May 15, 2024

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RE: Interagency Coordinating Committee on the Validation of Alternative Methods; Notice of Public Meeting; Request for Public Input (89 FR 38906)

Dear Dr. Kleinstreuer,

On behalf of the Humane Society of the United States (HSUS), Humane Society Legislative Fund (HSLF), and our members and supporters, thank you for the opportunity to comment on the important ongoing work of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). HSUS and HSLF continue to support ICCVAM and its member agencies' efforts to carry out the goals of the January 2018 publication, *A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States* (the Roadmap). We look forward to further progress in the development, acceptance, and use of new approach methodologies (NAMs) at all ICCVAM agencies.

We appreciate the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods' (NICEATM) continued efforts to increase the development, acceptance, and awareness of NAMs, accomplished through regular communications about these topics with interested stakeholders. Examples of NICEATM's recent efforts to modernize the way chemicals are assessed for safety while reducing animal use for this process include:

- The January 2024 ICCVAM Communities of Practice Webinar on Implementing Computational Approaches for Regulatory Safety Assessments.
- The October 2023 workshop and webinar series Trust Your Gut: Establishing Confidence in Gastrointestinal Models.
- The May 2023 workshop From Research to Readiness: Advancing Research and Regulatory Acceptance of Microphysiological Systems for Infectious Disease Applications.

Of particular importance is the publication of the March 2024 ICCVAM Validation Workgroup's report, *Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies* (Validation report). Updating the 1997 ICCVAM validation document was necessary to address the recognized problems with the previous validation process as being "lengthy and resource-intensive."[1] This comprehensive document helps align the ICCVAM validation process with the goals of the Roadmap.

HSUS and HSLF encourage ICCVAM and its member agencies to increase efforts on the suggested areas below.

Critical evaluation of data from animal models

It is important that any work to build confidence in NAMs also includes recognition of the limitations of data from animal studies. In the Validation report, ICCVAM recognizes that "the biological relevance to the species of interest and key exposure considerations should be acknowledged in assessing both the NAM and the existing reference

[1] ICCVAM Validation Workgroup. (2024): Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies. Retrieved from: https://ntp.niehs.nih.gov/sites/default/files/2024-03/VWG_Report_27Feb2024_FD_508.pdf

test method."[2] Without a proper understanding of inherent problems with variability and uncertainty of data from animal toxicity tests for human health risk assessment, NAMs will seemingly be held to a higher standard than traditional animal tests that have never received comparable scrutiny. NICEATM scientists have published studies in 2018 comparing animal and NAMs data for skin sensitization[3] and acute oral toxicity[4] tests. These studies not only help to build confidence in NAMs but point out some of the flaws with the traditional animal models. We urge NICETAM and ICCVAM member agencies to continue performing and publishing studies that not only establish the reliability of NAMs but highlight the limitations of traditional animal testing for chemical safety assessments as well.

For example, in a joint effort of HSUS, Environmental Protection Agency (EPA), and NICEATM, an *in silico* NAM, the Collaborative Acute Toxicity Modeling Suite (CATMoS) was recently shown to reliably predict EPA hazard categories III and IV of pesticide active ingredients when compared to *in vivo* data from rat studies. It also performed well in predicting discrete LD_{50} values of >2000 mg/kg for use in wild mammal risk assessment.[5] During development of the CATMoS model in which thousands of LD_{50}s curated from public sources were used, the variability in animal data was demonstrated for those 1885 chemicals that had more than one LD_{50} value available such that a data-derived 95% confidence interval around the LD_{50} of $\pm$ 0.24 $\log_{10}$ mg/kg was established.[6]

ICCVAM member agencies should conduct retrospective analyses on animal data that have been submitted to them by industry. Agencies should specifically examine what portion of those data was utilized in their regulatory decision making. In 2023, two retrospective analyses were published that looked at the reliability of dog study data, *Is the 90-day dog study necessary for pesticide toxicity testing?* and *Retrospective analysis of dog study data from food and color additive petitions.* The former article concluded that, for most pesticide risk determinations, the 90-day dog study provided no benefit beyond other available data when determining human safety.[7] The latter concluded that alternatives to animal tests should be developed for food and color additive safety assessments.[8] In a 2021 article, *Retrospective analysis of dermal absorption triple pack data*, scientists from NICEATM and EPA presented the results of their analysis of determining the human dermal absorption factor (DAF) for agrochemicals using the traditional "triple pack," which includes rat in vivo, rat in vitro, and human in vitro studies and comparing it to the DAF found by using each study individually. The retrospective analysis concluded that "for most of the formulations, the human in vitro method provided a similar or higher estimate of dermal absorption than the triple pack approach" and was supportive of "potentially using in vitro data alone for DAF derivation for human health risk assessment of pesticides."[9] These examples of retrospective analyses demonstrate the importance and value of critically evaluating the continued regulatory need for animal data.

