

August 19, 2015

Dr. Lori White, Ph.D., PMP
NTP Designated Federal Officer
Office of Liaison, Policy and Review
NIEHS/NIH
P.O. Box 12233, MDK2-03
Research Triangle Park, NC 27709

Sent via email to whiteld@niehs.nih.gov

Dear Dr. White,

The following comments are submitted on behalf of People for the Ethical Treatment of Animals (PETA) and the Physicians Committee for Responsible Medicine (PCRM) in response to the July 14, 2015 Federal Register Notice by the National Institutes of Health (NIH), “Scientific Advisory Committee on Alternative Toxicological Methods; Announcement of Meeting; Request for Comments.” The [preliminary meeting agenda](#) outlined several topic areas for public comments, and our responses below are divided among these sections.

Update on NICEATM and ICCVAM Activities

We have been pleased to see the new direction of the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) since Dr. Warren Casey was appointed director of NICEATM. He and his support staff at Integrated Laboratory Systems (ILS)-NICEATM have been spearheading many projects to reduce animal use. However, we see many opportunities to further reduce animal use within ICCVAM member agencies, and some of these are discussed below.

Federal Agency Updates

Timely acceptance of new methods and relevant revision of guidance documents

Timely adoption of new test methods by regulatory agencies is critical in order to achieve reductions in animal use. We ask that the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) encourage ICCVAM member agencies to institute plans for the rapid adoption of new test methods, clear guidance to industry on the acceptance of these methods (or preference for them), and a means to ensure that reviewers understand the new methods and the importance of the reducing animal testing. Four specific examples are provided below.

Skin sensitization testing example: In addition to the 2012 publication of the adverse outcome pathways for skin sensitization initiated by covalent binding to proteins,

Organisation for Economic Co-operation and Development (OECD) test guidelines for the direct peptide reactivity assay and the KeratinoSensTM assay were published in February 2015, and the test guideline on the human cell line activation test (h-CLAT) is expected to be published soon. Thus, sufficient information exists to allow for the use of available methods in a non-animal testing approach to predict skin sensitization in humans. Agencies should update guidance to clearly note that these methods are accepted or preferred over the animal tests. If these methods are not yet acceptable to regulatory agencies, work should be underway to address this limitation. For example, we applaud the work that the Environmental Protection Agency (EPA) is conducting in collaboration with pesticide industry companies and NGOs (including PETA and PCRM) to set up a database comparing the results of *in vitro* tests with *in vivo* tests in order to understand the applicability of the *in vitro* methods to the EPA's classification system. We urge other ICCVAM member agencies, such as the Consumer Product Safety Commission (CPSC) and the Food and Drug Administration (FDA), to ensure that the guinea pig maximization test and local lymph node assay are replaced with all due speed.

Draize testing example: Despite statements from FDA Center for Drug Evaluation and Research (CDER) officials in 2005 and onwards that the Draize skin and eye irritation test is not required or even desired, new drug applications continue to include these tests. One report noted that 94% of all skin irritation testing and 60% of all eye irritation testing conducted for new drugs approved between 2011 and 2014 reported use of the Draize test.¹ This may be due in part to the continued use of currently active guidance that implies the opposite, such as CDER's 2002 *Guidance for Industry: Immunotoxicology Evaluation of Investigational New Drugs*.² This guidance states that several *in vivo* methods, "along with the split adjuvant technique **and the Draize test**, are currently accepted by CDER for determining the sensitizing potential of drugs intended for topical use"[emphasis added]. CDER needs to conduct a review to determine how frequently the Draize test and other *in vivo* tests are submitted and to update or delete outdated guidance documents to reflect that the animal test is not required or requested.

Redbook example: We appreciate the initial steps that the Center for Food Safety and Applied Nutrition (CFSAN) has taken toward revising the Redbook. We look forward to updates from CFSAN on its plans to extend the Redbook revision to other guidance provided by CFSAN, including the many links contained within the Redbook to expanded external guidance. This includes, but is not limited to, the *Guidance for Industry: Questions and Answers About the Petition Process* and the *Bacteriological Analytical Manual*, which should be held to the same standard of review as the Redbook.^{3,4}

¹ Available at <http://ntp.niehs.nih.gov/iccvam/meetings/iccvam-forum-2015/crm-smt-508.pdf>

² FDA CDER. 2002. *Guidance for Industry: Immunotoxicology Evaluation of Investigational New Drugs*. Available at

<http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm079239.pdf>

³ FDA CFSAN. 2011. *Guidance for Industry: Questions and Answers About the Petition Process*. Available at

<http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/IngredientsAdditivesGRASPackaging/ucm253328.htm>

