THE HUMANE SOCIETY OF THE UNITED STATES

HUMANE SOCIETY LEGISLATIVE FUND™

September 11, 2024

Dr. Milene Brownlow
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Office of Policy, Review, and Outreach
Division of Translational Toxicology, NIEHS
P.O. Box 12233
Research Triangle Park, NC 27709

RE: Scientific Advisory Committee on Alternative Toxicological Methods; Notice of Public Meeting; Request for Public Input

Dear Dr. Brownlow,

On behalf of the Humane Society of the United States (HSUS), Humane Society Legislative Fund (HSLF), and our members and supporters, we appreciate the opportunity to provide comments in response to the August 2, 2024 Federal Register notice "Scientific Advisory Committee on Alternative Toxicological Methods; Notice of Public Meeting; Request for Public Input" 89 FR 63207. The SACATM meeting agenda outlines a few main topics for discussion, which we address individually here.

Session I ICCVAM Biennial Report

HSUS and HSLF continue to be supportive of the efforts of the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) member agencies to advance new approach methodologies (NAMs) and communicate with stakeholders about efforts to replace traditional animal tests across federal agencies. With the release of the March 2024 document, *Validation, Qualification, and Regulatory Acceptance of New Approach Methodologies*, we look forward to future biennial report updates showing the impact of a measured approach to validation that allows agencies and industry to take advantage of new technologies more quickly and to build confidence in NAMs. According to the report, "this confidence can be achieved through the implementation of flexible, fit-for-purpose validation strategies that consider the intended application of the NAM."[1]

[1] ICCVAM Validation Workgroup. (2024, March). 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

Session IIA Validation Updates

HSUS and HSLF were pleased to see the creation of the ICCVAM Method Developers Forum. This is a great opportunity for NAMs developers to present their technologies to regulators. The initial forum on carcinogenicity was an important first step, but these conversations need to continue. We recommend that ICCVAM member agencies organize follow-up activities where more specific uses for the individual methods can be explored. In addition, since so many NAMs are not stand-alone replacements for traditional animal tests, it would be beneficial for the method developers to work together with the regulators to build testing strategies that incorporate the best approaches. In addition, HSUS and HSLF strongly encourage ICCVAM member agencies to engage in direct dialogue with the method developers during the forum. This will help developers as well as the other stakeholders understand the needs and concerns of the individual agencies. We look forward to future ICCVAM Method Developers Forums and encourage the agencies to take additional steps to ensure these meetings ultimately lead to regulatory acceptance of NAMs.

Session IIB NAMs Pipeline – Future Directions

HSUS and HSLF are grateful for the leading role the National Institutes of Health (NIH) has taken the last few years in advancing human-relevant technologies, not least of which are the efforts of the NIH Advisory Committee to the Director Working Group to develop a set of recommendations that ultimately helped to inform the Complement Animal Research in Experimentation (Complement-ARIE) Program.[2] This dedicated funding for the development and validation of the NAMs is welcome. Notably, the 2023 National Academies report on *Nonhuman Primate Model Systems: State of the Science and Future Needs* found that "Continued development and validation of new approach methodologies (in vitro and in silico model systems) is critically important to support further advances in biomedical research."[3] One of the current areas of focus of Complement-ARIE funding is the establishment of validation and qualification centers through the Validation Network of Regulatory Implementation. We support this effort if it leads to the regulatory acceptance of complementary methods rather than new animal testing.

We also appreciate that the first round of Complement-ARIE prizes were awarded to a mixture of translational and applied research more suited to the use of NAMs for understanding physiology and disease processes, with less emphasis on regulatory application. Complement-ARIE has bold aims including to advance understanding of human disease and to provide standardized models for predictive toxicology in order to "transform the way that we do basic,

[2] NIH Office of Strategic Coordination-the Common Fund. (2024). Complement Animal Research in Experimentation (Complement-ARIE) Program. Retrieved from: https://commonfund.nih.gov/complementarie

[3] National Academies of Sciences, E., and Medicine;. (2023). Nonhuman Primate Models in Biomedical Research: State of the Science and Future Needs. The National Academies Press. https://doi.org/10.17226/26857

translational and clinical research."[4] The development and use of NAMs to replace animals across all areas of research and testing is valuable and necessary and aligns with public opinion.[5] However, there is a lack of transparency from the Food and Drug Administration (FDA) regarding acceptance of non-animal data such that it is not clear to sponsors where non-animal data are acceptable and where animal data may still be required. Regulatory input is crucial to ensure that investment in NAMs will result in methods that accelerate drug and chemical evaluation without reliance on live animal testing.

