General Considerations for the Use of New Approach Methodologies in Drug Development

Guidance for Industry

DRAFT GUIDANCE

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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

March 2026
Pharmacology/Toxicology

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General Considerations for the Use of New Approach Methodologies in Drug Development Guidance for Industry[1]

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

The purpose of this guidance is to provide drug developers with a validation framework for new approach methodologies (NAMs) used in drug development to improve predictive toxicology in humans and move away from reliance on animal testing.[2] NAMs include a broad range of methods such as complex in vitro, 2D in vitro, in chemico and in silico studies. This guidance describes the Center for Drug Evaluation and Research's (CDER's) general recommendations to consider for validating NAMs when nonclinical NAMs data are provided in support of a drug application or regarding an order issued under section 505G of the FD&C Act for an OTC monograph.[3] In 2022, Congress passed legislation clarifying that nonanimal alternatives can be used to support an investigational new drug (IND) application or a biosimilar biologics license application (BLA) in lieu of animal studies.[4] The Science Board to the Food and Drug Administration then provided comprehensive recommendations on how FDA could drive change

[1] This guidance has been prepared by the Office of New Drugs in the Center for Drug Evaluation and Research at the Food and Drug Administration.

[2] For purposes of this guidance, we use the term *drug* or *drug product* to refer to human drug and biological products regulated by CDER, unless otherwise specified.

[3] This includes OTC monograph orders issued under section 505G of the FD&C Act for nonprescription drugs intended for topical administration. Among other things, this guidance is issued in compliance with the requirement that FDA issue a new draft guidance on nonclinical testing alternatives to animal testing for OTC monograph drugs intended for topical administration under section 505G(r)(2)(B).

[4] See section 3209 of the Food and Drug Omnibus Reform Act of 2022, passed as part of the Consolidated Appropriations Act, 2023, which amended FD&C Act section 505 and Public Health Service Act section 351 to clarify that data from nonanimal testing may be sufficient to support an IND or BLA. The legislation also amended FD&C Act section 505(z) to add a definition of "nonclinical test."

with the adoption of scientifically validated NAMs.[5] Herein, CDER is providing this guidance to facilitate broader integration of NAMs in drug development and regulatory submission packages and in alignment with its *Roadmap to Reducing Animal Testing in Preclinical Safety Studies.*[6]

It is important to note that for a NAM to be considered for review in drug development, the test does not necessarily need to be validated. However, as FDA gains confidence in these tools, they could be formally adopted to reduce or replace specific animal tests. A fit-for-purpose NAM, even if not validated, may adequately address specific toxicological concerns. When a NAM is used it is always in the context of the weight of evidence (WOE) or, in other words, considering the specific concerns about that product based on prior knowledge and any preclinical toxicological information provided.

The recommendations in this guidance are intended to highlight scientific principles of study design and reporting that can be applied broadly in the validation of NAMs in drug development. This guidance is not intended to address specific NAMs and does not address the use of NAMs in drug discovery.

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

Under the regulations governing investigational new drug applications, drug sponsors must submit nonclinical data regarding their proposed product's pharmacology and toxicology before the proposed product can proceed to clinical trials.[7] This requirement helps FDA minimize risk and determine whether investigational drugs are reasonably safe to proceed in humans. These pharmacology and toxicology data may come from a range of methods, such as in vitro testing, in silico modeling, or combinations thereof. In this guidance, FDA intends to facilitate broader integration of NAMs in drug development and regulatory submission packages. Although animal toxicity studies have previously been relied upon to assess for potential risks to human health from drug products, the development and use of reliable NAMs furthers an important FDA priority to reduce and replace animal testing while advancing predictive toxicology using human-centric methods.

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[5] Potential Approaches to Drive Future Integration of New Alternative Methods for Regulatory Decision-Making, A Report to the Science Board to the Food and Drug Administration from the New Alternative Methods Subcommittee, October 2024, available at https://www.fda.gov/media/182478/download.

[6] Roadmap to Reducing Animal Testing in Preclinical Safety Studies, FDA, April 10, 2025, available at https://www.fda.gov/files/newsroom/published/roadmap_to_reducing_animal_testing_in_preclinical_safety_studies.pdf.

[7] 21 CFR 312.23(a)(8).

