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Via email to: niceatm@niehs.nih.gov

Subject: Update of the NICEATM-ICCVAM Five-Year Plan: Request for Comments

Our organization has repeatedly submitted comments to NICEATM, NIEHS, and ICCVAM over the past decade regarding ICCVAM's failure to fulfill its Congressional mandate. As a result of ICCVAM's leadership – or rather lack thereof – ICCVAM has been rendered moot as an entity that can validate and encourage the implementation of non-animal testing methods with U.S. regulatory agencies. As stated by Dr. Sandusky of the Physicians Committee for Responsible Medicine – and largely concurred with by Dr. Wilcox, a former FDA official, and industry officials and alternatives experts in the 2008 Washington Post expose of ICCVAM, "ICCVAM is being leapfrogged by the science."[1]

General Comments

The longstanding failures of ICCVAM were clearly documented in PETA's 2007 report, *Regulatory Testing: Why the U.S. Is So Far Behind Europe*;[2] in the 2008 front page *Washington Post* exposé of ICCVAM; in our 2010 detailed review of the 33 alternative methods for which ICCVAM has inaccurately claimed credit (attached); [3] and in our May 29, 2009 letter to Linda Birnbaum (attached).[4]

The ICCVAM Authorization Act of 2000 stipulates that one of the primary purposes of ICCVAM is to "ensure that new and revised test methods are validated to meet the needs of Federal agencies." Yet ICCVAM's activities have been repeatedly unresponsive to agency needs as well as in conflict with Congress' stated goal in creating ICCVAM, namely to modernize toxicity testing.

The primary agencies to which ICCVAM is relevant are the EPA and FDA, which require and actually carry out regulatory testing, and to a lesser extent OSHA, CSPC, and DOT,

[1] Washington Post, 12 April 2008. *In U.S., Few Alternatives to Testing on Animals*. Available: http://www.washingtonpost.com/wp-dyn/content/article/2008/04/11/AR2008041103733.html.
[2] PETA, 2008. *Regulatory Testing: Why Is the U.S. So Far Behind Europe?*
[3] Method Review by the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM)
[4] May 29, 2009 letter to Dr. Linda Birnbaum

which issue labeling restrictions on products. Recent examples of ICCVAM's failure to meet these agencies' needs include the following:

1. ICCVAM failed to appropriately review an industry-initiated and sponsored program to use completely non-animal methods for assessing eye irritation for anti-microbial pesticides. In response to ICCVAM's rejection of this approach, the EPA issued its own pilot program accepting data thus generated.

2. More than a decade ago, during the planning stages of ICCVAM, the EDSP was used as an example of a program that would benefit from the creation of ICCVAM, yet in the subsequent 10 years, ICCVAM has barely made a contribution. Several of the methods were reviewed through the Organization of Economic Cooperation and Development (OECD), and the EPA carried out its own validation exercises for the remainder of the assays. ICCVAM has validated only one new assay, the BG1Luc ER TA assay, and that took seven years to complete, an excessive amount of time for a test that already had a considerable amount of relevant data associated with it. Based on a very high concordance of this assay with the ER rat cytosol binding assay we urged ICCVAM to consider it as a replacement for the latter. Similarly, a high concordance of the BG1Luc ER TA assay with the uterotrophic assay suggested it as a replacement for this *in vivo* test, particularly if *in vitro* metabolizing systems were added. Yet, there is little evidence that ICCVAM has pursued investigation of either of these two potential animal saving suggestions. The second validation ICCVAM has undertaken in this area, i.e., for the CertiChem Inc. MCF-7 Cell Proliferation Test Method, began in 2006 and six years later still has not been completed..

Meanwhile, EPA recently released its Endocrine Disruptor Screening Program for the 21st Century (EDSP21) Work Plan. Subtitled *The Incorporation of In Silico Models and In Vitro High Throughput Assays in the Endocrine Disruptor Screening Program (EDSP) for Prioritization and Screening*, EDSP21 will use existing data, *in silico* models, and *in vitro* high throughput (HTP) assays to determine the relative order in which to screen chemicals, thereby reducing animal use. Its stated goal is the full replacement of *in vivo* screening assays with validated *in vitro* HTP assays, eliminating the use of animals for screening purposes, and again demonstrating that ICCVAM's approach to validation is not capable of keeping up with changing science or meeting agency needs.

