Meeting Report

ICCVAM Public Forum Meeting

June 25, 2014

Daniel M. Wilson, Ph.D., DABT

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) held a public forum meeting on June 25, 2014, at the William H. Natcher Conference Center at the NIH in Bethesda, Maryland. The purpose was to give stakeholders and members of the public an opportunity to provide suggestions to representatives of the federal agencies that comprise ICCVAM. The Public Forum was intended as inaugural meeting with more to come. ICCVAM's intention is to have at least three public meetings each year: (1) Community of Practices Webinar, (2) Stakeholder's Forum, and (3) the SACATM meeting. The agenda for the meeting is shown below:

1. Call to Order and Introductions
   Dr. Warren Casey, NICEATM
   Dr. Abby Jacobs, FDA, ICCVAM Co-Chair
   Dr. Anna Lowit, EPA, ICCVAM Co-Chair
2. ICCVAM Activities
   a. ICCVAM Committee Activities: *Dr. Lowit*
   b. Skin Sensitization Working Group Activities: *Dr. Joanna Matheson, CPSC*
   c. Open Forum
3. NICEATM Update
   a. NICEATM Update: *Dr. Casey*
   b. Discussion
4. ICCVAM Agency Updates
   a. FDA: *Dr. Jacobs*
   b. EPA: *Dr. Lowit*
   c. NIEHS: *Dr. Raymond Tice*
   d. Dept of the Interior: *Dr. Barnett Rattner*
   e. NIH: *Dr. Christine Kelley*
   f. Discussion
5. Public Attendee Presentations
   a. Physicians Committee for Responsible Medicine: *Ms. Kristie Sullivan*
   b. Center for Responsible Science: *Ms. Tammy Drake*
   c. Alternatives Research and Development Foundation: *Ms. Sue Leary*
   d. White Rabbit Beauty: *Ms. Jean Knight*
   e. Center for Alternatives for Animal Testing/Alternatives Research and Development Foundation: *Dr. Marty Stephens*
6. Wrap-up *Dr. Lowit*

Meeting Report

1) ICCVAM Activities
    a) ICCVAM Committee Activities - *Dr. Lowit*
        i) Draft 2013 document, "A New Vision and Direction for ICCVAM," describes a new direction for ICCVAM and covers three areas
            (1) Priority setting for immediate resources
            (2) Improved communications
            (3) Validation of Tox21 methods
                (a) Priorities for immediate resources
                    (i) More active Federal Agency role
                    (ii) Streamline the number of active projects
                    (iii) Establish working groups of agency experts
                    (iv) Maintain flexibility to maximize progress
                    (v) Methods need endorsement by at least one federal agency
                    (vi) Better collaboration with ECVAM
                (b) Progress on Priorities
                    (i) Acute oral and dermal toxicity
                        1. EPA has compiled oral and dermal LD50 studies
                        2. Assessed whether acute oral toxicity classifications can be used to predict dermal classifications
                        3. NICEATM developed an improved QA/QC'd acute toxicity dataset
                    (ii) Skin sensitization
                    (iii) Leptospira vaccine testing
                    (iv) Acellular Pertussis Vaccines as Alternatives to Murine Histamine Sensitization Test (HIST)
    b) Skin Sensitization Working Group Activities - *Dr. Joanna Matheson, CPSC*
        i) Criteria for acceptance of ECVAM validated methods
        ii) Cosmetics Europe skin sensitization test battery – 16 non-animal methods
        iii) Disposition of the NIOSH Electrophilic Allergen Screening Assay (EASA assay)
        iv) ECVAM has a long list of validated methods
            (1) DPRA
            (2) MUSST
            (3) h-CLAT
            (4) KeratinoSens
        v) There are "next stage" assays coming with metabolism included
        vi) Skin Sensitization AOP, very specific MIE = protein binding
        vii) ICCVAM's predictive battery proposal is to use a combination of the following:
            (1) Physiochemical parameters
            (2) *In Silico* methods
            (3) The three *in chemico* or *in vitro* assays validated by ECVAM
                (a) DPRA
                (b) h-CLAT
                (c) KeratinoSens

viii) ICCVAM has 123 compounds with all three assay results plus LLNA data for assessment of validation
ix) Statistical Networks
    (1) Bayesian networks – predict LLNA categories
    (2) Artificial Neural Networks
    (3) Support Vector Machine
x) Cosmetics Europe – has prioritized eight methods for testing 100 chemicals
xi) For EASA the only equipment needed is a spectrophotometer
xii) NICEATM has made its LLNA database available