Session III: Developmental Neurotoxicity (DNT)

HSUS and HSLF are concerned about the continued reliance on animal testing for evaluating developmental neurotoxicity (DNT), based not only on the time taken and financial costs, but also the huge numbers of animals required. There are significant scientific limitations related to the accuracy and relevancy of the data obtained from animal DNT studies, including data variability and the difficulty of translating developmental defects in animals to learning difficulties (as one example) in humans. For all these reasons, HSUS and HSLF decided to sponsor a workshop organized by FDA's Center for Food Safety and Applied Nutrition in November 2023, *State of the Science on Assessing Developmental Neurotoxicity (DNT) Using New Approach Methods (NAMs).* The stated purpose of this workshop was to "bring together experts in DNT to discuss the current state of the science on the development and use of alternative approaches to animal testing (e.g., in silico, in chemico, in vitro, and non-mammalian whole organisms) and considerations to optimize their application in regulatory decision-making." [6]

HSUS and HSLF also strongly supported the Environmental Protection Agency's (EPA) effort to replace the traditional DNT study with a battery of NAMs that focus on mode of action (MOA). In 2020, two NAMs developed by EPA's Office of Research and Development (ORD) were described in the agency issue paper, *Use of New Approach Methodologies to Derive Extrapolation Factors and Evaluate Developmental Neurotoxicity for Human Health Risk Assessment*: the microelectrode arrays (MEAs) and high-content imaging (HCI) assays. This battery of assays was "comprised of 57 assay endpoints in human and rat neural cells that evaluate proliferation, apoptosis, neurite outgrowth and maturation, synaptogenesis, and

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[4] NIH. (n.d.). Complement Animal Research in Experimentation (Complement-ARIE) – A Common Fund Proposal. Retrieved from: https://dpcpsi.nih.gov/sites/default/files/2024-01/1-1PM-OSC-Concept-Complement-ARIE-Rutter-Woychik-onepager-508.pdf

[5] Pew Research Center. (2018). Most Americans Accept Genetic Engineering of Animals that Benefits Human Health, but Many Oppose Other Uses. Retrieved from: https://www.pewresearch.org/science/2018/08/16/most-americans-accept-genetic-engineering-of-animals-that-benefits-human-health-but-many-oppose-other-uses/

[6] Joint Institute for Food Safety and Applied Nutrition. (2023). In-Person Workshop:State of the Science on Assessing Developmental Neurotoxicity (DNT) Using New Approach Methods (NAMs). Retrieved from: https://jifsan.umd.edu/events/2023_FDA_Workshop

neural network formation."[7] While these NAMs may not address every aspect of DNT, there have since been additional advancements. Blum et al. recently described the application of an in vitro battery for DNT for screening and risk assessment of chemicals[8] that was used for the preparation of a test guideline at the Organisation for Economic Cooperation and Development (OECD).[9,10,11] HSUS and HSLF encourage further exploration of the use of these NAMs-based integrated approaches for testing and assessment to spare the live animals used in DNT studies.

Session IV: Computational Resources

HSUS and HSLF strongly support the use of available computational tools and encourage NICEATM and ICCVAM agencies to continue to develop and improve upon them in collaboration with all stakeholders. In a recent joint effort between HSUS, the EPA and NICEATM, an in silico NAM, Collaborative Acute Toxicity Modeling Suite (CATMoS) was shown to reliably predict EPA hazard categories III and IV of pesticide active ingredients when compared to in vivo data from rat studies. CATMoS also performed well in predicting discrete LD_{50} values of >2000 mg/kg for use in wild mammal risk assessment.[12] The model also provides a data-derived 95% confidence interval around the LD_{50}, based on the variability in animal data determined during the development of the model,[13] which helps build confidence in the prediction when setting performance expectations for NAMs. We encourage the EPA to expedite development and distribution of a planned notice of availability to pesticide registrants for this NAM so that uptake can begin in the near future.

