June 25, 2009

Dr Mary S. Wolfe
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PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS

HEADQUARTERS
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Re: **74 FR 19562; April 29, 2009; National Toxicology Program (NTP); Office of Liaison, Policy and Review; Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)**

Dear Dr Wolfe:

People for the Ethical Treatment of Animals (PETA) is the world's largest animal rights organization, with 1.7 million members and supporters. We appreciate the continued opportunity to comment regarding the NICEATM/ICCVAM 5-Year Plan, in this instance in commenting on the *Draft Implementation Plan* (hereafter referred to as the "Draft Plan") by presenting oral comments at the meeting of SACATM June 25, 2009, and request the opportunity to submit formal written comments on the Draft Plan itself.

General Comments

We continue to believe that ICCVAM should focus its limited resources on methods that have applicability to more than one member agency.

We are encouraged to see that ICCVAM has created a Research and Development Working Group (RDWG) whose task is to help NICEATM/ICCVAM identify and promote research that incorporates new technologies. We and others, including SACATM's internal review committee for the 5-year plan, strongly advocated for a pro-active element to ICCVAM for bringing developing methods to the table for further development and validation. We will be anxious to learn the specifics about this committee including, for example, who the members of this committee, how they were selected, and a detailed plan for future activities.

For several of the Priority Areas, sections titled "Specific Objectives" and "Planned Activities for Implementation contain generic descriptions. It would be helpful if these sections contained some detail and context for the planned work, for example a summary of the state-of-the-art, how the planned activities will build on the existing foundation, and a description of the intended outcome of the activities. Similarly, descriptions of "Accomplishments" list past activities, such as "a peer review panel met" and "a report detailing the conclusions and recommendations resulting form this workshop is available." It would again be helpful if a summary of the outcome and conclusions of these activities was given, so that progress within a given area could be tracked.

A general comment about the envisioned workshops: ICCVAM workshops, both in the past and planned, regardless of topic, have the same generic goals and consist of the same generic elements. This organization does not inspire confidence that progress will be achieved; in fact,

as a case in point, these same goals and elements were used in the Workshop on Acute Chemical Testing: Advancing *In Vitro* Approaches and Humane Endpoints for Systemic Toxicity Evaluations, Feb 6 – 7, 2008. In this workshop, there was no discussion of the results of previous ICCVAM (or other) workshops on the same topic, no presentation of previous or ongoing work on the subjects, and no context for the questions asked. As a result the discussions were repetitive and did not substantially further the discussion topics. A more effective approach would be to tailor each workshop to the subject, beginning with the current state-of-the-art, inviting relevant experts that are at the forefront of the respective topics to be covered, and formulating discussion topics with defined goals in mind. Such an approach should be applied to the many workshops proposed in the Draft Plan.

General comments about Peer Reviews: Observers of two recent peer reviews, a review of Five *In Vitro* Test Methods Proposed for Assessing Potential Pyrogenicity of Pharmaceuticals and Other Products in February, 2007, and most recently an Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Strategies in May, 2009 (described in detail below), noted some similar procedural difficulties. In both cases, it was evident from the Peer Review Panel (PRP) discussion that the panel did not have a comprehensive view of the subject it was reviewing and apparently misunderstood its charge. In both cases, panel members appeared unaware of the validation and acceptance procedures, the PRP's role, or the ICCVAM process. It appeared as though panel members were provided no background information on the state-of-the-art of the current procedures and methods; panel members appeared to have unreasonable expectations regarding details of the alternative methods without a clear understanding of the limitations of the current animal-based tests. In addition, experts and stakeholders that were not allowed to interact with the panel, and were only allowed to comment ***after*** the panel had deliberated and made its recommendations. Panel members were not aware that they could ask questions of the experts present.

The peer review process could be greatly improved by providing the panel with appropriate background and context for review, along with a simple sent of focused questions for the review.

A note on expedited review (lesson learned from pyrogenicity): The European Center for the Validation of Alternative Methods (ECVAM) nominated five *in vitro* pyrogenicity methods to ICCVAM in June of 2005. Following an additional extensive and lengthy review that included a full peer review, ICCVAM issued its final recommendations in November 2008. A comparison of the letters written by then-Acting Director Wilson to US federal agencies[vii] and the ECVAM Scientific Advisory Committee statement, published in March of 2006,[viii] reveals the conclusions of each committee to be nearly identical. Delays such as this are a waste of precious time and resources. A much more expedited process is needed for reviewing methods that have already undergone extensive peer reviews.