EPA OPPT example: Within the EPA, the Office of Pesticide Programs (OPP) has provided regulations and guidance on several aspects related to potential ways to waive or otherwise derogate from guideline studies while registering pesticides. We encourage SACATM, ICCVAM, and the EPA to pursue greater involvement of EPA's Office of Pollution Prevention and Toxics (OPPT), as companies looking to manufacture new industrial chemicals may have an interest in conducting OECD-accepted *in vitro* methods. Timely, standardized updates on new strategies or technologies that the EPA may accept could be of great interest and utility to industry. Three examples include the OECD QSAR Toolbox, the fish embryo test (OECD TG 236), and the cell transformation assays (using primary Syrian hamster embryo [SHE] cells or the BALB/c 3T3 or Bhas 42 cell lines).

Agency training

It is critical that agency inspectors and regulatory submission reviewers be well informed about current alternatives to animal testing. Reviewers must be able to identify situations in which an alternative method can be used in place of an animal test and, in order for companies to feel confident submitting these tests to regulatory agencies, there must be less reviewer-to-reviewer variability regarding the acceptance of alternatives to animal tests. To achieve these goals, we recommend that agencies:

- provide regular training opportunities for their reviewers on available non-animal alternative methods; and
- share a current list of validated alternative methods (such as [this example](#)) so that reviewers and those conducting the tests are aware of, and are using, available alternatives.

Furthermore, there is a specific need for U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) inspectors and veterinary medical officers (VMOs) to be up-to-date on available alternative methods so that they can evaluate whether claims of a lack of alternatives to a painful procedure (a Category E experiment) are accurate. It is not uncommon for Category E justifications, submitted by USDA-registered facilities in an annual report, to state that alternatives are not available even when alternatives exist. Currently, there is no indication that the USDA is addressing this critical problem, and we would like to reiterate the urgent need for thorough training of all inspectors on available alternative methods.

PCRM and PETA would be happy to help coordinate a training program by suggesting experts who could conduct the training or by helping in any other way that would be useful. Both PETA and PCRM have sponsored a number of educational seminars in a variety of lengths, topics, and venues that reach scientists making regulatory decisions at agencies, including EPA, California EPA, and the European Chemicals Agency (ECHA). For example, PETA and PCRM, independently and through their association with the

⁴ FDA CFSAN. 2014. Bacteriological Analytical Manual. Available at <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm>

International Council on Animal Protection in OECD Programmes ([ICAPO](#)), have organized for experts from the Institute for In Vitro Sciences ([IIVS](#)) and the Laboratory of Mathematical Chemistry ([LMC](#)) to share information with the U.S. EPA on *in vitro* and *in chemico* test methods and the OECD QSAR Toolbox, respectively. We encourage interested agencies to reach out to PETA, PCRM, or Dr. Anna Lowit for more information.

Tracking the use of alternative methods for biologics testing (FDA and USDA)

It would appear that neither the FDA nor USDA track the number of instances in which firms are granted exemptions from codified vaccine potency assays (including Supplemental Assay Methods 217, 624-627, 612, and 613), codified safety tests (as permitted in Center for Veterinary Biologics (CVB) memorandum 800.116), or other *in vivo* methods that can be replaced or refined by the use of anesthesia, analgesia, and humane endpoints (CVB Notice 12-12). Since the records of these exemptions surely exist and can be quantified, we urge SACATM to recommend that ICCVAM work with representatives from the FDA and USDA to assess the degree to which these available alternative methods have been used in place of the *in vivo* standard requirements they were validated to replace.

Update on progress toward achieving workshop recommendations (FDA and USDA)

PETA and PCRM would like to see ICCVAM receive regular updates from the FDA and USDA on progress toward the recommendations put forth in various workshops organized by ICCVAM so that the information may be shared at SACATM meetings. For example, among the recommendations of the 2011 “International Workshop On Alternative Methods for Human and Veterinary Rabies Vaccine Testing: State of the Science and Planning the Way Forward,” organized by NICEATM and ICCVAM along with several international partners, participants agreed on the necessity of providing anesthesia, analgesia, and humane endpoints for animals used for rabies vaccine potency testing. The following year, the USDA codified this policy in the CVB Notice 12-12. We have requested that the FDA develop and publish an equivalent policy, but the agency has not indicated that it intends to do so. We ask that the FDA provide an update on its plans to respond to this particular workshop recommendation. More generally, we request that all agencies use SACATM and ICCVAM meetings as opportunities to provide regular updates on their response to workshop recommendations. To help foster this process, we encourage ICCVAM to commit to routine review of agencies’ progress toward achieving the recommendations of ICCVAM-sponsored workshops.