Clear and consistent communication with stakeholders

HSUS and HSLF urge all ICCVAM member agencies to clearly communicate with stakeholders about the acceptance of NAMs, ways to eliminate unnecessary animal testing, and appropriate processes to ensure consideration of new technologies in safety assessments. One of the Food and Drug Administration's (FDA) goals in 2023 through the New Alternative Methods Program was to "provide clear guidelines to external stakeholders developing alternative methods."[10] We look forward to seeing the agency follow through with this goal and believe the platform of

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[2] ICCVAM Validation Workgroup. (2024). *Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies.* Retrieved from: https://ntp.niehs.nih.gov/sites/default/files/2024-03/VWG_Report_27Feb2024_FD_508.pdf

[3]Kleinstreuer, Nicole et.al (2018). *Non-animal methods to predict skin sensitization (II): an assessment of defined approaches*. Critical Reviews in Toxicology, DOI: 10.1080/10408444.2018.1429386

[4] Kleinstreuer, Nicole et.al (2018). *Predictive models for acute oral systemic toxicity: A workshop to bridge the gap from research to regulation.* Computational Toxicology, DOI: 10.1016/j.comtox.2018.08.002

[5] Bishop et al. (2024). *Evaluation of in silico model predictions for mammalian acute oral toxicity and regulatory application in pesticide hazard and risk assessment*. Reg Tox Pharma 149 (105614). https://doi.org/10.1016/j.yrtph.2024.105614

[6] Karmaus et al. (2022). *Evaluation of variability across rat acute oral systemic toxicity studies*. Toxicol Sci 188(1): 34-37. https://doi.org/10.1093/toxsci/kfac042

[7] Bishop, P.L., Dellarco, V.L. and Wolf, D.C. (2023). *Is the 90-day dog study necessary for pesticide toxicity testing?* Critical Reviews in Toxicology, 53(4), pp. 207-228. doi: 10.1080/10408444.2023.2221987.

[8] Flannery, B.M., Turley, A.E., Anyangwe, N., Mattia, A., Whiteside, C., Hermansky, S., Schaefer, H.R., Tyler, T. and Fitzpatrick, S.C. (2023). *Retrospective analysis of dog study data from food and color additive petitions.* Regulatory Toxicology and Pharmacology, 145, 105523. doi: 10.1016/j.yrtph.2023.105523.

[9] Allen, D. G., Rooney, J., Kleinstreuer, N., Lowit, A. and Perron, M. (2021). *Retrospective analysis of dermal absorption triple pack data.* ALTEX - Alternatives to animal experimentation, 38(3), pp. 463–476. doi: 10.14573/altex.2101121.

[10] Food and Drug Administration. (2023). *Implementing Alternative Methods.* Retrieved from: https://www.fda.gov/science-research/advancing-alternative-methods-fda/implementing-alternative-methods#:~:text=New%20Alternative%20Methods%20Program,-FDA%27s%20New%20Alternative&text=FDA%20recognizes%20alternative%20methods%20also,program%20through%20FDA%20core%20operations.

ICCVAM can help facilitate communication on these new guidelines. ICCVAM's public forum is a unique opportunity for member agencies to not only provide information about the work they are doing to promote the development of NAMs, but also how they will realistically accept and implement them. Scientists working on NAMs should be able to obtain this information from ICCVAM's communications with stakeholders, including ongoing workshops, webinars, meetings, and website updates. Discussions of ways to avoid unnecessary animal testing and incorporate NAMs into testing plans should become a regular part of agency interactions with regulated industries. We also encourage ICCVAM member agencies to consider opportunities for incentivizing NAMs use and data submission for their regulated industries.

NAMs developers

To ensure acceptance of data from NAMs, it is important that the developers are granted an opportunity to present their new technologies to relevant regulatory agencies. Several ICCVAM member agencies have recently tried to address this need. In 2020, FDA announced the Innovative Science and Technology Approaches for New Drugs (ISTAND) Pilot Program with the stated goal of supporting "the development of novel approaches to drug development that may be acceptable for regulatory use."[11] FDA has also launched its Alternative Method Forum Series, which opens a dialogue between the agency and NAMs developers about the most recent human-relevant technological advances. In 2022, the Consumer Product Safety Commission released a guidance document *Guidance for Industry and Test Method Developers: CPSC Staff Evaluation of Alternative Test Methods and Integrated Testing Approaches and Data Generated from Such Methods to Support FHSA Labeling Requirements*, which "provides guidance to stakeholders (i.e., method developers and product manufacturers) on the process by which CPSC staff assesses whether alternative toxicological methods, integrated approaches, and the resulting data are appropriate for use."[12] HSUS and HSLF strongly support these programs and encourage agencies to devote additional funding and staff support to ensuring their success and measurable impact in reducing animal use.

