approach to dose setting and safety margin requirements across multiple regulatory jurisdictions leads to significant implications for U.S. innovation (Table 2). Divergent regulatory approaches can drive development to countries or jurisdictions that have a higher tolerance for risk when evaluating potentially life-saving new medicines. These differences may lead to program delays and higher costs that jeopardize moving promising drugs forward. Risk-averse approaches to data interpretation may slow innovation, increase reliance on animal studies, and shift some research outside the U.S. One clear opportunity for harmonization is deposition assumptions, which vary widely by region (e.g., 100% of nominal or fill dose assumed for humans in the U.S. versus emitted dose and fine particle fraction accepted elsewhere).

17 Nikula KJ, McCartney JE, McGovern T, Miller GK, Odin M, Pino MV, Reed MD. STP position paper: interpreting the significance of increased alveolar macrophages in rodents following inhalation of pharmaceutical materials. Toxicol Pathol. 2014;42(3):472–86. doi: 10.1177/0192623313507003.

TABLE 2. EXAMPLES OF VARYING GLOBAL REGULATOR APPROACHES (E.G., EMA, MHRA, PMDA, HC, TGA, ROW) FOR INHALATION TOXICOLOGY AND CLINICAL TRIAL EXPECTATIONS

- Clinical lung dose estimates
  - Use of a nominal approach (i.e., what goes into the device) by the FDA vs. what is estimated to be deposited in the respiratory tract by ROW
- FDA DPACC safety margin requirements are more stringent than ex-U.S. regulatory bodies
- Use of supportive NAMs data in WoE framework
- Considerations of histopathological finding severity and reversibility of histopathological findings

European Medicines Agency (EMA), Medicines and Healthcare products Regulatory Agency (MHRA), Pharmaceuticals and Medical Devices Agency (PMDA), Health Canada (HC), Therapeutic Goods Administration (TGA), Rest of World (ROW) drug regulation.

The current conservative approach to determining clinical dosing is a common concern among drug developers and is a major factor for initiating clinical development outside of the U.S.

4. Alternatives to Animal Testing

Presenters highlighted innovative approaches ranging from advanced preclinical models and human-relevant systems to novel monitoring strategies and discussed how state-of-the-art monitoring tools and NAMs could be integrated into a nonclinical lung safety package. The push for alternatives to animal testing is driven by growing recognition of the limitations of animal models in toxicology and the value of augmenting current practices, leading to a net benefit of using fewer animals to safely move drugs into humans. Discussions focused on lessons learned from developing alternative and human-relevant methods and how these innovations may transform the evaluation and monitoring of lung safety. Overviews of these innovations can be found in Table 3 and on the Foundation website at https://reaganudall.org/news-and-events/events/advancing-drug-development-reducing-reliance-animal-testing.

> **"...it's incumbent upon us as we're now in the 21st century to be thinking about where the new science is going, what new technologies are available to us. But importantly, if they're human-relevant, what advantage can they bring? How can we think about being ethical and sustainable in our endeavor to discover and develop new drugs..."**
>
> **— Lorna Ewart, Emulate, Inc.**

NAMs have the potential to impact the 3Rs of animal research: replacement, refinement, and reduction. Refinement and reduction apply once animal studies are required: refinement focuses on animal welfare, while reduction emphasizes experimental design to minimize animal numbers. When animals are needed, NAMs can reduce overall use by lowering the number of required studies, avoiding duplicate studies following specific findings, or eliminating testing in a species if it is found to lack human relevance.

NAMs sit at the intersection of science and regulatory standards and are not a one-to-one substitute for animal testing. Their value lies in enabling earlier monitoring through biomarkers or other indicators that can halt dosing or dose escalation before further toxicity testing is conducted with animals. Speakers also emphasized that many NAMs are not yet capable of fully recapitulating whole-organ or chronic pulmonary responses and are currently best suited as complementary tools within a broader WoE framework. Over time, NAMs are expected to evolve from augmenting animal studies to selectively replacing them. This is an incremental evolution, not a revolution, and applying novel technologies to redefine safe dose limits represents meaningful progress.

