ICCVAM
Interagency Coordinating Committee on the Validation of Alternative Methods

NICEATM
National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

ECVAM-ICCVAM/NICEATM Workshop on Validation of Toxicogenomics-Based Test Systems

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DEPARTMENT OF HEALTH & HUMAN SERVICES · USA

ICCVAM/NICEATM

Topics

- Regulatory Acceptance of Omics Data
- ICCVAM's Role in the Omics Arena
- ECVAM-ICCVAM/NICEATM Workshop on Validation of Toxicogenomics-Based Test Systems

Regulatory Acceptance of Omics Data (1) —Validation is Key—

- Toxicogenomics (TG)—state of the science:
  - Relatively early in its developmental stages though rapidly evolving
  - Technical challenges being addressed by research/regulatory arenas
  - FDA: soliciting –omics data (FDA Guidance on PG Data Submissions, March 2005); has developed algorithms to define whether submissions are voluntary or required
  - Unresolved issues:
    - standardization
    - validation
    - data interpretation and significance
    - extrapolation of results across species/populations
    - data transmission, processing and storage
- Prudent industry-driven decisions and credible regulatory decisions, the basis of which will derive from TG, will warrant:
  - Standardization of the technology
  - Validation of the technology
  - Validation of genomic-based biomarkers
  - Validation of predictive test methods based on the technology
  - Systematic compilation of data to develop the knowledge-base necessary to establish validity of TG technology and TG-based test methods

Regulatory Acceptance of Omics Data (2)

- The perception:
  - In view of all the promise projected for –omics technologies and test methods based upon the technology, regulatory arena should be eagerly soliciting -omics data for use in their regulatory decision-making processes
- The reality:
  - Although submission of such data are currently being encouraged (e.g., FDA Guidance on PG Data Submissions, March 2005) it may be premature to base regulatory decisions solely on such data
  - Regulatory agencies have, thus far, been relatively conservative in their use of toxico- /pharmaco- genomics data derived from a technology still viewed as relatively immature and evolving, and still requiring standardization and validation
  - Agencies soliciting TG data will, for now, likely utilize those data as an adjunct to, but not in place of, traditional toxicological tests

Regulatory Acceptance of Omics Data (3)

- The challenges:
  - To standardize, organize, evaluate, and correctly interpret the meaning of data generated together with other available interdisciplinary data
  - To design & employ advanced bioinformatic processes and algorithms for capturing, managing, processing, and statistically analyzing the plethora of data
  - To demonstrate *proof-of-concept*, i.e., biological relevance of -omics information and its correlation to traditional toxicologic/pharmacologic data
  - To validate both the technology and test methods based upon the technology that will generate those data that will be integrated into the body of information to be evaluated

Regulatory Acceptance of Omics Data (4)

- The challenges (cont'd):
  - To provide useful regulatory guidance to industry and regulatory reviewers that offers a framework for data submissions and incorporates flexibility that encourages (rather than inhibits) innovative research that fosters drug development and promotes human health (e.g., FDA Guidance on PG Data Submissions, March 2005)
  - To foster and maintain an open and candid dialogue (e.g. via workshops, panels, consortia) between industry and regulatory agencies to address questions regarding:
    - technological issues (e.g. protocol standardization, validation)
    - data management
    - data interpretation and usage
    - various topics of mutual interest and concern
  - To train agency reviewers on various aspects of the technology, quality control, data analysis across platforms, biomarker validation, format and content of data submissions
    - to facilitate appropriate and consistent data evaluation
    - to build industry and public confidence in reviewer capability
    - to ensure reviewer ability to make informed –omics-based regulatory decisions

ICCVAM's Role in the Omics Arena (1)

- What role can ICCVAM/NICEATM play in promoting the validation and regulatory acceptance of TG and TG-based test methods?
- Share knowledge derived from first-hand experience
  - conducting validation studies for "conventional" alternative toxicological methods
  - evaluating data submitted in support of validation claims
  - facilitating validation and national and international adoption of new, revised and alternative test methods
- ICCVAM guidelines can provide a framework and guidance for
  - satisfying validation and regulatory acceptance criteria
  - building a technical dossier to demonstrate validation of a test method
  - navigating the processes for regulatory acceptance and implementation