3. ICCVAM is carrying out an active campaign to prohibit the U.S. from adopting the Globally Harmonized System (GHS) of labeling put forth by the United Nations and the World Health Organization. Adopting GHS for skin and eye irritation would allow for the use of completely non-animal methods to assess skin irritation using methods that were validated for this purpose by the European Centre for the Validation of Alternative Methods (ECVAM). OSHA has already agreed to adopt the GHS for skin irritation. Yet the executive director of ICCVAM (who is also the director of the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods, or NICEATM) has decided that the U.S. should not adopt GHS and has been campaigning against adoption of GHS despite the fact that this is clearly a regulatory decision outside the purview of ICCVAM or NICEATM. ICCVAM's campaign against GHS also runs counter to OSHA decisions as well as the internal decision processes of both the EPA and the FDA. Adoption of GHS represents one

instance in which ICCVAM could actually make a positive impact and completely replace animal testing for skin and eye irritation – which would be in line with the individual assessments of the relevant regulatory agencies, but instead the ICCVAM director has chosen to once again operate in a manner completely contrary to his stated mandate.

4. ICCVAM has ignored the EPA and FDA nominations for members of ICCVAM's scientific advisory board, the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM). At a recent ICCVAM meeting, nominations from the EPA and FDA were ignored; the nominations for new members instead came directly from a list provided by the executive director of ICCVAM and were not subject to review by other members of ICCVAM. As a federal agency, SACATM's formation falls under FACA regulations, which specify the following:

> *Agency officials, members of Congress, the general public, or professional societies or current and former committee members may nominate potential candidates for membership. Selection of committee members is made based on the FACA's requirements and the potential member's background and qualifications. Final selection is made by the president or heads of agencies.*

5. ICCVAM's recent review of the local lymph node assay (LLNA) did not include a review of substances that would allow for its use by the FDA for pharmaceutical dermatologic formulations, and the FDA's response to ICCVAM's recommendations stated that the FDA "is eagerly anticipating a battery of *in vitro* tests to assess dermal sensitivity as a screen for human dermal sensitivity."[5] This response points again to a lack of consideration of agency needs in ICCVAM's review of the LLNA.

With the great strides currently being made in the development of non-animal testing methods, combined with the urgent need to put this science to use to protect workers and the public, the demand for rapid and effective validation of alternative methods has never been greater. While ICCVAM has taken seven years to review a single screening method, the EPA ToxCast program currently utilizes more than 500 screening methods. Under current circumstances it would take several millennia for ICCVAM to validate these for regulatory use. ICCVAM could contribute much more effectively than its past record demonstrates; however, it cannot do so under its current leadership.

Appropriateness of priority areas regarding ICCVAM activities

In identifying areas with the highest priority for alternative test methods use, ICCVAM cites the same priority areas as it did in its 2008 five year plan (our comments attached),[6] yet shows little if any progress in these areas over the last five years.

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[5] January 6, 2011 letter from Dr. Jesse Goodman, Food and Drug Administration, to Dr. William Stokes. Available at: http://iccvam.niehs.nih.gov/methods/immunotox/transmitJune10/FDA-Response.pdf.

[6] June 7, 2007 comments to Dr. William Stokes on the Draft NICEATM-ICCVAM 5-Year Plan (2008-2012) (attached)

Identification of activities most likely to have greatest impact or promise on reducing, refining or replacing animal use in priority areas

Acute eye irritation and corrosion

Identification of *in vitro* test methods for the evaluation of eye irritants and corrosives include EpiOcular™ and SkinEthic Human Corneal Epithelial (HCE)™ which are based on 3-dimensional models using human corneal epithelial cells. Currently, more than 100 products have been tested and reported in the literature using the EpiOcular™ tissue model. Both EpiOcular™ and SkinEthic HCE™ are currently undergoing prevalidation by ECVAM as an assay for ocular irritation. The replacement of *in vivo* animal models with reconstructed human tissue offers the possibility of more efficient and relevant systems for the identification of eye irritants and corrosives. Yet, if ICCVAM's history is any indication, the US will be many years delayed in the adoption of relevant non-animal methods for the identification of eye irritants or corrosives.

