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PETA
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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 26, 2013 Federal Register announcement by the National Institutes of Health (NIH), "National Toxicology Program Scientific Advisory Committee on Alternative Toxicological Methods; Announcement of Meeting; Request for Comments."

New ICCVAM Vision and Procedures

We are pleased that many of our suggestions previously submitted for the draft 5-year plan have been addressed in the draft of *A New Vision and Direction for ICCVAM*. The National Institute of Environmental Health Sciences (NIEHS) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) have acknowledged that real reductions in animal use are at present drastically below expectations and are making changes to rectify that situation, starting, most importantly, with a change in leadership. We applaud the appointment of Dr. Warren Casey as the Acting Administrator of the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Test Methods (NICEATM), and we are pleased that ICCVAM-participating agencies have spoken in support of working with NICEATM in this new strategic direction.[1]

ICCVAM priority setting

The stated intention to increase focus on mechanistic *in vitro* and *in silico* test methods along with a stronger interface of NICEATM with Tox21 will significantly accelerate identification and acceptance of alternative technologies. We fully support the emphasis being placed on testing batteries and use of testing tiers rather than a one-for-one replacement of *in vivo* with *in vitro* tests.

[1] ICCVAM. 2013. Committee response to the editorial. Available at http://iccvam.niehs.nih.gov/docs/about_docs/ShortLetter-RespBirnbaum-FD-508.pdf

We are pleased to see that more resources will be devoted to non-animal approaches that can replace and refine the use of animals but stress that, whenever possible, more emphasis should be placed on complete replacement of animals to fully meet the Tox21 vision. As Ticeand coleagues. point out, there are still many hurdles to overcome before *in vitro* systems are fully accepted replacements for current *in vivo* regulatory tests; however, one area in which NICEATM may be able to play a significant role is in the support and development of metabolizing systems, which are crucial to bridging the gap between *in vitro* and *in vivo* testing.[2] Development of new validation approaches for 21st century methods is also an extremely important area in which ICCVAM can play a major role.

The Committee's initial targeting of the following three projects with a reasonable likelihood of successful short-term implementation is wise; although details may arise in further discussion, specific measures for supporting these projects have yet to be publicly outlined.

ICCVAM priority: *Leptospira* vaccine potency

We commend ongoing work by the U.S. Department of Agriculture (U.S.DA) Animal and Plant Health Inspection Service (APHIS) to reduce the number of hamsters required for the maintenance of *Leptospira* challenge cultures, and we strongly encourage ICCVAM to broaden its focus to include a number of other pressing barriers to the implementation of additional replacement and refinement opportunities. Many of these barriers are referenced in the supplementary materials referenced for the SACATM meeting and elsewhere in published literature.[3] In discussing them we ask that ICCVAM formally include in its focus the opportunities and strategies described therein to further reduce the use of hamsters in *Leptospira* vaccine potency testing. For example, the established inadequacy of standard approaches to providing pain relief and humane endpoints for animals infected with this rapidly progressing disease merits close attention from ICCVAM and specific policies put in place to resolve the problem as long as the challenge assay is in use. Attention to pain relief and humane endpoints for *Leptospira* challenge experiments also raises the question of immediate, low-resource actions that ICCVAM can take to ensure a more consistent interagency approach to these considerations. For instance, we encourage SACATM to promote the expansion of the Center for Veterinary Biologics (CVB) Notice 12-12, which establishes a policy for the use of analgesia and anesthesia during and following required challenge assays, to the U.S. Food and Drug Administration (FDA).[4] ICCVAM can further address the most frequent violation of the Animal Welfare Act at animal research facilities—failure to search for available alternatives—by ensuring that facility inspectors are equipped with up-to-date training in recognizing the use of *in vivo* methods that can be replaced.[5,6]

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[2] Tice R. et al. (2013). Improving the Human Hazard Characterization of Chemicals: A Tox21Update. Environmental Health Perspectives. 121(7): 756-765.

[3] Dozier S, Brown J and Currie A (2011). Bridging the Gap Between Validation and Implementation of Nonanimal Veterinary Vaccine Potency Testing Methods. Animals 2011(1): 414-432.