ICCVAM's Role in the Omics Arena (2)

- ECVAM-ICCVAM/NICEATM Workshop on Validation of Toxicogenomics [TG]-Based Test Systems
- First of a planned series of workshops to address validation principles applicable to TG-based methods
- Co-organized/co-sponsored/co-chaired by ECVAM and ICCVAM/NICEATM
- Venue: ECVAM (European Centre for the Validation of Alternative Methods), Ispra, Italy, December 11-12, 2003
- Meeting report:
  - Summarizes discussions and presents workshop recommendations for future directions and priorities
  - *Environmental Health Perspectives* (in press)
  - Available at EHP on-line (17 August 2005): *Environ Health Perspect doi:10.1289/ehp.8247 available via http://dx.doi.org/*
  - Included in book prepared for this SACATM meeting (Tab 6)

Workshop Participants

- Raffaella Corvi, ECVAM (Co-chair)
- Leonard Schechtman, FDA/NCTR, ICCVAM (Co-chair)
- Hans-Jürgen Ahr, Bayer AG, Germany
- Silvio Albertini, Hoffmann-LaRoche, Switzerland
- David Blakey, Health Canada, Ottawa, Ontario, Canada
- Libero Clerici, PCE—Physico-Chemical Exposure, JRC, Italy
- Sandra Coecke. ECVAM
- George Douglas, Health Canada, Ottawa, Ontario, Canada
- Laura Gribaldo, ECVAM
- John Groten, TNO, Netherlands
- Berndt Haase, GeneLogic
- Karen Hamernik, EPA, ICCVAM
- Thomas Hartung, ECVAM
- Tohru Inoue, NIHS, Japan
- Ian Indans, Health Safety Executive, UK
- Daniela Maurici, ECVAM
- George Orphanides, Syngenta, UK
- Diana Rembges, PCE—Physico-Chemical Exposure, JRC, Italy
- Susanna-Assunta Sansone, EMBL—European Bioinformatics Inst., UK
- Jason Snape, AstraZeneca, UK
- Eisaku Toda, OECD, Paris, France
- Weida Tong, FDA/NCTR
- Joost van Delft, University of Maastricht, Netherlands
- Brenda Weis, NIEHS

William Stokes, NIEHS, NICEATM, Co-organizer

ECVAM-ICCVAM/NICEATM Workshop on Validation of Toxicogenomics [TG]-Based Test Systems (1)

- Objectives:
  - to discuss and define principles applicable to the validation of TG platforms as well as validation of toxicological test methods that incorporate TG technologies
  - to consider the applicability of ICCVAM/ECVAM validation principles and criteria to TG and TG-based test methods
  - to provide an opportunity for dialogue between technological experts, regulators, and the principal validation bodies
  - to identify those factors to which the validation process would be applicable
  - to establish the foundation that will facilitate future regulatory acceptance of scientifically valid toxicogenomics-based methods

ECVAM-ICCVAM/NICEATM Workshop on Validation of TG-Based Test Systems (2)

- Rationale
  - Promotion of dialogue now, as the technology is evolving and associated challenges are identified, would serve as the basis for the future validation of the technology when it reached the appropriate stage
  - Addressing critical validation issues early on and in parallel with the evolutionary and maturation phases of the technological development of TG-based methods, may help to:
    - pre-empt many potential pitfalls and data gaps encountered with retrospective method evaluations that could impede validation and adoption of this promising research and regulatory tool
    - facilitate buy-in and confidence in the technology by industry and the regulatory arena

ECVAM-ICCVAM/NICEATM Workshop on Validation of TG-Based Test Systems (3)

- Practical considerations for validation to proceed
  - New and innovative approaches will likely be necessary to evaluate the scientific validity and regulatory applicability of TG-based test methods
  - The validation process will likely be different and more complex than that for typical alternative methods, since both the predictive test system and the applied new technology itself will need to be validated
  - Regulatory acceptance of TG-based test methods and strategies will depend upon standardization and validation approaches rooted firmly in high quality science
  - TG technological advancements will continue to result in new/modified/revised TG-based test methods, which will necessitate
    - comparison of their performance with that of existing (e.g., *in vivo*) methods
    - flexible and adaptable validation processes commensurate with and able to accommodate technological advancement