NAMs hold great potential in terms of enhancing efficiencies in drug development and generating data to advance human health. For example, NAMs may offer additional tools for identifying potential drug toxicities or mechanisms of action for which there are currently no adequate models, and NAMs that incorporate human biology may provide more predictive and human-relevant information than traditional methods. Therefore, CDER encourages the use of NAMs in regulatory submissions,[8] especially when they improve the predictivity, reliability, and human relevance of nonclinical tests and therefore enhance the safety of subsequent clinical trials. Where nonanimal methods have been shown to accurately characterize the relevant risk, FDA encourages moving to scientifically validated nonanimal methods. CDER also encourages sponsors to consult with the applicable review division if there is uncertainty about the suitability of the method for regulatory use when considering use of a nonanimal testing method believed to be suitable, adequate, and feasible for a proposed regulatory use. We anticipate that early engagement will focus on indication-, disease-, organ-, and endpoint-specific considerations that should include interactions with review divisions. A variety of different types of NAMs data can be included as part of nonclinical submissions; this guidance provides general considerations regarding validation principles that are broadly applicable to all NAMs.

Although federal laws and regulations do not stipulate exactly which nonclinical tests sponsors must conduct nor in what model,[9] a common set of studies (e.g., general toxicology, primary pharmacology, and safety pharmacology) is generally conducted before clinical trials in humans are initiated. As a drug development program continues, other nonclinical tests may be needed to support clinical trials (e.g., carcinogenicity studies, developmental and reproductive toxicity studies). These studies can be done in a variety of models, and they provide insight regarding several key issues in drug development, such as mechanism of action, safe starting and maximum tolerated doses.

While many of these investigational new drug-enabling studies have traditionally been conducted in animals, CDER routinely receives and reviews data from studies using NAMs. For NAM data to be useful for regulatory decision-making, however, the data must be reliable and there must be confidence in the methodology used. This confidence is typically obtained through scientific consensus generated by the application of validation criteria to the design, performance, and interpretation of the studies.

[8] See, for example, the guidances for industry *Oncology Therapeutic Radiopharmaceuticals: Nonclinical Studies and Labeling Recommendations* (August 2019) and *Severely Debilitating or Life-Threatening Hematologic Disorders: Nonclinical Development of Pharmaceuticals* (March 2019), the ICH guidance for industry *M3(R2) Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals* (January 2010), and the draft guidance for industry *Monoclonal Antibodies: Streamlined Nonclinical Safety Studies* (December 2025). When final, this guidance will represent the FDA's current thinking on this topic. For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

[9] See id. ("The kind, duration, and scope of animal and other tests required varies with the duration and nature of the proposed clinical investigations.")

We note that this guidance focuses solely on considerations for the validation of NAMs and does not address qualification of drug development tools (including NAMs), which is addressed in other guidance.[10] *Validation* is a process by which the accuracy, reliability, and relevance of a procedure are established for a specific context of use (COU).[11] *Qualification* is a determination that a drug development tool and its proposed COU can be relied upon to have a specific interpretation and application in drug development and regulatory review.[12] While qualification is useful, validation is critical to establishing the reliability of NAM data for specific situations. CDER will review data from both qualified and validated NAMs used in a drug development program to determine whether its use is appropriate in the particular context.

Regardless of whether a NAM is qualified, its validation is warranted to help determine the quality of the data produced and how the results should be interpreted for regulatory decision-making. Sponsors should take into account the validation considerations described in this guidance to ensure a given NAM is fit-for-purpose. The Organisation for Economic Co-operation and Development (OECD) test guidelines include additional validated methods that CDER has recognized as acceptable in lieu of in vivo studies in the past. For example, certain NAMs that evaluate eye irritation, skin irritation, and skin sensitization have been accepted as fit-for-purpose. CDER has also accepted NAMs using in vitro reconstructed human epidermis assays to predict risk for human skin sensitization to replace the use of animals (e.g., mice in the local lymph node assay). Although not all these factors are required for acceptance, skin sensitization NAMs have been found acceptable because there are well-defined adverse outcome pathways,[13] in vitro and in silico strategies to evaluate the ability of NAMs to predict human

[10] See the guidance for industry and FDA staff *Qualification Process for Drug Development Tools* (November 2020). We update guidances periodically. For the most recent version of a guidance, check the FDA guidance web page at https://www.fda.gov/regulatory-information/search-fda-guidance-documents.

[11] Interagency Coordinating Committee on the Validation of Alternative Methods, 2024, Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies: A Report of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Validation Workgroup, https://ntp.niehs.nih.gov/sites/default/files/2024-03/VWG_Report_27Feb2024_FD_508.pdf.