Acute skin toxicity

Progress has been made in the area of replacing animal models for acute skin toxicity testing. ICCVAM has recommended "four *in vitro* corrosivity test methods for use … in an integrated testing scheme for dermal corrosion and irritation."[7] These methods include Corrositex® (InVitro International), Rat Transcutaneous Electrical Resistance (TER) assay, EpiSkin™ and EpiDerm™. ECVAM endorsed EpiSkin™ and EpiDerm™ as replacements for rabbit skin corrosivity tests in April and May 1998, respectively. However, ICCVAM has recommended that all samples which produce negative results in the *in vitro* corrosivity tests be tested *in vivo* for confirmation of results, refusing to take weight-of-evidence and other non-animal methodologies into account and increasing repetitive testing and animal suffering.

Further, ECVAM did most of the work on the validation studies which quickly resulted in an OECD test guideline,[8] with ICCVAM participating mostly in an advisory role. The validation materials listed on ICCVAM's website are all from ECVAM, with the exception of ICCVAM's comments:

- Eskes et al. The ECVAM (European Committee on the Validation of Alternative Methods) International Validation Study on In Vitro Tests for Acute Skin Irritation: Selection of Test Chemicals. ATLA 35:603-619 (2007) [PDF]
- Spielmann et al. The ECVAM International Validation Study on In Vitro Tests for Acute Skin Irritation: Report on the Validity of the EPISKIN and EpiDerm Assays and on the Skin Integrity Function Test ATLA 35:559–601 (2007) [PDF]
- Summary Report of the ECVAM Skin Irritation Validation Study (SIVS) (October 2006) [PDF]

[7] Biennial Progress Report 2008-2009, ICCVAM.
[8] TG 439 (2010): *In Vitro* Skin Irritation: Reconstructed Human Epidermis Test Method. This TG covers the EPISKIN™-RHE (Skin Ethic), EpiDerm-SIT (MatTek) and SkinEthic RHE (Skin Ethic) methods.

- Report From the Chemicals Selection Subcommittee to the Management Team on Potential Reasons for the Misclassification Of Chemicals in the EPISKIN and EpiDerm Assays [PDF]
- SIVS Phase II: Analysis of the primary endpoint MTT and the secondary endpoint IL1-α — Report from the study biostatistician to the management team [PDF]
- Performance Standards for Applying Human Skin Models to In Vitro Skin Irritation Testing (May 2007) [PDF]
- SIVS Phase I: Interim Analysis [PDF]
- August 2008 letter to Dr. Jerry Smrchek, U.S. National Coordinator for the OECD Test Guidelines Program, regarding ICCVAM comments on the December 2007 draft Test Guideline [PDF]
- NICEATM-ICCVAM Comments on the December 2007 draft Test Guideline [PDF]
- Statement of the ECVAM Scientific Advisory Committee (ESAC) on the Validity of In Vitro Tests for Skin Irritation [PDF]
- View ESAC Statements on the ECVAM website - Select "Validated Methods"
- ECVAM Peer Review Panel Report [PDF]
- View ECVAM Reports on the ECVAM website - Select "Download Study Documents"

Acute oral toxicity

Although ICCVAM has recommended consideration of the use of "one of two *in vitro* basal cytotoxicity test methods [neutral red uptake endpoint assay using (1) 3T3 murine fibroblasts or (2) normal human epidermal keratinocytes] to estimate starting doses for acute oral systemic toxicity testing"[9] evaluation of testing models which completely replace the need for *in vivo* animal testing has been glaringly absent since it was recommended by an ICCVAM workshop more than a decade ago.