ECVAM-ICCVAM/NICEATM Workshop on Validation of TG-Based Test Systems (4)

- Breakout Groups, each comprised of technical experts, regulators, and validation specialists
- Focus areas:
  1. Biological aspects: Biological validation of toxicogenomics-based test methods for regulatory decision-making
  2. Technological aspects: Technical and bioinformatics aspects related to validation
  3. Regulatory aspects: Validation issues as they relate to regulatory acceptance and utilization of TG-based test methods

ECVAM-ICCVAM/NICEATM Workshop on Validation of TG-Based Test Systems (5)

- Issues addressed
  - Differences in platforms
  - Impact of changes of arrays/sets of genes
  - QA and GLP compliance
  - Technology standardization
  - Degree of variability considered acceptable
  - Technology transferability
  - Assessment of intra- and inter- laboratory reproducibility
  - Reference materials that would serve to standardize the methods and demonstrate relevance to *in vivo* biological responses
  - Data evaluation and analysis procedures
  - Generation and maintenance of databases
  - Validation approaches that should be considered
  - "Yardstick(s)"/standards against which TG responses should be measured
  - The need for comparing *in vitro* and *in vivo* TG data
  - Adequate demonstration of biological (*in vivo*) relevance
  - Regulatory considerations for acceptability of the technology for use in the regulatory decision-making process

TG Validation Focus Area 1: Biological Aspects

- Strategies for developing and validating TG-based test methods were considered
- Two strategies proposed that would support regulatory decision-making:
  1) Phenotypic anchoring of gene expression changes to identify molecular mechanisms and candidate biomarkers of toxicity (e.g. single genes, proteins), thereby enhancing existing toxicity prediction strategies
  2) Identification and validation of predictive gene expression signatures of toxicity

TG Validation Focus Area 1: Biological Aspects

- Discussion points:
  - Initial focus on relatively simple biomarkers [e.g. single or small sets of genes or proteins; early (prognostic) biomarkers of response]
    - Conduct TG-based tests and associated conventional toxicological tests in parallel to generate comparative data, characterize biological variability, and provide mechanistic data to help in establishing biological relevance in toxicological context
    - Markers should be reproducible, capable of distinguishing toxic from non-toxic responses, and robust enough to withstand technological advances and differences in microarray platforms
  - This approach was considered appropriate in view of the state of the science and the need for a clear understanding of the toxicological relevance of the gene expression signals detected by this technology in order to achieve regulatory acceptance

TG Validation Focus Area 1: Biological Aspects

- Additional considerations—eventual needs:
  - Longer-term focus should be on use of gene expression signatures (i.e. *patterns* of gene expression rather than the measuring single or small sets of genes) in toxicity prediction
  - To determine TG's predictive capability of effects of unknown compounds
  - To characterize system performance
  - Screening vs. full replacement testing—
    - To determine the extent of validation needed (may differ)
  - To perform parallel comparative *in vivo* and *in vitro* studies to identify potential *in vitro* surrogates for *in vivo* systems
  - Once adequately validated, TG has greater likelihood of being integrated into a hierarchical approach of safety/hazard/risk assessment

TG Validation Focus Area 2: Technological Aspects

- Three types of validation were identified:
  - Array Manufacturer/Provider – QA/QC validation
    - microarray sequence verification
    - robustness of array platform
    - fabrication in accordance with GMPs
  - Experimental toxicologist and array manufacturer or provider – "routine validation"
    - sequence verification
    - use of common reference RNA standards – to facilitate comparison between array platforms
    - initial implementation of "Best Practices" for TG, ultimately giving way to GLP compliance
  - Validation triggered by introduction of procedural change(s)
    - e.g., changes in microarray technology, experimental design
    - distinction between major and minor changes to help determine the extent of validation necessary