Clarify requirements for animal testing and regularly update guidance documents

A main barrier to the replacement of animal tests with NAMs is the lack of understanding about what data from animal tests are or are not required by government agencies for safety testing. This is a primary contributing factor to the unnecessary animal testing industry too often performs. A 2020 article that analyzed acute toxicity "six-pack" data submitted in support of new drug applications in the U.S., revealed that, despite the existence of updated guidance documents stating that lethal dose studies were no longer needed, pharmaceutical companies continued to submit these animal data to the FDA Center for Drug Evaluation and Research (CDER).[13] In 2015, FDA published guidance for the nonclinical safety evaluation of reformulated drugs[14] that allows the use of NAMs for assessing skin and eye irritation. However, this has yet to be taken up more widely, so that drug companies continue to submit animal data for acute toxicity studies.[15] On May 15, 2024 HSUS and HSLF submitted a petition to FDA asking the agency to update its pharmaceutical regulations to clarify that animal testing is not required, publish a guidance document of available and accepted NAMs, and update all guidance documents with language encouraging industry to use those NAMs instead of animal tests when applicable.[16]

Guidance documents for all regulated sectors should be regularly updated to reflect the most recent human-relevant science. Updates should be swiftly made on all member agencies' websites and documents should be easily found by stakeholders searching for them. Outdated guidance documents should be removed to avoid confusion. As NAMs become accepted for specific tests, guidance documents that encourage animal use for those tests

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[11] Food and Drud Administration. (2024). *Innovative Science and Technology Approaches for New Drugs (ISTAND) Pilot Program.* Retrieved from: https://www.fda.gov/drugs/drug-development-tool-ddt-qualification-programs/innovative-science-and-technology-approaches-new-drugs-istand-pilot-program

[12] Consumer Product Safety Commission. (2024). *Guidance for Industry and Test Method Developers: CPSC Staff Evaluation of Alternative Test Methods and Integrated Testing Approaches and Data Generated from Such Methods to Support FHSA Labeling Requirements.* Retrieved from: https://www.cpsc.gov/s3fs-public/Guidance-for-Industry-and-Test-Method-Developers-CPSC-Staff-Evaluation-of-Alternative-Test-Methods-and-Integrated-Testing-Approaches.pdf?VersionId=6EJxcMXMu4PzZEQFQivF3AUZODrMRK5J

[13] Manuppello J, Sullivan K, Baker E. Acute toxicity "six-pack" studies supporting approved new drug applications in the U.S., 2015-2018. Regul Toxicol Pharmacol. 2020 Jul;114:104666. doi: 10.1016/j.yrtph.2020.104666. Epub 2020 Apr 23. PMID: 32335206.

[14] Food and Drug Administration. (2015). *Nonclinical Safety Evaluation of Reformulated Drug Products and Products Intended for Administration by an Alternate Route Guidance for Industry and Review Staff Good Review Practice.* Retrieved from: https://www.fda.gov/media/72246/download

[15] Manuppello J, Sullivan K, Baker E. (2020). *Acute toxicity "six-pack" studies supporting approved new drug applications in the U.S., 2015-2018.* Regul Toxicol Pharmacol. 2020 Jul;114:104666. doi: 10.1016/j.yrtph.2020.104666. PMID: 32335206

[16] The Humane Society of the U.S. & Humane Society Legislative Fund. (2024). *Citizen Petition.*

should be promptly updated to avoid any unnecessary animal testing. These updates should also include an emphasis that the accepted NAM should always be used in place of the animal test to not only avoid unnecessary animal suffering, but to also demonstrate the member agencies' commitment to prioritizing the use of NAMs as directed by the ICCVAM Authorization Act of 2000 to "promote and encourage the development and use of alternatives to animal test methods."[17] With this in mind, we strongly urge EPA to rapidly develop guidance for waiving the *in vivo* acute oral toxicity test for new pesticide active ingredients and replacing it with the CATMoS model whenever possible, and broadly communicate the availability of this NAM to industry stakeholders. Too often, the availability of NAMs for use by the regulated industry has trailed far behind the science that demonstrated their effectiveness and fit-for-purpose. We urge all ICCVAM members to speed the dissemination of information regarding NAMs once they have been proven to be acceptable to increase uptake and use by industry.