Specific Comments

Challenge #1: Conduct and Facilitate Alternative Test Method activities in Priority Areas

Biologics Testing

The Goal and Specific Objectives in this section lack sufficient description to evaluate; however, there are a number of initiatives in this area that ICCVAM should take into account and build from when planning its activities, particularly the workshop mentioned.[123] Specifically, note should be taken of the progress made by the European Centre for the Validation of Alternative Methods (ECVAM) to validate the following: ELISA test for batch potency testing of tetanus vaccines for human use, Toxin Binding Inhibition (ToBI) test for batch potency testing of tetanus vaccines for human use, and ELISA test for batch potency testing of erysipelas veterinary vaccines, Newcastle Disease Virus (NDV) for veterinary use.

In addition to implementing the ECVAM-validated methods, the European Directorate for the Quality of Medicines and HealthCare (EDQM) is seeking to make progress on the following vaccine potency tests: Pertussis, Tetanus, Diptheria, HepA, HepB, HPV-VLP, smallpox, Yellow Fever, IPV, TBE, among others. If ICCVAM were to hasten the replacement of animal-based potency tests on these as well as other vaccine potency tests in the U.S., a great deal of animal testing would be avoided.

EDQM has also made allowances for companies to avoid target-animal safety test (TAST) for batch safety testing of vaccines for veterinary use after an appropriate number of safety tests have been completed for consecutive batches. The elimination of the target animal safety test for vaccine safety testing in the U.S. would harmonize with EU regulations thus allowing for a greater number of animal tests that would be avoided.

Leptospirosis: It is not clear what the need is for ICCVAM review of Leptospirosis vaccine potency tests being used by the USDA; these methods have been deemed appropriate and are already in use by the USDA, and there is no other agency need for these tests. USDA Supplemental Assay Methods (SAM) 624, 625, 626, and 627 allow for the use of the sandwich ELISA method for serovars *pomona, canicola, grippotyphosa,* and *icterohaemorragiae* for *Leptospira interrogans* vaccines. The successful implementation of these analytical methods (in lieu of the hamster test) has been verified by USDA as well as the pharmaceutical industry.[456]

Ocular Toxicity Testing

As an accomplishment of 2009, ICCVAM describes the Independent Scientific Peer Review Panel Meeting: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Strategies, which was held May 19- 21, 2009. This peer review was ostensibly in response to the submission of an approach to assess ocular irritation by a consortium of manufacturers of

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[1] Hendriksen, C. Refinement, Reduction, and Replacement of Animal Use for Regulatory Testing: Current Best Scientific Practices for the Evaluation of Safety and Potency of Biologicals. 2002. ILAR Journal (43) S43-S48.
[2] Cussler, K. et al. Humane Endpoints in Vaccine Research and Quality Control. 2002. Altern. Lab Anim. 30(1):93-108
[3] Halder, M. et al. *ECVAM's Activities on Biologicals*. 2002. ATLA 30:125-128.
[4] United States Department of Agriculture Center for Veterinary Biologics Testing Protocol (SAM 624) Supplemental Assay Method for *in vitro* Potency Testing *of Leptospira interrogans* Serovar *pomona* Bacterins
[5] United States Department of Agriculture Center for Veterinary Biologics Testing Protocol (SAM 625) Supplemental Assay Method for *in vitro* Potency Testing *of Leptospira interrogans* Serovar *canicola* Bacterins
[6] United States Department of Agriculture Center for Veterinary Biologics Testing Protocol (SAM 627) Supplemental Assay Method for *in vitro* Potency Testing *of Leptospira interrogans* Serovar *icterohaemorrhagiae* Bacterins

antimicrobial cleaning products (AMCP) and the Institute for In Vitro Sciences (IIVS). The consortium had been working for several years to develop and evaluate a completely non-animal method to assign ocular hazard categories required for EPA registration of AMCPs and the consortium kept ICCVAM apprised of its activities from very early in the process.

