July 15, 2025

Dr. Helena Hogberg
Acting Director of NICEATM
National Institute of Environmental Health Sciences
P.O. Box 12233, K2-17
Durham, NC USA 27709

RE: Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM); Notice of Public Meeting; Request for Public Input

Dear Dr. Hogberg,

Cruelty Free International appreciates the opportunity to submit written comments ahead of the 2025 ICCVAM Public Forum. ICCVAM plays a vital role in fostering cross-agency collaboration to accelerate the adoption of new approach methodologies (NAMs) and drive progress in regulatory science away from testing on live animals.

We welcome the strong leadership shown by U.S. agencies in recent months to accelerate the shift away from outdated animal-based testing approaches and toward modern, human-relevant science. The recent FDA-NIH Workshop marked a pivotal moment in this broader shift, and many of the themes reflected key priorities across our work at Cruelty Free International – particularly those highlighted in our Replace Animal Tests (RAT) List.

We intend to provide an updated oral presentation on our RAT List during the public forum, highlighting new developments since we first introduced it to ICCVAM and SACATM in 2021. Below we identify priority areas where ICCVAM-led collaboration, expert group formation, and cross-agency coordination could help cement this transition, ensuring it results in sustained, measurable reductions in animal use.

NIH Policy Shift and Alignment with HEARTS Act

Cruelty Free International warmly welcomes the NIH's transformative announcement that all new NIH funding opportunities will require consideration of NAMs, and that NIH will no longer solicit proposals exclusively reliant on animal models. This policy shift aligns directly with the Humane and Existing Alternatives in Research and Testing Sciences (HEARTS) Act (HR 1291), which we have long supported, and represents a foundational step toward replacing animal-based research with modern, human-relevant science.

To ensure meaningful implementation, we recommend ICCVAM support the development of clear NIH guidance detailing the nature and extent of NAM considerations expected in grant proposals. Such guidance will help prevent superficial or tokenistic inclusion of NAMs and encourage genuine integration of non-animal approaches in funded research.

Recommendations for New ICCVAM Working Groups

Building on the priorities identified in our RAT List and recent agency commitments, we urge ICCVAM to establish focused working groups targeting high-impact animal tests where internationally validated non-animal methods exist but remain underused:

- **Batch testing for biologicals**: Focused efforts are needed to completely phase out tests such as the Laboratory Animal Batch Safety Test (LABST), Target Animal Batch Safety Test (TABST), and the Abnormal Toxicity Test (ATT, also known as the General Safety Test or Innocuity Test); support the adoption of NAMs; and enhance coordination with international initiatives led by the European Medicines Agency (EMA) and the World Health Organization (WHO). For example, reported challenges in implementing VICH guidelines with respect to the waiving of animal tests in the U.S. highlight the need for ICCVAM to provide clear guidance and facilitate interagency and international coordination to overcome barriers and accelerate adoption of alternatives to animal tests. In parallel, ICCVAM should also focus on prioritizing the phase-out of batch potency tests for leptospirosis, rabies, and other vaccines where validated in vitro methods exist, encouraging broad regulatory acceptance, and addressing implementation challenges with manufacturers.

- **Antibody production**: the production of animal-derived antibodies raises serious welfare concerns and often shows batch variability, impacting reliability. Phage display technology offers a non-animal approach that produces antibodies with higher quality, stability, and reproducibility, while also being faster and more scalable. In 2020, the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) recommended the use of non-animal-derived antibodies generated using phage display technology and urged their acceptance and use. Building on this, an ICCVAM working group should lead efforts to promote broader adoption of non-animal-derived antibodies in the U.S., including by publishing a formal recommendation similar to the EURL ECVAM statement. Additional activities could include organizing a workshop and/or training webinars to address misconceptions and highlight the scientific and practical benefits of non-animal-derived antibodies, and developing a national resource listing key manufacturers and suppliers.