[4] United States Department of Agriculture, Center for Veterinary Biologics (2012) Veterinary Services memorandum 12-12, "Use of humane endpoints and methods in animal testing of biological products." Available at http://www.aphis.usda.gov/animal_health/vet_biologics/publications/notice_12_12.pdf

[5] U.S.DA Animal Care. 2000. Employee Survey on the Effectiveness of IACUC Regulations. Accessed September 3, 2013. Available at http://www.aphis.usda.gov/animal_welfare/downloads/iacuc/iacucaugust.pdf

ICCVAM priority: acute oral and dermal toxicity testing

The draft document says that ICCVAM is working with NICEATM to assess the utility of *in vitro* assays (e.g., 3T3 NRU) for predicting oral LD50 values. In February 2008, the Committee recommended to its agency partners that, while data from these test methods should be used in a weight-of-evidence approach for determining starting doses for *in vivo* studies, these methods were not sufficiently accurate to replace animals for regulatory hazard classification purposes. These recommendations were based on a validation study conducted by NICEATM and the European Centre for the Validation of Alternative Methods (EURL-ECVAM) that was completed in January 2005.[7]

Recently, EURL-ECVAM published a new recommendation on the 3T3 NRU assay based on a follow-up validation study to assess the capacity of the assay to identify specifically non-classified chemicals.[8] Concluding that the assay has a high sensitivity and hence a low false negative rate, EURL-ECVAM recommended that data from the assay should always be considered as an initial testing step prior to conducting animal experiments. ICCVAM should update its recommendation to its agency partners based on an evaluation of the results of this follow-up validation study.

ICCVAM priority: skin sensitization

The draft document states that "ICCVAM is developing a strategy for how it plans to evaluate skin sensitization test methods and to make progress towards skin sensitization testing without the use of intact animals." We welcome this work and make several comments later in this document. Here, we only wish to point out that there is an activity underway at OECD related to recommending Integrated Testing Strategies (ITS) for skin sensitization, and these activities should be coordinated to avoid duplication.[9]

Improving communications with stakeholders

We welcome increased coordination between ICCVAM member agencies and the U.S. National Coordinator for the OECD Test Guidelines Programme. As the Committee is well aware, there is

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[6] U.S.DA Office of the Inspector General, Western Region. 2005. Audit Report: APHIS Animal Care Program Inspection and Enforcement Activities. Accessed September 3, 2013. Available at http://www.usda.gov/oig/webdocs/33002-03-SF.pdf

[7] NIH (2006) Background Review Document (BRD): Validation of Neutral Red Uptake Test Methods NIH/In Vitro Cytotoxicity Test Methods for Estimating Acute Systemic Toxicity. Publication NO. 07-4518, November 2006. Available at http://iccvam.niehs.nih.gov/methods/acutetox/inv_nru_brd.htm

[8] EURL ECVAM (2013) Recommendation on the 3T3 Neutral Red Uptake (3T3 NRU) Cytotoxicity Assay for the Identification of Substances Not Requiring Classification for Acute Oral Toxicity. Report EUR 25946, April 2013. Available at http://ihcp.jrc.ec.europa.eu/our_labs/eurl-ecvam/eurl-ecvam-recommendations/3t3-nru-recommendation

[9] Casati et al (2013) EURL ECVAM Strategy for Replacement of Animal Testing for Skin Sensitisation Hazard Identification and Classification. Publications Office of the European Union. JRC Publication Number JRC79446. Available at: http://publications.jrc.ec.europa.eu/repository/handle/111111111/27708.

often a lag between OECD adoption and U.S. adoption of new alternative methods. For example, in 2010 the OECD adopted Test Guideline 439: In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method. While this method can completely replace rabbit skin testing in combination with *in vitro* skin corrosion methods in most regulatory contexts, it still has not yet been accepted by U.S. regulatory agencies. We suggest that ICCVAM take advantage of this new collaboration to ensure that translational issues that may arise are determined and addressed in a more timely manner.

As discussed above and below for skin sensitization, there are efforts underway at OECD to draft test guidelines for some *in vitro* methods for this endpoint. This new cooperation between the U.S. National Coordinator and ICCVAM will undoubtedly help to ensure that the drafted test guidelines meet the needs of U.S. agencies. However, we would like to point out that there are other working groups of the OECD that have expertise and are leading programs in alternative methods and strategies. These groups are mostly contained within the Task Force on Hazard Assessment (TFHA) and other subsidiary bodies of the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology[10] and include the following:

Working Party on Manufactured Nanomaterials
QSAR Toolbox Management Group
Advisory Group for Molecular Screening and Toxicogenomics (AGMST)

It is within the AGMST that the OECD's AOP program is administered, and within the TFHA that efforts to create ITS are likely to occur. As alternative strategies become more integrated into the larger OECD Environment, Health, and Safety Programme, it will be useful to actively coordinate, and participate within this larger collaborative environment, again, to ensure efficient uptake of new methods and strategies coming out of the OECD process and to prevent duplication of work between NICEATM and other international entities. As involved stakeholders, we commit to helping to strengthen these collaborative efforts.