[12] See section 507(e)(7) of the Federal Food, Drug, and Cosmetic Act (FD&C Act) (21 U.S.C. 357(e)(7). Section 507 of the FD&C Act establishes FDA's drug development tool (DDT) qualification program. FDA has established DDT qualification processes for biomarkers, clinical outcome assessments, and animal models, and can review other types of tools under the ISTAND program. More information on the DDT qualification program is available at https://www.fda.gov/drugs/development-approval-process-drugs/drug-development-tool-ddt-qualification-programs, and more information on the ISTAND program is available at https://www.fda.gov/drugs/drug-development-tool-ddt-qualification-programs/innovative-science-and-technology-approaches-new-drugs-istand-pilot-program.

[13] OECD, 2014, The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins, OECD Series on Testing and Assessment, No. 168, OECD Publishing, https://doi.org/10.1787/9789264221444-en.

hazard potential and/or potency,[14] and validated test guidelines for NAMs used in the testing of chemicals.[15]

III. VALIDATION CONSIDERATIONS

This section describes general validation principles intended to enhance the interpretability and reliability of NAMs studies submitted in support of regulatory decision-making. We focus on the following four key features of any validation approach: (1) COU, (2) human biological relevance, (3) technical characterization, and (4) fit-for-purpose.

A. Context of Use

The COU should clearly define the intended use and regulatory purpose of the NAM to address a specific drug development decision context. An adequate COU will address a data gap and fulfill one or more drug development objectives. For a COU to be useful, it has to answer a scientific and/or drug development question allowing CDER to use that information to support a regulatory decision. The following are examples of COUs that can support regulatory decision-making: (1) supporting the extent of patient monitoring in clinical trials, (2) supporting dosage selection (whether it is first in human or whether it is for later phases of development and chronic use), (3) addressing a mechanistic understanding of an adverse event seen in animals and/or humans (such as identifying off-target effects or exaggerated pharmacology), (4) justifying not using an animal species because it does not add value to regulatory decision-making, and (5) helping to support a WoE approach and predicting risks typically not measured in humans.

B. Human Biological Relevance

The human biological relevance of a NAM refers to the relationship between the information generated from the NAM and how that information could impact the assessment of the drug in the context of human testing and prediction of toxicities that cannot be measured in clinical trials. Adequate demonstration of the biological relevance of a NAM provides confidence in the methodology.[16] The bullet points below summarize key recommendations for establishing the human biological relevance of a NAM:

- Describe the physiological features that will be assessed by the NAM. This includes

[14] Kleinstreuer NC, Hoffmann S, Alépée N, Allen D, Ashikaga T, Casey W, Clouet E, Cluzel M, Desprez B, Gellatly N, Göbel C, Kern PS, Klaric M, Kühnl J, Martinozzi-Teissier S, Mewes K, Miyazawa M, Strickland J, Van Vliet E, Zang Q, and Petersohn D, 2018, Non-Animal Methods To Predict Skin Sensitization (II): An Assessment of Defined Approaches, Crit Rev Toxicol, 48(5):359–374. https://doi.org/10.1080/10408444.2018.1429386.

[15] OECD (2023), Guideline No. 497: Defined Approaches on Skin Sensitisation, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, https://doi.org/10.1787/b92879a4-en.

[16] Interagency Coordinating Committee on the Validation of Alternative Methods, 2024, Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies: A Report of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Validation Workgroup, https://ntp.niehs.nih.gov/sites/default/files/2024-03/VWG_Report_27Feb2024_FD_508.pdf.

describing the relevance of the cell types used, species of origin, and how the anatomical and physiological characteristics involved in toxicology findings may be mimicked in the NAM platform.

- For example, for a NAM intended to assess neurotoxicity, the choice of neural cellular composition (e.g., glial, endothelial, stromal cells) and/or cellular architecture (e.g., monoculture, coculture, spheroid, organoid) may affect biological and functional baseline characteristics (e.g., morphological, transcriptomic, neural activity) and should be carefully considered when selecting an appropriate model.

- For example, for a NAM intended to assess hepatotoxicity, the model should contain the relevant cell types (e.g., hepatocytes, stellate cells, Kupffer cells) and biological features (e.g., albumin production, metabolic competence) to recapitulate the hepatocellular physiology observed in vivo.

- For example, for a NAM intended to assess respiratory toxicity, the model may be a tissue reconstructed from primary human lung cells that capture key mechanisms of toxicity (e.g., loss of membrane integrity) and cellular properties (e.g., beating cilia) of the in vivo human situation.

- Demonstrate how relevant toxicological findings can be reliably evaluated in the NAM.

  - For example, for an in vitro NAM intended to predict liver toxicity, indicate whether the assay simultaneously and accurately measures markers such as albumin and urea secretion over an extended period to replicate a repeated exposure scenario. Similarly, indicate whether the NAM provides functional assessments of typically measured endpoints such as CYP450 protein expression, alanine transaminase or aspartate transaminase release.