1. ICCVAM had been asked by EPA and the consortium to assess the general question of whether the proposed testing strategy would "assure EPA, with a reasonable degree certainty, that the Agency can make labeling decisions for antimicrobial cleaning products that appropriately inform the user?"
    a. ICCVAM had agreed to an expedited review; the extensive peer review therefore came as a surprise to the consortium.
    b. ICCVAM did not contact any of the participants in the consortium's effort to present the logic behind the proposal to the Peer Review Panel.
2. As part of the review, ICCVAM took it upon itself to review the validation status of the "low volume eye test" (LVET) method, which is a refinement of the Draize rabbit test and is a method that provided some of the data for the consortium's validation studies.
    a. The request additional review was unexplained since:
    b. European Centre for Validation of Alternative Methods (ECVAM) is currently reviewing this method
    c. ECVAM has compiled a comprehensive Background Review Document
    d. Only a subset of the data available to ECVAM is available to ICCVAM.
    e. The Draize test is known to significantly over predict the human response therefore
    f. the LVET method was specifically designed to be less sensitive that the traditional Draize test and more predictive of humans.
3. The ICCVAM peer review panel concluded that it was necessary to change the scoring system of the LVET to replicate exactly the Draize results.
    a. The Panel recommended a full validation study be done using approximately 50 chemicals to compare the LVET with the traditional Draize,
    b. enough data already exists to compare the two methods. In addition, the Consortium provided both animal and *in vitro* data on more than 60 antimicrobial (or similar) cleaning products (which represent the major proportion of all AMCPs on the market) yet the Panel concluded that there were not enough data to make a determination.

It was evident from the Peer Review Panel discussion that the panel did not have a comprehensive view of the subject it was reviewing and apparently misunderstood its charge[7]. The stakeholders that were present, including representatives from participants in the consortium, were only allowed to comment ***after*** the panel had finished its discussion and made its recommendations. The Panel itself was not instructed that it could ask questions of the consortium members; therefore any real debate or discussion was prohibited between consortium members and the Panel.

In the meantime, due to the lack of progress of ICCVAM on this topic, the EPA has independently initiated a pilot program which will allow, under certain conditions, for the proposed non-animal testing strategy to be used to register AMCP with the EPA.

[7] This is a continual concern within the ICCVAM process, and it has been raised by us on at least two other occasions, most notably with regard to the ICCVAM review of five *in vitro* pyrogenicity methods in February 2007. See below.

While we also applaud the use of NIH Small Business Innovation Research (SBIR) grants to fund the development and validation of non-animal methods, we question the appropriateness of the use of the SBIR mechanism for the particular topics mentioned here: the use of an alternative corneal holder and the effect of modifying test method components on accuracy and/or reliability. These topics have been in the ICCVAM plan for years and are relatively simple straightforward assessments, yet are proposed for SBIR initiatives in 2009/2010, in which case no work would actually be done until 2011/2012 at the earliest.

Acute Toxicity Testing

The first three Specific Objectives listed in this section are the same objectives that were to have been addressed in the previous two ICCVAM workshops. ICCVAM first considered *in vitro* methods for estimating actuate toxicity in 2000. Following an initial workshop, ICCVAM published a report suggesting follow-up: "Continued development and optimization of such systems (as gut absorption, BBB passage, key kinetic parameters, and metabolism) for this application should be encouraged and should receive regulatory support" as well as concluding "…if the commitment to conducting a formal validation study was strong enough, the scientific resources could be harnessed for this effort with facility and the *in vitro* tests studied proved good enough, a replacement test battery might be achieved in as short a time as 2-3 years."[8] To the best of our knowledge, none of the suggestions have been taken up. In 2008, ICCVAM finally issued recommendations to agencies that cytotoxicity methods could be used to ***set starting doses*** for acute toxicity testing. As listed as an accomplishment for 2008, ICCVAM held a second workshop addressing these same issues. In spite of these workshops, ICCVAM has made no progress toward replacing the use of animals in acute toxicity testing since it began working on this issue in 2000.

Under "Planned Activities for Implementation," the second point, work with stakeholders to promoted the collection and submission of *in vitro* and *in vivo* data in order to "advance the development and validation" of more predicitive *in vitro* test methods and more humane endpoints is too vague to be evaluated as a plan. What, exactly, is to be done, and how will it be accomplished? It is not clear how the third point, namely, participation in a group evaluating biotransformation using human cells, will accomplish any of the Specific Objectives listed in this section.

Endocrine Disruptors Testing

One of the stated purposes for creation of ICCVAM was to validate methods for the EPA's Endocrine Disruptor Screening Program (EDSP), yet not a single assay that is currently on the EDSP list has been evaluated by ICCVAM. While it is a laudable goal for ICCVAM to review and validate appropriate assays, ICCVAM's inaction in this area has driven the EPA to conduct its own validation exercises for methods not validated by Organisation for Economic Co-operation and Development Test Guidelines Programme (OECD) to be included in the Tier 1 battery of the Endocrine Disrupter Screening Program (EDSP).[9]

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[8] National Institutes of Health. 2001. Report of the International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity. NIH Publication No: 01-4499 (http://iccvam.niehs.nih.gov/docs/acutetox_docs/finalrpt/finalall0801.pdf)

[9] For a summary of test method validation and links to Peer Review Reports, see: http://www.epa.gov/endo/pubs/assayvalidation/status.htm (accessed 23 June, 2009).