Examples of new technologies include:

- Digital biomarkers
- Microphysiologic System (MPS) & human tissue models
- Lung-on-chip model
- Serum or Bronchoalveolar Lavage Fluid (BALF) biomarkers of lung injury
- Computed Tomography (CT) imaging

TABLE 3. AREAS OF NAMs INNOVATION TO REDUCE RELIANCE ON ANIMAL TESTING FOR SAFETY/ TOXICOLOGY

AREAS OF USE	NAMs POTENTIAL USE
Species variation	• MPS — may be used to further evaluate and resolve conflicting results in animal studies and compare to human tissues, gaining further confidence to be able to progress into human trials. • Imaging — may be able to establish species-specific radiographic correlates to humans; lung imaging could be incorporated into animal studies of lung toxicity to allow cross species comparisons of drug-related lung findings between rats, dogs, and humans.
Redefining safe dose limits	• Building predictive in vitro assays. • Using NAMs to better show disposition and localization of the drugs to help better define clinical doses.
Translation	• Translating preclinical lung findings into clinically monitorable endpoints. • Mapping preclinical signals to early clinical biomarkers to justify first-in-human studies. • Establishing radiographic correlates between animal findings and clinical imaging endpoints as a key strategy to improve confidence in early human studies, even in the presence of ambiguous or species-specific nonclinical signals.
Histopathology	• Spatial transcriptomics – used to understand the molecular mechanisms driving histopathology. – provides a gene signature level of drug-induced lung toxicity. – could be a step forward towards identifying a mechanism to monitor drug-induced lung injury in humans identified by the histopathology. – implications for dosing: may be able to reduce the required 10-fold margin.

Imaging **Advanced imaging, in conjunction with functional tools, can be used to assess lung structure and function**	• Functional/structural imaging endpoints can de-risk early human studies. • CT Lung Imaging – Advances in CT lung imaging have reduced reliance on tissue biopsies in the diagnosis and treatment of lung disease. – Incorporation of CT lung imaging into drug development and drug safety monitoring provides a method to improve monitoring for lung toxicity and reduce reliance on animal data. – CT scanning is widely available, and radiation doses are low enough to allow for repeat assessments. – CT is a sensitive test of drug-induced interstitial lung disease. – Radiographic measures and patterns of pulmonary drug toxicity can be quantified and normal ranges are established. – CT, together with computational modeling, may be used in a patient-specific fashion to model how the inhaled drug will actually deposit in the lungs, though may not yet be scalable to address toxicity. • The use of imaging provides an argument against the conservative concept of nonmonitorability of pulmonary findings in animal studies. – The concern that inhaled drugs can cause lung inflammation or tissue damage that are not easily monitored in human clinical trials may be addressed by incorporating CT lung imaging to tests for structural changes that impact function. – Practices in oncology that rely on CT monitoring of drug-induced lung toxicity for multiple classes of anti-cancer drugs can be adopted for respiratory drugs being developed for diseases with high morbidity and/or mortality. – Monitorability using biomarkers of imaging has improved greatly, and when presented in the case of reversibility, can be used to move programs forward.
Data Aggregation	• Data mining to identify potential biomarkers. • Translational digital biomarkers: Digital biomarkers can expand the translational relevance of animal research and reduce animal numbers. • The combination of *in vivo* data, *in vitro* data, and human-based epidemiological data can add to the WoE approach and reduce reliance on animals.

5. Future Actions

Advancing drug development while reducing reliance on animal testing will require coordinated change across the research and regulatory ecosystem. Presenters and panelists outlined strategies to reduce animal use in lung toxicology and address barriers in pulmonary drug development to improve patient access. Key themes emerging from the presentations and discussions included sustained engagement with patient groups to define priorities and treatment gaps, greater data sharing, addressing challenges with traditional animal testing, integrating established approaches with NAMs and targeted clinical monitoring. Increased regulatory harmonization will benefit both drug developers and regulatory authorities.

Ongoing Dialogue with Patient Groups

Patient groups must be included in innovation, safety discussions, and risk–benefit evaluations. Strengthening shared understanding of pulmonary disease and the unmet needs among all stakeholders is essential. Greater regulatory flexibility is also needed to support access to experimental therapies through clinical trials in the United States, so that U.S. patients are not left out of the research process.

Data Sharing and Maximizing Use of Available Data

By collaboratively sharing data from animal studies, screening assays, historical *in vivo* studies, and *in vitro* models, while protecting proprietary interests, the pulmonary research community can advance more efficiently. Federated data sharing enables secure analysis across distributed datasets without transferring sensitive information.