- Describe how the biological mechanisms investigated in the NAM are applicable to outcomes measured in human clinical trials.

  - For example, for an in vitro NAM investigating developmental toxicity, describe how the NAM mimics the key stages of fetal development during specific developmental windows to predict clinical outcomes when administered to women of childbearing potential.

C. Technical Characterization

The technical characterization of a NAM, as is the case for animal studies, is essential to establish scientific confidence in the data obtained using the platform and to ensure it is sufficiently robust, reliable, and reproducible to quantify specific endpoints. Aligned with the OECD Guidance Document on Good In Vitro Method Practices (GIVIMP)[17], the bullet points

[17] OECD Guidance Document on Good In Vitro Method Practices (GIVIMP), OECD Series on Testing and Assessment, Dec. 2018, available at https://www.oecd.org/en/publications/guidance-document-on-good-in-vitro-method-practices-givimp_9789264304796-en.html.

below summarize some of the key recommendations for characterizing certain types of NAMs:

- Describe the test method details such as dose and frequency of dosing, test substance preparation and storage, detection methods and instrumentation used, compatibility of materials with the assay measurements or test substances, and any steps or processes that could introduce assay variability.

- Describe the statistical methods and criteria applied for data analysis and interpretation, such as for determining positive, negative, or inconclusive results.

- Demonstrate the predictive performance of the NAM for the specific COU (e.g., sensitivity, specificity).

- Provide details about the working duration of the assay, a period over which the assay will provide stable results using the supplied assay materials. Assay results should be shown to be stable throughout the working duration and as materials are resupplied over time (e.g., new batches of cells, reagents).

- Document and describe the cell type used (e.g., primary, immortalized, stem cell derived), cell/tissue isolation and/or differentiation methods, cell source (e.g., commercial, patient-derived, catalog and batch number), and species.

- Describe biological variability (e.g., genetics, donor variability) and donor cell phenotype (e.g., strain, sex, age) as they relate to the COU.

- Define and justify the appropriate selection of reference compounds, such as positive and negative controls, to demonstrate the scientific validity and performance of the NAM.

- Define the cell culture medium, including the use and concentration of reagents, specific growth factors, serum, or antibiotics. Document maintenance conditions such as passage number, incubator conditions, and surface coatings.

- Provide the following study details to address specific considerations for certain platforms and devices, such as organ chips:

  - Technical considerations such as flow, matrix, shear stress, and media.

  - Critical characteristics of scaffolds and matrices, such as biocompatibility, fabrication method, mechanical properties, architecture, biochemical properties, compatibility with substrate, and how these influence both experimental variability and biological relevance.

  - Evidence to rule out (1) possible interaction between the test article and the platform or device, such as leaching of materials from an organ-on-a-chip, which could cause toxicity or alter cells; (2) absorption of the test article in the cell culture wells, which could decrease the concentration reaching the target; or (3) issues with

biocompatibility of the biomaterial (e.g., tissue or cells) in contact with the material interface.

D. Fit-for-Purpose

A NAM is fit-for-purpose if it assists CDER in regulatory decision-making. The bullet points below summarize some recommendations for demonstrating a NAM to be fit-for-purpose:

- When traditional comparator methods are available, provide data demonstrating that the NAM characterizes risk at least as well as an established method, including performance assessments comparing the two (e.g., sensitivity, specificity, positive and negative predictivity, false positive, and negative rates).

- Discuss the benefits and limitations of the NAM, including aspects of the study design or conduct that may affect its reliability, reproducibility, and/or ability to inform a risk assessment for its intended COU.

- Describe how findings from the NAM contribute to the overall determination of risks to human health and/or safety of the drug.

A fit-for-purpose NAM can provide information intended to fulfill one or more of the following three drug development objectives:

- **Replaces** or offers an alternative approach to traditional methods, for example, provides equivalent or better proof of activity or safety information compared with the traditional method.

  - For example, an in vitro NAM might be used to replace an animal study.

- **Fills a data gap**, for example, provides additional proof of activity or safety information on drugs when traditional nonclinical models are unavailable or insufficient.

  - For example, an in vitro NAM might help to understand a mechanism of toxicity or mechanism of action seen in humans which cannot be modeled in animals due to species-specific differences.

- **Confirms and/or complements** findings from traditional methods, for example, provides information equivalent to that of traditional nonclinical models.

  - For example, an in vitro NAM might justify why certain animal species may not yield useful information.