- **Pyrogenicity testing:** The European Pharmacopoeia plans to delete the rabbit pyrogen test (RPT) from all monographs by 2026, reflecting significant progress in regulatory acceptance of non-animal methods such as the Monocyte Activation Test (MAT) and the Recombinant Bacterial Endotoxin Test (rBET). Building on this momentum, an ICCVAM working group should lead efforts to accelerate replacement of the RPT in the U.S. Workgroup activities could include seeking deletion of the RPT from all U.S. legal requirements; conducting a nationwide survey to assess current use of the RPT; and organizing workshops and/or training webinars to promote adoption and regulatory acceptance of available non-animal pyrogenicity tests. We urge ICCVAM to build on progress made in the EU and encourage similar advancements in the U.S.

- **Botulinum toxin potency testing**: Validated cell-based assays (CBAs) provide significant animal welfare and scientific advantages over the mouse LD50 test, a procedure known to cause severe animal suffering. However, the LD50 test remains in use because it is still considered the official reference method in many regions, and regulatory frameworks often require the CBA to be validated against the LD50 for every specific product. Many CBAs are only validated for final product testing and not consistently applied across all production stages. Combined with limited regulatory pressure and incentives, these factors contribute to ongoing reliance on the LD50. We recommend ICCVAM facilitate a survey of manufacturers and regulators to better understand barriers to CBA adoption and promote collaborative strategies – similar to recent EMA initiatives – to accelerate transition away from the LD50 test.

- **Marine biotoxin testing**: The traditional mouse bioassay for marine biotoxin detection causes significant animal suffering and has been replaced in many regions by validated in vitro and analytical methods that are more humane, sensitive, and reliable. However, its continued use in some regulatory contexts reflects slow uptake and inconsistent implementation of these approaches. Building on our previous recommendations, we urge ICCVAM to establish a dedicated working group to identify and address scientific, regulatory, and practical barriers to the adoption

of non-animal methods. This group could also survey stakeholders to better understand persistent challenges and design targeted strategies that support broader regulatory recognition and industry transition to non-animal methods.

- **Skin sensitization**: OECD-approved defined approaches combine several in vitro assays to reliably produce data on skin sensitisation; these approaches have been validated and are ready for broad regulatory acceptance. We encourage ICCVAM to reactivate its skin sensitization workgroup to promote and support increased, widespread adoption of these non-animal methods across all relevant sectors, and to help address remaining validation needs to enable their use in additional contexts, such as for medical devices.

Several of these topics were explicitly identified during the FDA-NIH Workshop as areas where immediate action could reduce animal use, underscoring the timeliness of ICCVAM-led expert groups to coordinate agency efforts and eliminate outdated practices. These priorities align closely with ICCVAM's 2018 Strategic Roadmap goal to encourage the further adoption of existing non-animal methods by federal agencies and regulated industries. These points also reflect the core aim of our RAT List: to phase out animal tests where suitable non-animal approaches exist, enabling rapid, measurable reductions in animal use while fostering ongoing innovation.

Dedicated ICCVAM working groups are essential to overcoming barriers to increased adoption and accelerating regulatory acceptance of non-animal methods – especially in areas like these where non-animal approaches already exist but implementation is delayed due to inertia, unclear guidance, enforcement gaps, lack of confidence in the methods and insufficient global harmonization. Coordinated action will help address these challenges and drive faster adoption of humane non-animal methods, ultimately benefiting animal welfare, public health, and scientific advancement.

Expert Group on Second Species Testing

International regulatory guidance for pharmaceuticals, such as ICH M3 (R2), generally requires toxicity testing in two animal species: one rodent and one non-rodent (commonly dogs or primates). However, recent scientific literature and regulatory experience suggest that a single-species approach may be sufficient in many cases, especially for longer-term studies. At the same time, public concern over the use of dogs and primates continues to grow. We recommend that ICCVAM establish an expert group to provide a forum for interagency dialogue on this issue – facilitating data sharing, identifying potential waiver opportunities, and coordinating input into the revision and updating of existing guidelines. Building on the FDA's recent roadmap, which includes a commitment to lead efforts to update ICH guidelines, we recommend prioritizing revision of ICH M3 (R2), which currently offers no flexibility to waive the second-species requirement for small molecule pharmaceuticals. ICCVAM and its partners could encourage ICH to undertake a targeted review of the second-species requirement – similar to the ICH S1 review on carcinogenicity testing – to assess whether a more flexible, science-driven approach is now warranted. A revised guideline should examine and support conditions under which single-species approaches are scientifically justified and could be more broadly accepted.

