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Dear Dr. Kleinstreuer:

The following comments are being submitted on behalf of People for the Ethical Treatment of Animals (PETA) in response to the April 9 Federal Register notice by the National Institutes of Health (86 FR 18547).

First, we would like to thank the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) for its commitment to the development and application of new approach methods (NAMs). ICCVAM's activities have resulted in valuable strides toward the advancement of animal-free methods that will better predict human and environmental health outcomes. We look forward to ICCVAM's continued progress, particularly in the areas identified below.

Implementing Available, Animal-Free Methods

We appreciate ICCVAM member agencies' work toward advancing NAMs that protect human health and the environment. For example, last year, the Environmental Protection Agency (EPA) published its NAMs Work Plan, and it has made substantial progress toward achieving the work plan goals over the past year. The Office of Pollution Prevention and Toxics, Office of Pesticide Programs (OPP), and Office of Research and Development continue to conduct research or provide input on *in vitro* method development, participate in data curation and analysis projects, present webinars, and publish on collaborative work, among other efforts. A recent example of a positive outcome from a stakeholder working group is the journal article "Human-Relevant Approaches to Assess Eye Corrosion/Irritation Potential of Agrochemical Formulations," which was coauthored by the EPA, the National Toxicology Program's Interagency Center for the Evaluation of Alternative Toxicological Methods, the European Commission's Joint Research Centre, an NGO, and a not-for-profit testing organization. With the aim of using health-protective testing approaches, this paper evaluates the existing *in vitro*, *ex vivo*, and rabbit test methods with respect to human biology, rather than relying on the conventional approach to method validation, which requires a direct comparison to an unreliable reference standard. Pairing nicely with this publication is the EPA's commissioning of a National Academies of Sciences, Engineering, and Medicine report on the variability and relevance of mammalian toxicity testing and expectations for NAMs used in human health risk assessment. This work is a critical component of achieving one of the goals in the EPA's work plan—establishing scientific confidence in NAMs—and is important to the regulatory community as a whole. We applaud the EPA for leading efforts that will advance the use of health-protective NAMs at the agency and other agencies as well. The EPA's progress

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demonstrates the power of focusing on relevant and reliable science and transparent engagement with stakeholders.

Complementing its 2017 Predictive Toxicology Roadmap, the Food and Drug Administration (FDA) recently launched a webpage on "Advancing Alternative Methods at FDA" and released a report on "Advancing New Alternative Methodologies at FDA." We support the FDA's goal of using NAMs to identify toxicity and efficacy earlier in the product-development process while expediting the availability of treatments to patients, and we see several clear actions that will help the FDA achieve its goal, two of which we describe here:

- Despite its scientific, technical, and ethical limitations, the mouse bioassay (MBA) is commonly used to test for marine biotoxins in shellfish in order to fulfill FDA data needs. More specific and sensitive methods are available—and are required in some countries—but the MBA is still commonly used in the U.S. The FDA has an opportunity to put the sentiments of its Roadmap into practice by engaging with stakeholders to facilitate the rapid adoption of these more scientifically robust and humane methods. The FDA's leadership in this area would align with its Roadmap goals, and it's particularly feasible because the alternative methods are already established.
- In 2001, the FDA formally requested information and comments on animal-free models as replacements for the rat caries test—a testing requirement unique to the U.S.—to demonstrate the availability of fluoride in over-the-counter (OTC) dentifrice formulations (66 FR 52418; reopening of comment period at 67 FR 11704). In the 20 years since the FDA's initial request, studies have continued to demonstrate the human relevance of the available animal-free methods, yet there have been no updates to the OTC anticaries drug products monograph. Since animal-free methods already exist and the U.S. is the only known country to require the rat caries test, this provides another optimal opportunity for the FDA to make progress toward its Roadmap goals of advancing human-relevant science.

Antibodies

Agencies can reduce animal use while increasing the reproducibility and reliability of testing by evaluating their use of antibodies. Animal-derived antibodies are notoriously unreliable—often showing poor specificity and failing to recognize their targets—and have been labeled as a major driver of the "reproducibility crisis" in research. Numerous peer-reviewed publications have highlighted the consequences of using animal-derived antibodies, including misleading data that can have considerable public health implications and that cost laboratories worldwide hundreds of millions of dollars.[1–8] Animal-free, sequence-defined recombinant antibodies can be used in all applications in which antibodies are needed, as outlined in the EU Reference Laboratory for alternatives to animal testing's 2020 recommendation on non–animal derived antibodies. As new *in vitro* methods are being developed, it's an opportune time to ensure that antibodies used in them are animal-free, in addition to revisiting antibody use in existing test methods. Because of the large number of antibodies used in research and testing, agencies could choose to address this area using a phased approach, starting with sequencing or otherwise replacing antibodies produced using the ascites method.

Defining Metrics

We commend the steps that the EPA has taken to establish a webpage containing quantitative information on the acceptance of data waivers and acute *in vitro* eye and skin assays. This EPA OPP webpage lists, by year, the number of waivers granted and *in vitro* skin and eye tests conducted per endpoint. This information is very useful and would benefit from the addition of the number of waivers that aren't granted and animal tests that are conducted for skin and eye testing. These additions would

convey the overall scope of testing and put the numbers in context so that progress could be measured. We hope to see other ICCVAM member agencies similarly compile quantitative information on the use and acceptance of non-animal testing approaches relative to tests on animals.

The ICCVAM Metrics Workgroup's (MWG) February 2021 report, "Measuring U.S. Federal Agency Progress Toward Implementation of Alternative Methods in Toxicity Testing," was published in response to the Government Accountability Office's (GAO) recommendation for ICCVAM and its member agencies to propose metrics to help them better monitor progress in reducing animal use and to report their progress to the public. The MWG report notes that no one set of metrics can be used by all ICCVAM member agencies and recommends that each agency develop its own metrics that are relevant and practical to its unique situation and to communicate those metrics transparently to the public online.

To address the recommendation of the GAO, agencies must build on this report by developing strategies to compile quantitative information on their use and acceptance of non-animal testing approaches relative to tests on animals. At a minimum, each agency can report the number of *in vitro* and *in vivo* toxicity tests conducted internally or commissioned by the agency and the number submitted by companies to meet agency requirements or recommendations. Where applicable, this number can also include the number of tests waived based on scientific justification. To improve their ability to prioritize activities, agencies should track animal use by categories, e.g., endpoint and purpose. This is a practicable next step that has been shown to be feasible in the collection of statistics within the European Union and the U.K.

While the specific approach used will depend on the agency, metrics are listed below that may be considered as each agency establishes its own system for monitoring the use and acceptance of NAMs and reduction in animal use:

- The number of *in vivo* tests and NAMs conducted in house and funded or commissioned to be conducted externally
- The number of *in vivo* tests and NAMs submitted by companies
- The number and species of animals used (*in vivo*) or spared (via NAMs) by each test method or strategy
- The purpose of the testing—for example, whether a test is conducted for research and development, toxicity testing, or efficacy testing—and whether a test was conducted by a company to meet an explicit regulatory requirement or a recommendation of that agency
- The number of NAM submissions rejected by the agency
- The number of waivers accepted and rejected

Thank you for considering our comments.

Sincerely,

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