<u>New paradigms for regulatory acceptance</u>

Taking Advantage of International Validation/Regulatory Acceptance Efforts

We have always welcomed efforts to increase the involvement of ICCVAM in EURL ECVAM, JACVAM, and other validation entities worldwide. However, we have been disappointed by the lack of clear benefits from such mutual discussions. We encourage ICCVAM and its member agencies to consider how they may be more effectively involved in validation studies led by other entities, including through the contribution of test chemicals, serving as participating laboratories, or providing statistical analyses or existing data. With more of a stake in international validation efforts, perhaps the results will be more applicable to U.S. agency needs.

[10] http://www.oecd.org/env/ehs/organisationoftheenvironmenthealthandsafetyprogramme.htm

Regarding methods which have already been endorsed by EURL ECVAM or another validation entity, as a first step, we urge ICCVAM member agencies to undertake a relevance assessment for each method in the near future. If any methods are determined likely to be relevant to any ICCVAM agency, efforts should be made to quickly determine whether data from the methods can be accepted (including ongoing discussion within the United Nations Subcommittees of Experts on Transport of Dangerous Goods and GHS regarding the implementation of GHS for transportation testing and labeling purposes). If so, this can be communicated to the relevant agency and regulated communities. If not, a document can be created that outlines the steps necessary for acceptance of data from the method. This process will transparently update member agencies and the public and may inspire stakeholder involvement to overcome identified barriers.

Biological Variability

The traditional parallel *in vivo/in vitro* validation study, or even a retrospective validation, is beset by one major flaw: the comparison of results from tests using (often) human cells and whole non-human animals may doom the new method unnecessarily. A lack of human data does not allow for an assessment of the true predictive accuracy of a new method to the species of interest: humans. This limitation has been understood for some time, if not adequately addressed.

Over time, toxicologists have come to realize another major consideration: biological variability. Tests conducted using an animal, such as the Draize eye irritation test, can lead to different classification categories upon repeated performance of the test. This differential nature has also been found to be true for the Local Lymph Node Assay (LLNA): some materials have EC3s (effective concentration for a Stimulation Index of 3 in proliferation of lymph node cells) with coefficients of variation of between 38 and 48%.[11] It is imperative that these considerations be taken into account and communicated often to peer review panels and advisory boards to prevent unrealistic expectations, such as a 0% false negative rate.

Agency Regulations

We were glad to see acknowledgement at the very end of this paper that agencies can do more to revisit their existing regulations to find "test neutral" policies that, once promulgated, can reduce significantly the number of animals used in testing.

We wish to point out that agency regulations can also be an active barrier to the uptake of new methods. Many agency regulations, especially for hazard assessment, labeling, and communication, were first written according to the animal study protocol and are reflective of results from an animal study. It can be difficult to "match" the non-animal method to characteristics describing results one can only get from an animal, especially for *in vitro* assays that often give mechanistic information.

[11] Bayesian Integrated Testing Strategy to Assess Skin Sensitization Potency: from Theory to Practice, Jaworska J, Dancik, Y, Kern P, Gerberick F, and Natsch A. J Applied Tox (Epub 2013 May 14)

Furthermore cutoff points on a continuum are usually arbitrary and not science-based. Yet, alternative methods must correctly "bin" substances within these arbitrary cutoff values or are found lacking.

Therefore we suggest NICEATM and ICCVAM agencies explore activities with the aim of de-coupling such regulations from specific animal tests and consider ways to make agency regulations more receptive to data from non-animal methods.

SACATM Agenda Item: Update on NICEATM Activities

Endocrine Disruptor Screening Program

ICCVAM's activities in this area should be driven by EPA's need to meet the stated goals in its EDSP21 workplan and the EDSP Comprehensive Management Plan. A critical factor will be both regulatory and public acceptance of the new methods as they become available for use. ICCVAM can facilitate validation of these methods at different levels, e.g., prioritization vs. screening vs. adverse effects testing. Moving away from the long-term, inter-laboratory validation process to development of a performance standard-based system should be a priority for ICCVAM in the next 5 years.

EPA's work on an estrogen pathway expert system, which was the topic of a FIFRA Science Advisory Panel (SAP) meeting in January 2013, is a major step in meeting the agency's EDSP21 goals. Development of an androgen pathway system will likely follow similarly along the lines of the estrogen approach. However, it has come to our attention that work for the thyroid pathway has not progressed to the degree of either the estrogen or androgen pathways. The thyroid pathway is considerably more complex, and only a few events in the pathway have proposed test systems. EPA acknowledges that considerably more work is needed for the thyroid pathway; this work may be an area to which NICEATM/NIEHS can direct resources and support to assist in meeting EDSP21 long-term goals.