2) NICEATM Update – Warren Casey
   a) ICCVAM is not a validation organization. ECVAM is more efficient – they will manage formal validation activities and USA ICCVAM will provide support for data analysis
   b) The Workshops on Aquatic Models and Tox21 was held at NC State University in May, 2014, was a success. Assays for induction of vitellogenin in zebrafish were a topic of interest. There was also discussion of research on cross-species read-across for rat oral LD_{50}.
   c) Efforts on validation of Tox21was mentioned with the example of converting the BG1-ER transactivation assay to a high-throughput assay being a success, with reference in Nature Science Genetics.
   d) Validation efforts are examining Manual to HTS; HTS only; HTS to Manual assay conversions
   e) Another example discussed was a skin sensitization pathway moving from Haptenation to T-cell proliferation with representative computational and in vitro data being used at different points in the pathway. Models include skin permeability, in vitro flags for electrophilic chemical activity, and QSAR models for skin sensitization populated with LLNA data.
   f) Development of an *in vitro – in vivo* extrapolation (IVIVE) model was discussed in relation to perturbations of estrogen signaling pathway. The model uses *in vitro* HT gene expression concentration data relative to ADMET Predictor estimations of bioavailability.
   g) ICCVAM is assessing practicable approaches for using in vitro and in silico predictions of xenobiotic metabolism. Currently, ADMET Pedictor™ is being used for a number of high-throughput predictions of metabolism.
   h) UNC has built a QSAR model for skin sensitization and has compared its utility relative to finding analogues using the OECD Toolbox with skin sensitization data. For some parameters the UNC Consensus model fared better.
   i) Joanna Jaworska's group published an approach (ALTEC, 2014) for an Integrated Testing and Decision Strategy for skin sensitization using Open Source Software.
   j) ICCVAM/NICEATM has built a High Quality Reference Data set for Uterotrophic Assay data covering approximately 1200 studies
   k) There is an upcoming Workshop on Regulatory Use of Adverse Outcome Pathway's (AOPs), Sep, 2014, in Bethesda, MD
   l) ICCVAM put a notice in the Fed Register seeking data and information on approaches to identify inhalation hazards using non-animal methods

3) ICCVAM Agency Updates

a) FDA - Dr. Abby Jacobs gave an overview of 3Rs implementation in several FDA Centers
   i) CDER
      (1) International Harmonization (ICH)
         (a) Reduced repetition of studies and animal use in drug development
         (b) Does not require acute lethality tox testing
         (c) Combines endpoints so no stand-alone assays for local toxicity
         (d) Established exposure and dose limits for toxicity studies
         (e) Allows exploratory clinical studies reduces animals needed to support clinical studies
         (f) Defer repro-toxicity studies until later in development for biologics
         (g) Allows the 3T3 photo-cytotoxicity assay instead of animals
         (h) Eliminated photo-carcinogenicity testing
         (i) Waive carcinogenicity studies for most biologics
         (j) Drafting criteria for waiving carcinogenicity testing for small molecules
         (k) Endorses use of SAR for impurities
         (l) Considering an *in vitro* battery to sometimes replace one species for regulatory use in reproductive toxicity testing
         (m) Considering reuse of animals normally discarded before pre-postnatal studies
      (2) Does not use the Draize test for skin or eye irritation testing – uses alternatives—accepts the BCOP assay
      (3) Accepts non-animal pyrogenicity assays
      (4) Contributes to DARPA and NCATS "human on a chip" initiatives
      (5) Supports work on a dermal sensitization non-animal battery
      (6) Supports work on pathway-based assays
   ii) CBER
      (1) Continued leadership in international efforts to replace Murine Histamine Sensitization Test (HIST) for Acellular Pertussis Vaccines
      (2) Conducting research with USDA on non-animal potency testing for rabies vaccine
      (3) Accepts non-animal endotoxin testing
      (4) Final Guidance for "Preclinical Assessment of Investigational Cellular and Gene Therapy Products" has a specific section on alternatives (III.B.8) and product area specific 3R's approaches
   iii) CFSAN
      (1) Represents FDA on Tox 21
      (2) Contributes to DARPA and NCATS "human on a chip" initiatives
      (3) Supports work on Adverse Outcome Pathways (AOPs) for As
      (4) Supports work on a dermal sensitization non-animal battery
      (5) Uses SAR and read-across when applicable
b) EPA – Dr. Anna Lowit, Office of Pesticide Programs, gave an overview of EPA's 3Rs activities
   i) Incorporation of an alternate testing framework for classifying eye irritation potential for labeling antimicrobial pesticide products