The European ACuteTox project recently reported the identification of a number of *in vitro* assays that were able to flag substances as neurotoxicants and nephrotoxicants. These in vitro assays could be used to alert on tissue specific toxicity for substances that are identified as toxic with 3T3 cells. ICCVAM has done nothing to address the metabolism limitation of *in vitro* models for acute oral toxicity. Evaluation of *in vitro* models based on human liver cells, which are responsible for this metabolism, may result in the replacement of animal models for acute oral toxicity. HepaRG™ cells (Life Technologies, Inc.) are an example of an alternative model for acute oral toxicity. HepaRG™ cells are capable of metabolizing compounds and remain sensitive to toxicity of metabolites. Rather than undertake a proactive review of this method as a replacement for animal testing, ICCVAM is admittedly allowing the burden to fall on ECVAM for validation and review of HepaRG™ cells.[10]

Importantly, the requirement to ascertain acute toxicity has been removed from the International Council on Harmonization (ICH) M3 guidelines for non-clinical safety studies

[9] Biennial Progress Report 2008-2009, ICCVAM.
[10] Ibid

for human clinical trials of pharmaceuticals.[11] The current revision of these guidelines states that when acute toxicity information is available from any study, separate single-dose studies are not recommended. With regard to whether acute toxicity testing is still necessary to predict the consequences of human overdose, Chapman et al.[12] report a consensus among representatives from poison centers, the pharmaceutical and chemical industries, and regulatory bodies that the information it provides is of little value. This is partly because high doses of chemical substances often elicit non-specific effects in animals that have no relevance to incidences of human overdose. In addition, acute toxicity testing typically does not provide information on adverse and functional effects, target organ toxicity, and toxicokinetics that is considered by poison centers to be most useful.

The remaining driver for the conduct of acute toxicity studies is for the classification and labeling of chemicals.[13] However, the European Commission's ACuteTox project, the first attempt to create an integrated testing strategy based solely on in vitro and in silico methods, analyzed the consistency in classification of the 97 chemical substances included in the project. The analysis showed that based on the ranges of their reported LD50 values, only approximately 50% of the substances fall unequivocally into a single class (with at least 90% probability). Approximately 40% fall within the limits of two adjacent classes and the remaining 10% fall into three or more different classes,[14] leading the authors to recommended revision of the GHS and CLP systems.

Once again, ICCVAM's lack of activity in this arena has rendered its role moot.

Biologics and vaccines testing

The lack of progress in the area of biologics and vaccines testing is exceptionally troubling when one considers the ten million animals who are used and killed each year for general vaccine quality control tests, such as batch potency and safety tests.

With the ever mounting demand for botulinum toxin potency testing for cosmetic, wildlife monitoring and food safety, the number of animals killed in order to determine the LD50 of each batch of toxin also continually increases. PETA submitted comments (attached) [15] related to the botulinum toxicity testing suite that BioSentinal, Inc. developed and submitted for validation by ICCVAM. We recommend using a combination of existing validation data from BioSentinal and the collaborating pharmaceuticals so that a sensitive, *in vitro* assay would be poised to replace the LD50 assay currently in use. For some time, the SNAP25

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[11] ICH, 2009. Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals M3(R2). Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Multidisciplinary/M3_R2/Step4/M3_R2__Guideline.pdf

[12] Chapman, K. et al. 2010. The Value of Acute Toxicity Studies to Support the Clinical Management of Overdose and Poisoning: A Cross-Discipline Consensus. *Regulatory Toxicology and Pharmacology*, 58:354–359.

[13] Seidle, T. et al. 2010. Cross-Sector Review of Drivers and Available 3Rs Approaches for Acute Systemic Toxicity Testing. *Toxicological Sciences*, 116(2): 382–396.

[14] AXLR8, 2011. Alternative Testing Strategies Progress Report 2011. Available at: http://axlr8.eu/assets/axlr8-progress-report-2011.pdf.