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[7] EPA. (2020). Agency Issue Paper: Use of New Approach Methodologies to Derive Extrapolation Factors and Evaluate Developmental Neurotoxicity for Human Health Risk Assessment. p.10

[8] Blum J, Masjosthusmann S, Bartmann K, Bendt F, Dolde X, Dönmez A, Förster N, Holzer AK, Hübenthal U, Keßel HE, Kilic S, Klose J, Pahl M, Stürzl LC, Mangas I, Terron A, Crofton KM, Scholze M, Mosig A, Leist M, Fritsche E. Establishment of a human cell-based in vitro battery to assess developmental neurotoxicity hazard of chemicals. Chemosphere. 2023 Jan;311(Pt 2):137035. doi: 10.1016/j.chemosphere.2022.137035. Epub 2022 Oct 31. PMID: 36328314.

[9] OECD (2023), Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In-Vitro Testing Battery, OECD Series on Testing and Assessment, No. 377, OECD Publishing, Paris, https://doi.org/10.1787/91964ef3-en.

10 Bal-Price, Anna et al. (2018)Strategies to improve the regulatory assessment of developmental neurotoxicity (DNT) using in vitro methods. Toxicology and applied pharmacology vol. 354 (2018): 7-18. doi:10.1016/j.taap.2018.02.008

[11] Fritsche E, Barenys M, Klose J, et al. (2018). Current Availability of Stem Cell-Based In Vitro Methods for Developmental Neurotoxicity (DNT) Testing. Toxicol Sci. 2018;165(1):21-30. doi:10.1093/toxsci/kfy178

[12] Bishop, P. L., Mansouri, K., Eckel, W. P., Lowit, M. B., Allen, D., Blankinship, A., Lowit, A. B., Harwood, D. E., Johnson, T., & Kleinstreuer, N. C. (2024). Evaluation of in silico model predictions for mammalian acute oral toxicity and regulatory application in pesticide hazard and risk assessment. Regul Toxicol Pharmacol, 149, 105614. https://doi.org/10.1016/j.yrtph.2024.105614

[13] Karmaus, A. L., Mansouri, K., To, K. T., Blake, B., Fitzpatrick, J., Strickland, J., Patlewicz, G., Allen, D., Casey, W., & Kleinstreuer, N. (2022). Evaluation of Variability Across Rat Acute Oral Systemic Toxicity Studies. Toxicol Sci, 188(1), 34-47. https://doi.org/10.1093/toxsci/kfac042

HSUS and HSLF urge NICEATM and ICCVAM member agencies to explore the ability of CATMoS to accurately predict acute oral toxicity in other chemical spaces. We would like to see broader adoption of this predictive tool and note that Dr. Kleinstreuer, as part of the August 28 webinar on CATMoS,[14] mentioned its potential applicability for assessing parasiticides regulated by the FDA's Center for Veterinary Medicine. We urge NICEATM and ICCVAM member agencies to work together to broaden the context of use for this important in silico tool.

As with CATMoS, it will be essential to ensure that as new computational models are developed, that their ability to satisfy regulatory needs is carefully considered. The ultimate goal should be that these tools are accepted as part of integrated testing strategies and a weight of evidence approach that can serve as replacements for new animal tests.

Conclusion

As we have commented in past years, HSUS and HSLF continue to encourage significant, dedicated funding for NAMs development at all ICCVAM member agencies, international cooperation to ensure NAMs are accepted around the globe, and the need to critically evaluate the utility of animal data. We welcome the opportunity to work with NICEATM or any ICCVAM agency to replace the use of animals with scientifically sound testing strategies. Thank you for the consideration of our comments.

Sincerely,

[redacted]

Vicki Katrinak
Director, Animal Research and Testing
Animal Research Issues
The Humane Society of the United States

[redacted]

Danielle Palermo
Specialist, Regulatory Affairs
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Humane Society Legislative Fund

[14] Kleinstreuer, N. (2024, August 28). CATMoS model for acute oral toxicity and evaluation of its potential use in a regulatory context. Available at https://www.youtube.com/watch?v=wsSalaSik6U