ii) OPP Guiding Principles for Data Needs – Focus on providing consistency in identification the critical data needed for risk assessment and a weight of evidence evaluation to determine data needs or to review waivers
iii) Pesticides data waivers
    (1) Document implemented by Hazard and Science Policy Council (HASPOC)
    (2) EPA considers waivers for developmental, reproductive, DNT and chronic/carcinogenicity toxicity studies; Special protocol studies (e.g., acute inhalation for fumigants, CCA studies, shorter duration) instead of standard guideline protocols; and Pharmacokinetic studies in lieu of toxicity studies
    (3) Over the past 3 years, HASPOC discussed 285 chemicals, reviewed 540 waivers, required 81 studies, waived 459 studies; equating to >60,000 animals not used and over $40 million savings to industry
iv) OPP is in a collaborative project for alternative batteries for skin sensitization, dermal irritation and skin irritation
v) Adverse Outcome Pathway (AOPs) applications include: developing and implementing QSAR, *in vitro* HTS methods; forming category read-across methods including species extrapolation; and establishing dosimetry and biomarkers for studies, epidemiology, and biomonitoring
vi) Pyrethroids and Pyrethrins were an AOP application example
    (1) OPP had been routinely requiring DNTs for all pyrethroids
    (2) Sep 2009, OPP issued a letter noting the DNT wasn't providing the type of data to assess comparative lifestage sensitivity
    (3) Dec 2010, agreement on
        (a) –*in vitro* studies: HT Na channels & neurolemma
        (b) –*in vivo* acute, behavioral studies
        (c) –PBPK models
vii) Application of AOPs for EDSP
    (1) AOPs being drafted for Estrogen, Androgen, and Thyroid pathways
    (2) The US-EPA ToxCast program uses automated high-throughput assays to screen for changes in biological activity that may suggest toxicities effects and potential adverse health effects. ToxCast may eventually limit the number of required laboratory animal-based toxicity tests.
viii) Rapid advances to implementing the 3R's into regulatory testing and alternative approaches but more work needed. Mining existing data provides a robust source of information for assessing data needs. Collaboration across sectors is critical. Harmonization and coordination across state, federal, and international regulatory agencies is important and will be a potential road-block.

c) NIEHS – Dr Ray Tice gave an overview of NIEHS's Alternatives activities
    i) Tox21 (Toxicology in the 21st Century) formed by a Memorandum of Understanding on high-throughput screening, toxicity pathway profiling, and biological interpretation of findings between NHGRI, NIEHS/NTP, EPA and FDA
    ii) Tox21goal to identify patterns of compound-induced biological response to characterize toxicity pathways, facilitate species extrapolation, model low-dose extrapolation, prioritize compounds for testing, and develop predictive models