[15] June 9, 2011 letter to Dr. Lori White

Endopeptidase Assay has been listed by European Pharmacopoeia (Ph. Eur.) as a replacement to the mouse LD50 assay for botulinum toxin potency testing yet, ICCVAM neglected to recommend this replacement test, as well.[16]

Human vaccine batches are tested in animals to verify potency and safety. The European Pharmacopoeia has listed nine methods that allow the reduction of animal testing and one *in vitro* method that replaces animals in testing the potency of human vaccines; of these, two have been validated and endorsed by ECVAM. In stark contrast, ICCVAM has reviewed and recommended one alternative testing method as a refinement of the rabies vaccine potency test. There has been little effort to reduce or replace animal testing on the part of ICCVAM for vaccine potency or safety, and NGOs have had to step in to the vacuum created by ICCVAM.[17]

Pyrogenicity

As stated in previous comments to ICCVAM (attached),[18] while we appreciate the effort to expand the use of the Monocyte Activation Test in order to replace the rabbit pyrogen test (RPT), we are concerned about the rabbit use proposed for the validation study. BioTest has suggested a validation study that includes the RPT and LAL along with the MAT.[19] Inclusion of these assays in parallel is an attempt to address the ICCVAM recommendations for future studies enumerated in the 2008 Test Method Evaluation Report (TMER), section 2.3.[20] BioTest also proposes to include endotoxin and non-endotoxin standards (lipotechoic acid and crude preparations from gram positive bacteria), a pro-inflammatory substance, parenteral pharmaceuticals, biologics, and devices. However, we question the need for parallel LAL and RPT testing given the inability of the LAL to detect non-endotoxin pyrogens and the abundance of existing LAL and RPT reference data available for comparison and extrapolation.

If all the reference standards and classes of products proposed are tested in rabbits, this study could lead to significant animal use. The number of animals who would be consumed by parallel testing is one of the reasons that RPT studies were not conducted as part of the original validation study performed by the European Center for the Validation of Alternative Methods (ECVAM).[21] Another reason cited by ECVAM is the fact that it is common practice to validate pyrogen tests for every given product. Rather than conducting a massive and

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[16] European Pharmacopoeia, 5th Edition, 2005.

[17] Dozier, S. et al. 2011. Bridging the Gap Between Validation and Implementation of Non-Animal Veterinary Vaccine Potency Testing Methods. *Animals*, 1:414-432

[18] White, 2011

[19] April 7, 2011 letter from BioTest AG to Dr. William Stokes. Available at: http://iccvam.niehs.nih.gov/methods/pyrogen/MAT-2011/CoverLtr-MAT-07Apr2011HK.pdf

[20] National Toxicology Program (NTP); NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM); Availability of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Test Method Evaluation Report: Validation Status of Five In Vitro Available at: http://www.federalregister.gov/articles/2008/11/24/E8-27790/national-toxicology-program-ntp-ntp-interagency-center-for-the-evaluation-of-alternative

[21] European Commission, Directorate General, JRC: Statement on the Validity of In-Vitro Pyrogen Tests. March, 2006

animal-intensive validation study, ECVAM opted for a smaller study to demonstrate the general applicability and validity of the methods for regulatory purposes, leaving validation of the assays for additional pyrogens and product classes up to manufacturers. This sensible approach should be applied here to prevent the duplicative use of rabbits in an ICCVAM validation followed by a product specific validation. Parallel studies should not be conducted. Instead, ICCVAM and BioSentinel should take advantage of RPTs currently taking place for regulatory purposes and facilitate product specific validation of the MAT. Collection of this data could, over time, fulfill data needs for validation of the MAT.

Leptospirosis

Lastly, USDA Supplemental Assay Methods (SAM) 624,[22] 625,[23] 626,[24] and 627[25] 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 approved and adopted by USDA as well as the pharmaceutical industry. Since the USDA is the agency that oversees the use of Leptospira vaccine potency testing and it has announced that this project is completed with respect to method validation, ICCVAM should therefore not be involved in this issue.

Sincerely,

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Attachments:

Method Review by the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM)
May 29, 2009 letter to Dr. Linda Birnbaum
June 7, 2007 comments to Dr. William Stokes on the Draft NICEATM-ICCVAM 5-Year Plan (2008-2012)
June 9, 2011 letter to Dr. Lori White

[22]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
[23] 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
[24] United States Department of Agriculture Center for Veterinary Biologics Testing Protocol (SAM 626) Supplemental Assay Method for *in vitro* Potency Testing *of Leptospira interrogans* Serovar *grippotyphosa* Bacterins
[25] 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