Better use of existing data can reduce animal use by avoiding unnecessary duplication, with appropriate safeguards such as anonymization. Broad agreement that data sharing benefits all stakeholders is essential. Aggregated laboratory animal data can help validate translational approaches, while AI-driven, large-scale omics platforms can predict toxicities and reduce reliance on repeated *in vivo* studies. Clear mechanisms should also be developed to communicate successes to the public and other stakeholders.

Coexistence of Animal Testing, NAMs, and Elements of Clinical Monitoring

Advancing NAMs requires building robust bridging data to support the transition from animals to new approaches. The use of modern monitoring tools and NAMs has the potential to reduce redundant animal testing.

Successful adoption of NAMs will depend on expanded organ model development, use of large datasets, and clear demonstration that these approaches are equal or superior to current methods. Models must be fit for purpose, reliable, reproducible, and capable of generating human-relevant mechanistic data. NAMs can play a large role in identification of serum or lung lavage/tissue biopsy biomarkers of toxicity.

Leveraging modern clinical technologies, specifically CT imaging, can strengthen pulmonary safety and toxicology bridging, and support advancement of new therapies into clinical trials.

Overall progress will require a hybrid approach that balances NAMs with targeted animal testing.

Regulatory Flexibility and Harmonization with Other Regulatory Bodies

Harmonization could begin with standardizing pulmonary research terminology and dose definitions to avoid confusion. Aligning the use of nonclinical data according to the MRSD 2005 guidance to determine starting doses for clinical trials and allowing clinical data to determine escalation and maximum dose in clinical trials is a critical step for respiratory drug development. Failure to evolve toward harmonized, science-driven approaches may continue to shift early clinical development outside the U.S., with implications for patient access, innovation, and the U.S. role in shaping global regulatory approaches to pulmonary drug development.

According to speakers and panelists, the best available science should be used to estimate deposited doses in animals and humans to reduce uncertainty. The NOAEL should not include speculation about the origin or progression of a lesion or theoretical human patient extrapolation. Defining a NOAEL should be based only on the observed findings in the model used for toxicity testing. Safety margin calculations should support early human clinical studies and not have predetermined set points that limit clinical dosing. This separation acknowledges that nonclinical models are best suited to inform safe entry into human studies, whereas maximum tolerated or pharmacologically active doses should be established using clinical data supported by real-time monitoring tools.

A harmonized approach should include adoption of a WoE framework that integrates multi-species data, mechanistic understanding of toxicity, and clinically monitorable endpoints. This approach can maintain safety while advancing therapies, particularly for serious lung diseases, by better balancing risk, innovation, and U.S. competitiveness.

Additional suggestions for moving forward:

- Reevaluate outdated assumptions, historical practices, and overly conservative approaches that may limit pulmonary drug development.
- Consider more flexible and creative nonclinical and clinical study designs.
- Leverage the ability to monitor lung toxicity in humans using existing clinical tools, enabling simpler and shorter animal studies with fewer animals.
- Move toward the use of NAMs, alongside *in vivo* and traditional methods, to better contextualize risks, especially for toxicities with unclear relevance to humans.

Ongoing professional development is essential to support adoption of these changes. Country and jurisdictional differences in evaluation and application of clinical and nonclinical data highlight the need for harmonization. Regulatory engagement could move towards emphasizing integrated, WoE safety assessments, in line with international regulators increasingly recognizing the value of NAMs.

> **"We're helping to partner with industry to bring new medicines forward while also protecting patient safety...we now also have this renewed focus or newly emphasized focused on reducing animal testing and we're trying to weave that in where scientifically justified without compromising either of those other two goals. [NAMs developers] hone in on the context of use for the assays they're developing, where they fit into this picture and [address] those validation questions. [This means] assessing their tools and the data they generate...through the eyes of a regulator...[focusing on] relevance and the technical aspects of the assay,... data integrity,...transparency and data sharing..."**
>
> — **Dr. Andrew Goodwin, Director, Division of Pharmacology-Toxicology for Immunology and Inflammation, FDA**

Prioritizing Short- and Long-term Goals

Define short- and long-term, high-impact goals and prioritize work based on patient relevance rather than animal models alone. Focus on context of use where specific models address specific questions before pursuing broader validation.