Public Tools to Support Adoption of NAMs

We welcome NIH's and NICEATM's commitment to developing searchable public resources, such as CAMERA, that list accepted NAMs along with their regulatory contexts. To complement these efforts, ICCVAM should consider publishing a regular, publicly accessible status report – similar to EURL ECVAM's reports in Europe – that tracks the validation, regulatory acceptance, and adoption of non-animal methods

across U.S. agencies. Such a report would enhance transparency, inform stakeholders, and accelerate regulatory acceptance and industry uptake of NAMs.

AI-Driven Comparison of NAM and Animal Data

The FDA's initiative to apply artificial intelligence to analyze proprietary data from both animal tests and NAMs represents a transformative opportunity to systematically evaluate the predictive value of traditional animal testing. This type of critical assessment is long overdue. For decades, animal tests have been treated as the gold standard despite rarely undergoing the same level of formal validation demanded of NAMs. We have consistently called for objective evaluations of animal test performance, and we strongly welcome this effort to apply modern tools to legacy methods. We encourage ICCVAM to engage with the FDA in this work and consider how insights from these AI-driven analyses could inform test validation, regulatory guidance, and prioritization of replacement efforts. The outcomes of this initiative could be pivotal in building a more transparent, evidence-based, and human-relevant safety assessment framework.

Tracking Progress: Animal Use Data and Reduction Metrics

We emphasize the critical importance of comprehensive and detailed tracking of animal use across all relevant federal agencies. Without robust and standardized data collection, high-level commitments risk becoming symbolic rather than driving real change. With its multi-agency membership, ICCVAM is well positioned to coordinate harmonized data collection and reporting efforts. We encourage ICCVAM to facilitate the coordination of detailed, standardized data collection and reporting, broken down by species, test type, purpose, and other key categories to enable thorough analysis. This approach would provide clear insight into where progress is being made and where animal use remains high, helping to identify priority targets for further reduction. Drawing on successful models such as the EU's ALURES database, ICCVAM can promote transparency and equip regulators, industry, and the public with reliable data to measure and drive meaningful reductions in animal testing.

Terminology and Scope of "NAMs"

The recent FDA-NIH workshop confirmed ongoing inconsistencies in how NAMs are defined and used across agencies. For example, one agency referred to "new alternative methods," while others use "new approach methodologies." Definitions also vary regarding what qualifies as a NAM – some emphasize full replacement of animal testing, while others include reduction or refinement approaches that still involve some animal use. To promote clarity and consistency, we recommend that ICCVAM lead efforts to establish a unified definition of NAMs that prioritizes the full replacement of live animal use, while recognizing reduction and refinement only where replacement is not yet feasible. Achieving this clarity is essential to harmonize interagency initiatives and improve transparency in public communication.

International Harmonization and Leadership

We encourage ICCVAM to continue working closely with international bodies such as EMA, ICH, VICH, and OECD. ICCVAM is well-positioned to facilitate alignment between U.S. and global regulators and should actively champion updates to international guidelines that support and accelerate the adoption of non-animal methods.

The FDA-NIH Workshop demonstrated a unified and urgently needed vision for replacing animal testing with more ethical, scientifically advanced methods. ICCVAM has a key role to play in helping coordinate this transition across agencies and sectors. With multiple agencies now signalling a willingness to reduce reliance on animals, ICCVAM's leadership will be essential in ensuring these commitments translate into sustained and measurable progress. We look forward to continued collaboration and stand ready to support efforts to replace the animal tests listed in our RAT List and beyond.

Thank you for the opportunity to submit these comments.

Sincerely,

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