iii) To date, Tox21 has screened10K library in 3 x at 15 concentrations in qHTS assays focused on nuclear receptors and induction of cellular stress response pathways. The data is public via PubChem
iv) The 1000 Genomes Project is using1086 Human Lymphoblastoid cell lines representing nine racial groups to screen 179 of the Tox21 compounds for toxicity using by measuring ATP production with the CellTiterGLO® kit
v) The NIEHS-NCATS-UNC DREAM Toxicogenetics Challenge used Crowd-Sourcing to better predict toxicity with a data set of genomic, cytotoxicity and chemical property data from 1000 cell lines and approximately 200 compounds. Quantitative Biomedical Research Center, UT Southwestern Medical Center, Dallas, TX, won.
vi) Phase III focusing on more physiologically relevant cell systems (e.g., human stem cell derived differentiated cell populations), including cell types that incorporate metabolism/allow for longer-term exposures; increase the use of computational models to predict metabolism/toxicity; increase the testing of compounds in alternative animal models; and develop a HT transcriptomics platform for human, rat, mouse, zebrafish, and *C. elegans.*
vii) Small business awards – went to Stem Cell Companies
    (1) MTAs with Cellular Dynamics and Molecular Dynamics
viii) Ran Simulations Plus for 8400 compounds in Tox21
ix) HT Transcriptomics platform in human, rat, mouse, zebrafish and C Elegans
    (1) Choosing 1500 genes
    (2) Picking the genes and making them public within several months

d) Dept of the Interior - Dr Rattner gave an overview of DOI's Alternatives activities
    i) Limited regulatory authority on chemicals
    ii) U.S. Geological Survey U.S. Fish and Wildlife Service
        (1) Toxicological research (hypothesis testing)
        (2) Environmental contaminant biomonitoring
        (3) Natural Resource Damage Assessment
        (4) Registration of "shot" used in hunting
        (5) Registration of chemicals used in aquaculture
    iii) Registration of non-toxic shot using existing information, risk assessment and no toxicity testing
    iv) Screening and Testing Candidate Fishery Management Chemicals
        (1) Toxicants and therapeutics screened using a tiered approach:
            (a) Physiochemical data to identify SAR
            (b) Developing *in vitro* fish cell lines to replace *in vivo* testing
            (c) Genomic analyses attempting to identify biomarkers of toxicant-sensitive and resistant species

e) NIH (Christine Kelley)
    i) Current Landscape for Drug Development
        (1) Risky Enterprise—Long Time Frame, High Attrition, Expensive, and Inefficient.
    ii) *Need for more predictive pre-clinical models for drug development*
        (1) Low efficacy and high toxicity account for approximately 70% of Phase II and 87% of Phase III clinical attrition.

(2) Improving the predictivity of pre-clinical models is a high priority.
(3) NIH, DARPA, and FDA recently made large investments in the development of innovative predictive pre-clinical in vitro models. Should reduce or perhaps eventually eliminate the need for animal models.

iii) Microphysiological Systems Program (Human-on-a-Chip)
(1) GOAL: Develop an *in vitro* platform that uses human tissues to evaluate the safety, toxicity, and efficacy of therapies.
(2) Ten human physiological systems will be functionally represented
(3) Physiologically relevant, genetically diverse, and pathologically meaningful
(4) Modular, reconfigurable platform
(5) Tissue viability for at least 4 weeks
(6) Community-wide access

iv) Need for validation
(1) Documented evidence that provides a high degree of assurance that a specific assay will consistently produce a result that meets its specifications
(2) NIH and the American Institute for Medical and Biological Engineering held a series of workshops on Validation and Qualification of New *in vitro* Tools and Models for the Pre-clinical Drug Discovery Process
(3) Goal to draft practical guidelines for technology developers on principles and practices for the validation and qualification of in vitro systems/technologies
(4) Steering Committee—NIST, FDA, NIH, Industry, Academia
(5) Series of workshops to address requirements for validating human-on-a-chip technologies

4) Public Attendee Presentations
a) Physicians Committee for Responsible Medicine
Ms. Kristie Sullivan
b) Center for Responsible Science
Ms. Tammy Drake
c) Alternatives Research and Development Foundation
Ms. Sue Leary
d) White Rabbit Beauty
Ms. Jean Knight (via teleconference)
e) Center for Alternatives for Animal Testing/Alternatives Research and Development Foundation
Dr. Marty Stephens