Tox21

ICCVAM's past role with Tox21 was minimal at best. We are pleased to see that expanded collaboration between ICCVAM and involved agencies is anticipated. ICCVAM should, to the greatest extent possible, support development of Tox21 methods and play a significant role in their eventual validation.

SACATM Agenda Item: Adverse Outcome Pathways (AOPs)

As participants in the OECD, we appreciate the leadership role the U.S. has taken thus far on the international stage in promoting the AOP concept and developing specific AOPs. We also welcome indications that NICEATM is becoming involved in these efforts. While traditional OECD involvement has been filled by EPA, we encourage efforts to include NICEATM

leadership in OECD distribution lists to ensure the work that is being done at NICEATM and NIEHS is fed into the OECD AOP process.[12]

One major task to ensure the efficient development of AOPs is the recording of the pathway linkages and evidence for these linkages. An idea that has begun to gain traction is a "Wiki"-type platform that would allow individual scientists working throughout the world and in all fields to contribute to their linkages and evidence to the development and recording of various AOPs. This vision was first articulated by the late founder of the International QSAR Foundation, Dr. Gil Veith. Effectopedia is now in beta form and continues to be constructed by Dr. Veith's collaborators.[13] There is also an AOP Wiki currently being tested by the OECD through its AOP efforts. NICEATM can play an important role here: through NIEHS and NIH, it has the ability to access literally hundreds of thousands of active life sciences researchers. Participation from a much broader community than regulatory toxicology is needed in order to accomplish the ambitious goal of moving towards a pathway-based approach to predictive toxicology. We suggest consideration of how these significant human resources can contribute to this effort, perhaps through communication or funding mechanisms already in place within NIH.

SACATM Agenda Item: Skin Sensitization

We appreciate ICCVAM's and NICEATM's work in finding alternatives to the current animal tests—the LLNA, Guinea Pig Maximization Test, and the Buehler test—for dermal sensitization and look forward to learning more during the SACATM meeting. We have a few suggestions for ensuring efficient and effective uptake of replacement methods for this endpoint.

We understand that EURL ECVAM is currently in the final stages of validation for several *in vitro* methods designed to be used in a weight-of-evidence approach for determination of dermal sensitization potential. These include the Direct Peptide Reactivity Assay (DPRA), the human Cell Line Activation Test (h-CLAT), the Myeloid U937 Skin Sensitisation Test (MUSST), and KeratinoSens, which was validated independently and is under peer review at EURL ECVAM. Test guidelines for these methods are now in development at the OECD; that process is expected to take 2 to 3 years. While there has been ICCVAM member involvement in the validation, we are concerned that there may not have been enough chemicals in the validation study specifically relevant to U.S. EPA's regulatory purview (i.e., pesticides and pesticide formulations). We request that ICCVAM and NICEATM investigate whether this is the case and, if so, facilitate catch-up validation immediately so that no time is lost between the adoption of new OECD test guidelines and the ability of the EPA, or other U.S. agencies, to accept data from these *in vitro* methods for this endpoint.

We are keen to see the results of the NICEATM work to integrate Bayesian network models into ITS for this endpoint; Jaworska et al. (2013) offer some promising results for integrating high- and medium-throughput assay information in a more efficient and human approach.[11] We urge maximum cooperation on the part of ICCVAM member agencies with this effort.

[12] See also comments in above sections regarding OECD coordination.
[13] http://sourceforge.net/projects/effectopedia/ and http://effectopedia.org

First, we encourage the EPA to release the Data Evaluation Records (DERs) it has with pesticide-specific sensitization information so that this data can be compared to *in vitro* data within NIEHS and elsewhere. We also encourage industry to contribute data it holds.

However, we would also caution that the effort keep in mind regulatory agencies' needs. For example, EPA does not routinely rank sensitizers of varying potencies, except in certain cases; therefore a simple "yes/no" answer may suffice in most cases. On the other hand, the CPSC requires labeling of "strong" sensitizers; this obviously requires some indication of potency.[14]

In light of ICCVAM's new vision and the many proposed changes, we urge the Committee to elaborate the draft vision with defined goals and timelines for the priorities it has identified upon completion of this SACATM meeting. We support ICCVAM's reorientation around priority setting, improving communication, and exploring new approaches to validating and implementing nonanimal and refined toxicological test methods, and we look forward to collaborating in the future.

Sincerely,

[Redacted]

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[Redacted]

Kristie Sullivan, MPH
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[14] However, see above; we recommend ICCVAM member agencies consider harmonizing labeling approaches to simplify adoption of new methods.