On a related note, we see that a representative from the FDA is on the scientific committee of the IABS conference (September 16-18), “3Rs alternatives and consistency testing in vaccine lot release testing.” We encourage ICCVAM representatives from the USDA to participate in this conference as well. We would appreciate comments from both agencies on their current thinking regarding the adoption of the consistency approach as well as an update on any steps the agencies have taken to implement consistency measures in their policies. A standardized approach to production

consistency is anticipated to be a key component in reducing the use of animals in testing all biologics.

In vitro botulinum toxin detection method development (FDA)

In addition to the ELISA-based methods described in the *Bacteriological Analytical Manual* and in other guidance, FDA has been involved in the development of several *in vitro* methods for the detection of botulinum neurotoxins (BoNT). We would appreciate an update from the FDA on the progress of these validation studies, including those being carried out with the support of the U.S. Department of Homeland Security.

There has been a recent effort to validate a novel, automated bioluminescence-based *in vitro* assay—the BLB-Test—for use with a broad range of complex matrices that include patient specimens, environmental samples, food and beverages, animal feed, and other substrates.⁵ We would like to know if the FDA has been involved in this validation study and would like to receive any information the FDA can share on the progress of promoting the development and use of alternative BoNT assays.

Acute systemic toxicity testing

The development of alternatives for acute systemic toxicity testing is an area of significant research interest. For example, the Department of Defense (DoD)-commissioned National Research Council (NRC) report on the “[Application of Modern Toxicology Approaches for Predicting Acute Toxicity for Chemical Defense](#)” was recently published. The NRC-convened committee highlighted the concerns with using animals for predicting acute systemic toxicity testing and the importance of using non-testing approaches (including the use of existing data, structural alerts, grouping, and (Quantitative) Structure Activity Relationships (Q)SARs) with high- and medium-throughput *in vitro* assays in a tiered testing strategy. The report emphasized the importance of understanding the mechanism of action leading to acute systemic toxicity in order to design a human-relevant testing strategy. The DoD report is timely considering the additional efforts to replace, reduce, and refine the use of animals in acute systemic toxicity testing underway or recently completed. We hope to see the DoD implement the NRC’s strategy within the agency and DoD’s collaborations with other organizations to promote the use of these modern testing methods. We also encourage ICCVAM to continue to focus on acute systemic toxicity testing as a priority area, (in follow-up to its 2001 “[Report of the International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity](#)”), and to continue working closely with EURL ECVAM and other interested stakeholders on this effort.

International Activities, Opportunities, and Challenges

Lack of global harmonization is a barrier to the widespread implementation of available alternative methods, and inconsistencies have been noted in test method guidance from

⁵ Available at https://www.crackit.org.uk/portable-bioluminescence-test-botulinum-neurotoxin?utm_source=newsletter&utm_medium=email&utm_campaign=april%2015%20newsletter

U.S. regulatory agencies, the OECD, U.S. Pharmacopeia (USP), European Pharmacopoeia (Ph. Eur.), the International Organization for Standardization (ISO), among others. For example, regarding pyrogenicity testing, U.S. regulatory agencies theoretically accept the monocyte activation test (MAT) on a case-by-case basis, but ISO omits the use of the MAT, and USP guidance encourages avoidance of MAT. In contrast, Ph. Eur. encourages firms to use the MAT, unless there is a scientific justification for using another method. In our discussions with companies required to generate pyrogenicity data for their products, this variability in guidance is a frequently cited rationale against using the MAT.

To promote global harmonization, we encourage ICCVAM and NICEATM to engage additional agencies and organizations that are stakeholders in the development and promotion of regulatory test methods. For example:

- As a member of the International Cooperation on Alternative Test Methods (ICATM), we would like to see ICCVAM encourage this group to advance international harmonization efforts. We believe it would be helpful to have a forum for public participation in at least a portion of ICATM meetings.
- Representatives from standards organizations, such as USP and ISO, should be invited to future ICCVAM Public Forums and SACATM meetings to keep their leadership aware of alternative methods in development. Standardized test methods used in the U.S., including those maintained by USP and ISO, are revised infrequently and consequently do not always acknowledge alternative methods that have been validated and approved for use by U.S. agencies. This creates serious conflicts for regulated industries, as it is not uncommon for U.S. agencies to indicate that they accept the results of a given validated alternative method, and yet the same agencies refer to USP or ISO standards that do not discuss alternative methods. Considering the global influence of U.S. and E.U. policies on regulatory testing programs, involving standards organizations in the ICCVAM and SACATM discussion process would provide an ideal platform to ensure that all stakeholders share access to the most current thinking on various alternative methods.