Near-term goals include reevaluating human and nonclinical deposition factors by using contemporary models and empirical data and replacing default 100% “nominal” human lung deposition assumptions and predefined nonclinical deposition factors. Additional near-term goals include 1) refinement of adversity determinations in inhalation toxicity studies, 2) implementation of imaging or biomarker endpoints, and 3) reassessing predetermined clinical/nonclinical safety margins. Longer-term goals include broader integration of NAMs and use of large datasets to improve predictive value of model systems for human safety.

6. Conclusion

Reducing reliance on animal models is a priority, but progress occurs in defined stages and contexts. Significant efforts are underway to improve product development while decreasing animal use, including addressing current changes in animal testing requirements and improving the efficiency of animal studies that remain necessary. A consistent theme across discussions was that advances in clinical monitoring provide a path to reduce reliance on animal models while maintaining or improving patient safety. Specifically in pulmonary safety assessment and pulmonary drug development the two-pronged approach of advancing NAMs while moving towards a contemporary, data-driven weight of evidence regulatory framework can reduce animal use and bring more promising drugs to patients.

The next step is to determine the right venue to continue these conversations and move forward one step at a time.

Appendix

Advancing Drug Development by Reducing Reliance on Animal Testing

Case Example: Preclinical Animal Models in Lung Toxicology

THURSDAY, FEBRUARY 26, 2026
10 am – 4 pm (eastern) | Hybrid Meeting

10:00 am	**Welcome & Opening Remarks** Susan C. Winckler, RPh, Esq. *CEO, Reagan-Udall Foundation for the FDA* **Steven Kozlowski, MD** *Chief Scientist, Office of the Chief Scientist, Office of the Commissioner, FDA*
10:15 am	**Use of Animal Models in Preclinical Lung Toxicology Safety Studies: Current Expectations and Limitations** **Speakers** • Matt Reed, PhD, DABT, *Principal, Coleus, LLC* • Jeff Tepper, PhD, DABT, *Consultant, Tepper Nonclinical Consulting*
11:00 am	**Industry Experience in Current Environment** **Speakers** • William Thelin, PhD, *Senior Vice President, Aer Therapeutics* • Per Åberg, MSc, *Senior Director, Clinical Pharmacology and Safety Sciences, AstraZeneca* • Jorrit Hornberg, PhD, MSc, Vice *President, Global Head of Safety Sciences, AstraZeneca* • Aidan Curran, PhD, *Principal, Curran Nonclinical Consulting*
11:45 am	**Panel Discussion: Impact of Current Environment on Product Development and Patients** **Reactor Panelists** • Teresa Barnes, *Chief Executive Warrior, PF Warriors* • Karin Hoelzer, DVM, PhD, *Senior Director, Patient Advocacy, Biotechnology Innovation Organization (BIO)*

12:30 pm	**Lunch**
1:15 pm	**Innovations in Lung Toxicology Safety Studies: New Approaches in Preclinical Models & Clinical Monitoring** **Speakers** • Mary McElroy, PhD, MBA, *Head, Discovery Pharmacology and Toxicology, Charles River Laboratories* • Alexandra Maertens, PhD, *Assistant Professor, Bloomberg School of Public Health, Johns Hopkins University* • Megan LaFollette, PhD, *Executive Director, 3Rs Collaborative* • Emily Richardson, PhD, *Biology Group Leader, CN-Bio* • Rachel Eddy, PhD, *Imaging Scientist, Clinical Development, VIDA* • John Fahy, MD, MSc, *Professor of Medicine, Division of Pulmonary and Critical Care Medicine, University of California-San Francisco*
2:45 pm	**What the Future Might Look Like: Regulatory Harmonization and Global Alignment** **Panelists** • Lorna Ewart, *PhD, DSc, Chief Scientific Officer, Emulate, Inc.* • Andrew Goodwin, PhD, *Director, Division of Pharmacology-Toxicology for Immunology and Inflammation, FDA* • David Jones, FRSB, FBTS, *Consultant, ApconiX* • Tim McGovern, PhD, *Principal Consultant, White Oak Regulatory Tox, LLC* • Steven Rowe, MD, *Chief Scientific Officer, Cystic Fibrosis Foundation*
3:55 pm	**Closing Remarks** Susan C. Winckler, RPh, Esq.
4:00 pm	**Adjourn**

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