Meanwhile, ICCVAM's review of the LumiCell estrogen receptor bioassay, which began over four years ago, has not been completed and the EDSP is continuing without this assay or the CertiChem, Inc., MCF-7 cell proliferation assay that is also under review by ICCVAM.[10]

Challenge #2: Incorporating New Science and Technology

Nanomaterials Testing

Again, the workshop plan description is too generic to evaluate; however, any workshop in this area should take into account and invite participants from the large international efforts already underway. A positive element of the plan for the one-day symposium to define activities within ICCVAM agencies is the explicit request for agencies to "identify current of new members with expertise specific to nanomaterials" to participate in the workshop. It would be beneficial to include such criteria in all ICCVAM activities.

High Throughput Screening

While the first part of the Specific Objective, "Facilitate the review of the usefulness and limitations of defined HTS approaches" would seem to be an appropriate action for ICCVAM, it is not clear what ICCVAM intends by the second part: "and also assist in the identification of assays and endpoints that are relevant for alternative test methods that have already been adopted." It is also not clear how the planned activates relate to or will accomplish the Specific Objective. A major issue for the incorporation of HTS data in the regulatory process is to define when and were the data can be applied; this would involve detailed conversations with regulators akin to those initiated at the recent NAS Symposium on Toxicity Pathway-Based Risk Assessment: Preparing for Paradigm Change, held in Washington, DC on May 11 – 13, 2009 (which was attended by members of NTP but not of ICCVAM).

Furthermore, evaluation of HTS and other battery approaches (such as the EDSP Tier 1 screening battery) is likely to require a different assessment paradigm than ICCVAM has developed for assessing individual tests; if ICCVAM is to be prepared to evaluate this rapidly evolving technology, this Implementation Plan should articulate the development of such an assessment strategy.

Challenge #3: Fostering acceptance and Appropriate Use of Alternative Test Methods

NICETAM-ICCVAM Website

The current version of the website is quite an improvement in terms of ease of navigation and access to documents and timelines. A significant contribution to the use of the information contained within ICCVAM's documents would be to extract the data into searchable data bases like the ToxRefDB (or perhaps even incorporate the data into this NTP database). ICCVAM's website should also inter-link with the extensive website on implementation of the NRC's Toxicity Testing

[10] Environmental Protection Agency. Agency Information Collection Activities; Submission To OMB for Review and Approval; Comment Request; Tier 1 Screening of Certain Chemicals Under the Endocrine Disruptor Screening Program (EDSP); EPA ICR No. 2249.01, OMB Control No. 2070-New [Federal Register Notice: April 15, 2009 (Volume 74, Number 71, pages 17477-17479)]

for the 21st Century currently being constructed by the EPA's Pesticide Program Dialogue Committee.

Not mentioned: A prominent role for ICCVAM could be to facilitate the use of alternative methods within agencies via its members; agency representatives should have the ability to ensure implementation of ICCVAM-recommended methods within their agency.

Challenge #4: Developing Partnerships and Strengthening Interactions with ECCVAM Stakeholders

As no specifics are presented in this section, the same comments we provided for the Five-year Plan itself are appropriate for this section of the Implementation Plan:

> "This Chapter represents yet another missed opportunity. The draft Plan contains only descriptions of past approaches to developing partnerships and fostering interactions, with several promises to continue these same approaches, all of which again which have achieved very limited success over the past decade. The point of requesting a 5 year plan is to *re-strategize*, to develop *new* approaches to *improve* and *strengthen* interactions. Again, several suggestions were provided in the animal protection community's December 2006 comments, none of which have been incorporated into the draft Plan."

In conclusion, we hope ICCVAM will build on the suggestions contained in these comments to provide a more concrete and detailed implementation plan for its next five years. In addition, we also hope there will be an opportunity to submit formal comments on the Draft Implementation Plan.

Sincerely,

[Redacted]

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[vii] Letter from Samuel Wilson to Elias Zerhouni. Dated 23 October 2008. Available at: http://iccvam.niehs.nih.gov/methods/pyrogen/transmitNov08/ZerhouniLtrPyroF.pdf; Accessed 12 December 2008.
[viii] ESAC Statement on the Validity of In-Vitro Pyrogen Tests. Published 21 March 2008. Available at: http://ecvam.jrc.it/. Accessed 16 December 2008.