TG Validation Focus Area 2: Technological Aspects

- Additional considerations:
  - MIAME-Tox compliance (Minimal Information About a Microarray Experiment—Toxicology)
  - Use of performance standards & reference substances to:
    - Evaluate minor vs. major procedural changes
    - Conduct "re-validations"—using flexible approaches that ensure reliability and accuracy of a modified method but not needlessly hamper technological advancement
    - Periodically reassess a test method's performance (accuracy and reliability) of the modified test method relative to the antecedent method
  - Long-term goal sought:
    - Creation of an internationally compatible informatics platform of integrated scientific data from various disciplines with a structured standard format that would facilitate data analysis and data comparisons and be available for regulatory evaluation purposes

TG Validation Focus Area 3: Regulatory Aspects (1)

- Validation issues identified, re. regulatory acceptance of TG-based test methods and TG-derived data
  - Scientific validity of such methods for their proposed uses is pivotal for them to be considered reliable and acceptable for informed regulatory decision-making purposes
    - Validation will help establish the potential of such methods in contributing to regulatory assessment processes, e.g. as screens, supplementary or complementary endpoints to traditional test methods, or as replacements for traditional test methods
    - Validation will help secure confidence in the prediction capabilities of the technology
  - The need for attendant validation as the technology advances in such a way as to avoid the need for a complete revalidation with every new innovation (e.g., a modular approach to validation)
  - The institution of validation strategies that do not stifle innovation and technical progress

TG Validation Focus Area 3: Regulatory Aspects (2)

- Validation issues identified as needing further exploration
  - Intra- and inter- laboratory reproducibility
  - Biological relevance
  - Applicability of current ICCVAM and ECVAM validation and acceptance criteria to TG technology and to TG-based test methods
  - Regulatory criteria that will determine the acceptability and implementation of the technology for regulatory assessment purposes
  - Prospect of development and validation of *in vitro*-based TG methods as surrogates for those *in vivo*:
    - for potential applicability in the regulatory arena
    - as pre-screens or for complementary/adjunct testing
    - for potential contributions to animal welfare

TG Validation Focus Area 3: Regulatory Aspects (3)

- Training of regulatory reviewers is viewed as vital
  - Regulators will need to develop a level of comprehension of such factors as:
    - the TG technology
    - test methods based on the technology
    - the data generated
    - the quality and reproducibility of those data
    - the limitations of the technology
    - data analysis and interpretation
    - the relationships of those data to toxicological outcomes of interest (biological relevance)
    - incorporation of TG information into safety/hazard/risk assessments
    - possible incongruities between TG-derived data and conventional toxicity data

TG Validation Focus Area 3: Regulatory Aspects (4)

- Strategies for ensuring regulatory community preparedness
  - Intra- and inter- agency training courses and workshops
  - Establishment of Centers of Excellence and dedicated laboratories focused on various –omics and related informatics areas, e.g.,
    - FDA/NCTR Center for Functional Genomics
    - FDA/NCTR Center for Toxicoinformatics
    - NIEHS National Center for Toxicogenomics (NCT)
  - Hiring scientists with the needed expertise
  - Cultivating communication links between the regulatory community and external scientists to share experiential and theoretical knowledge

TG Validation Focus Area 3: Regulatory Aspects (5)

- Harmonization of TG-based test methods — prerequisites:
  - Standardization of test methodologies and test components (platforms)
  - Validation of specific test protocol(s) developed for a specific purpose(s), as conducted by International Validation Bodies (IVBs), e.g., ECVAM, ICCVAM/NICEATM, JaCVAM
  - Collaboration between IVBs and OECD
    - To ensure appropriate crafting of harmonized OECD TG-based test guidelines
    - To ensure test guidelines are based upon standardized, adequately validated, practical procedures
    - To ensure test guidelines permit consistent scientific results and congruent regulatory judgments

Discussion Question for the SACATM

- Lead Discussants: Drs. Safe, Sonnenschein, Curren
- Do you have any comments on the recommendations and priorities outlined in the publication "Validation of Toxicogenomics-Based Test Systems: ECVAM-ICCVAM-NICEATM Considerations for Regulatory Use"?
  - Do you support those recommendations?
  - Do you envision other (additional) recommendations?
- Manuscript (SACATM book, Tab 6), tables 1, 2, and 3:
  - Recommendations: Focus on Biological Systems (Table 1)
  - Recommendations: Focus on Technology (Table 2)
  - Recommendations: Focus on Regulatory Acceptance of TG-Based Methods (Table 3)