Metrics, Measuring Success in the 3Rs

The AWA does not require the number of rats, mice, birds, or cold-blooded animals to be reported to the USDA, resulting in an unrealistic view of the number of animals used in testing. In a recent study published in the *Journal of Medical Ethics*, the authors report that 98.8% of animals used in NIH-funded research were from species not counted under the AWA, with a 72.7% increase in the use of these animals from 1997 through 2012.⁶ Thus, while USDA reports show a decrease in animal use, the total number of animals used has actually dramatically increased over the past 15 years. The total animal numbers, including mice and rats used in NIH-funded institutions, is reported to the NIH, but this data is not compiled, analyzed, or published.

⁶ Goodman J.R., Chandna A., Roe K. Trends in Animal Use at U.S. Research Facilities. *Journal of Medical Ethics*. 2015;41:7 504-505

In order to measure success in the 3Rs, accurate reporting of the number of animals used in testing is necessary. Reporting will also allow for the evaluation of whether existing alternatives are being used to the fullest extent and for the identification and prioritization of tests for which alternatives need to be developed or validated. We encourage SACATM to advise ICCVAM to work with NIH, industry, and member agencies to publish the numbers of animals of all species used to test specific endpoints.

As an example, ECHA's report on "The use of alternatives to testing on animals for REACH" is published every three years to fulfill ECHA's obligation under Article 117(3) of the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation. The reports include an in-depth statistical analysis of endpoint data from registration dossiers submitted to ECHA, provide an overall quantitative picture of options used by registrants, identify trends, and highlight areas where uptake of alternatives could be improved.⁷

A common objection to the requirement to report all animal numbers is that it would cause a significant burden on regulators who would need to calculate animal numbers from data reported in a PDF format. We suggest that agencies standardize the reporting format—for example, by updating to an electronic reporting system—so that summary reports are simpler to mine for specific data.

Creating a 3Rs Roadmap and Strategy for the U.S.

We would like to see SACATM promote the development of a comprehensive 3Rs strategy for the U.S. that supports the ongoing development and acceptance of alternative methods across regulatory agencies in a timely manner. Ideally, such a strategy would include the establishment of a 3Rs center charged with its implementation and serving as a focal point for method validation and training.

Through public and private funding of multi-year projects, such as ACuteTox, ReproTect, and SEURAT-1, the E.U. leads the world in alternative methods development. The U.S. has led the world in high throughput assay development and data interpretation, including the first example of integrating these new approaches into regulatory decision-making with the EPA's EDSP program. A U.S. 3Rs center would allow often disparate efforts by various agencies to become more efficient and focused and would provide a point of contact for better collaboration with E.U. activities.

In general, there is a need to focus on the application of new methods to replacing existing *in vivo* requirements. Regulatory agencies require toxicity testing, with both animal and non-animal methods available for many of the required endpoints. As is required in the E.U., it is critical that U.S. agencies transition to requiring the use of available non-animal alternatives when they exist rather than continuing to accept animal tests.

⁷ Available at: <http://echa.europa.eu/web/guest/about-us/the-way-we-work/plans-and-reports>

Report on Workshops and Meetings: *In Vitro* Methods to Predict Inhalation Toxicity, Good Cell Culture Practices: iPSC, Organ on a Chip

The PETA International Science Consortium Ltd. appreciated NICEATM's contributions to the February 24-25 expert working group [meeting](#) on the design of an *in vitro* test to assess the inhalation toxicity of nanomaterials. It was a productive meeting, which has led to the funding of a leading nanotechnology laboratory to design a method to predict the development of pulmonary fibrosis in humans. The long-term goal of this project is to develop an integrated testing approach that could reduce and replace the use of the 90-day rodent inhalation test by the U.S. EPA and other regulatory agencies. We look forward to continuing to collaborate with NICEATM and ICCVAM member agencies on this and other projects.

PETA and PCRM have had numerous productive partnerships with NICEATM and ICCVAM member agencies and hope that these will continue in the future. We would also like to offer our services to assist in implementing any of the above suggestions (or to assist in any other areas to reduce animal use). Please feel free to reach out with any comments or questions.

Sincerely,

Kristie Sullivan, MPH
Director
Toxicology and Regulatory Testing Issues
PCRM
ksullivan@pcrm.org
P: 510-923-9446
F: 202-527-7235

Amy J. Clippinger, Ph.D.
Associate Director
Regulatory Testing Department
PETA
AmyJC@peta.org
P: 610-701-8605
F: 757-628-0786