Setting clear timelines for replacing animal testing that include measures for accountability

HSUS and HSLF encourage ICCVAM member agencies to set clear timelines for how they plan to carry out their goals of replacing animals with NAMs in safety testing. The Fiscal Year 2024 Federal Appropriations report language directed FDA to provide Congress with a detailed report of its plans to create the New Alternative Methods Program and specifically called for timelines for implementing the priorities of the program.[18] EPA also published its own timeline for NAMs via the New Approach Methods Workplan,[19] updated in December 2021. This important document sets out the agency's plan to replace animal testing with NAMs with clear deliverables and timelines. EPA "identifies tangible steps to pursuing and achieving a reduction in the use of vertebrate animals for toxicity testing and related research while ensuring that the Agency's regulatory, compliance, and enforcement activities, including chemical and pesticide approvals and Agency research, remain fully protective of human health and the environment."[20] Such timelines create accountability for agencies' work on progressing NAMs development and acceptance and keep progress on track. All ICCVAM member agencies should release or update strategic plans to reduce animal use and reliance, create timelines for progress, produce metrics for tracking uptake of NAMs, and provide the incentive needed to ensure NAMs are fully incorporated into regulatory decision-making. These plans communicate agency priorities while also offering opportunities for input and collaboration among all stakeholders.

Increase international harmonization

Reducing animal use while promoting the development and acceptance of NAMs in the United States should be accompanied by efforts to seek alignment with regulatory schemes in other countries. It is imperative that the leadership at NICETAM and the member agencies of ICCVAM maintain consistent communications with regulatory agencies outside of the U.S. and continue to participate in international organizations such as the Organisation for Economic Co-operation and Development (OECD), International Cooperation on Alternative Test Methods (ICATM), and International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). Consistent communication with regulators in other countries will allow sharing of lessons learned and ensure global harmonization across product sectors, therefore maximizing efforts to reduce animal use worldwide.

Increase funding for NAMs

The eventual full replacement of animals with NAMs necessitates shifting federal funding and resources from the former to the latter. HSUS and HSLF encourage all ICCVAM member agencies to prioritize making this shift a reality. Because these new, non-animal technologies provide more human-relevant information often at a lower cost, shifting funding will increase the impact of agency dollars, without compromising human or environmental safety. HSUS and HSLF were pleased to see the creation of the National Institutes of Health Common Fund's Complement Animal Research In Experimentation (Complement-ARIE) program with the goal to "speed the development, standardization, validation, and use of human-based NAMs."[21] We appreciate this forward-thinking

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[17] ICCVAM Authorization Act of 2000, Pub. L. No. 106-545, § 4, 114 Stat. 2721.
[18] Agriculture, Rural Development, Food and Drug Administration, and Related Agencies Appropriations Bill (2024). 118th Congress
[19] Environmental Protection Agency. (2021). *New Approach Methods Work Plan.* Retrieved from: https://www.epa.gov/system/files/documents/2021-11/nams-work-plan_11_15_21_508-tagged.pdf
[20] *Ibid.*
[21] National Institutes of Health. (2024). *Complement Animal Research in experimentation (Complement-ARIE).* Retrieved from: https://commonfund.nih.gov/complementarie

approach to addressing challenges in human health while investing in the development and ultimate regulatory acceptance of NAMs.

HSUS and HSLF were also gratified that in Fiscal Years 2023 and 2024, Congress included $5 million and $6.5 million respectively for FDA to be used to "Reduce Animal Testing through Alternative Methods."[22,23] We encourage FDA to ensure this funding is used judiciously to maximize a reduction in animal testing. We encourage agencies to advocate for dedicated funding for NAMs development and acceptance and we will continue to urge Congress to provide appropriations for the prioritization of these agency efforts.

Conclusion

HSUS and HSLF welcome the opportunity to work with NICEATM or any ICCVAM agency to help achieve the common goal of replacing animals with human-relevant test methods and strategies. Thank you for the consideration of our comments.

Sincerely,

(signature redacted)

(signature redacted)

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[22] Agriculture, Rural Development, Food and Drug Administration, and Related Agencies, Appropriations Act (2023). 117th Congress. Congressional Directives 49. Retrieved from: https://www.appropriations.senate.gov/imo/media/doc/Division%20A%20-%20Agriculture%20Statement%20FY23.pdf

[23] Agriculture, Rural Development, Food and Drug Administration, and Related Agencies, Appropriations Act (2024). 118th Congress. Congressional Directives 30. Retrieved from: https://docs.house.gov/billsthisweek/20240304/FY24%20Ag%20Conference%20JES%20scan%203.2.24%20(1).pdf.