

**Appendix A2**  
**Summary Minutes from Peer Review Panel Meeting on**  
**February 6, 2007**

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**Meeting Summary**

**Independent Peer Review Panel Meeting  
 Five *In Vitro* Test Methods Proposed for Assessing Potential Pyrogenicity of  
 Pharmaceuticals and Other Products  
 National Institutes of Health (NIH), Natcher Conference Center  
 Bethesda, MD**

**February 6, 2007  
 8:30 a.m. – 5:00 p.m.**

**Panel Members:**

Karen Brown	DRL Pharma and Pair O'Doc's Enterprises
Brian Crowe	Baxter Vaccine AG
Nancy Flournoy	University of Missouri-Columbia
Ihsan Gursel	Bilkent University
Ken Ishii	ERATO, Japan Science & Technology Agency, Osaka University
Jack Levin	University of California-San Francisco
Albert Li	<i>In Vitro</i> ADMET Laboratories
David Lovell	University of Surrey
Melvyn Lynn	Eisai Medical Research
Anthony Mire-Sluis	AMGEN, Inc.
Jon Richmond	UK Home Office
Peter Theran	MSPCA
Kevin Williams	Eli Lilly

**ICCVAM and ICCVAM Pyrogenicity Working Group (PWG) Members:**

Mustafa Akkoyunlu	FDA/CBER
Peter Amin	FDA/CBER
Kimberly Benton	FDA/CBER
Joseph George	FDA/CBER
David Hussong	FDA/OPS
Abigail Jacobs	FDA/CDER
Jodie Kulpa-Eddy (ICCVAM Vice Chair)	USDA/APHIS
Robert Mello	FDA/CDER
Richard McFarland (PWG Chair)	FDA/CBER
Penelope Rice	FDA/CFSAN
William Stokes	NIEHS
Raymond Tice	NIEHS
Daniela Verthelyi	FDA/CDER
Marilyn Wind (ICCVAM Chair)	CPSC
Jiaqin Yao	FDA/CDER

**Public Attendees:**

Allen Dearry	NIEHS
Basil Golding	FDA/CBER
Thomas Hartung	ECVAM
Coty Huang	FDA/CBER
Sue Leary	ARDF
Thomas Montag	ECVAM
Michael Myers	FDA/CVM
Steven Myers	Becton, Dickinson & Company
Seishiro Naito	NIID
Michael Scott	FDA/CVM
Kristie Stoick	PCRM
Michael Timm	University of Copenhagen
Rachel Waltman	USDA/APHIS

**NICEATM Staff:**

David Allen	ILS, Inc.
Elizabeth Lipscomb	ILS, Inc.
Linda Litchfield	ILS, Inc.
Debbie McCarley	NIEHS
James Truax	ILS, Inc.
Douglas Winters	ILS, Inc.

Abbreviations: APHIS = Animal and Plant Health Inspection Service; ARDF = Alternatives Research and Development Foundation; CBER = Center for Biologics Evaluation and Research; CDER = Center for Drug Evaluation and Research; CFSAN = Center for Food Safety and Applied Nutrition; CPSC = Consumer Product Safety Commission; CVM = Center for Veterinary Medicine; ECVAM = European Centre for the Validation of Alternative Methods; ERATO = Exploratory Research for Advanced Technology; FDA = U.S. Food and Drug Administration; ILS = Integrated Laboratory Services; MSPCA = Massachusetts Society for the Prevention of Cruelty to Animals; NIEHS = National Institute of Environmental Health Sciences; NIID = National Institute for Infectious Diseases; OPS = Office of Pharmaceutical Science; PCRM = Physicians Committee for Responsible Medicine; USDA = U.S. Department of Agriculture

**Call to Order**

Dr. Karen Brown (Panel Chair) called the meeting to order at 8:30 a.m. and introduced herself. She then asked all Peer Panel members, National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) staff, members of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the ICCVAM Pyrogenicity Working Group (PWG) in attendance, the European Centre for the Validation of Alternative Methods (ECVAM) liaison to the PWG, and members of the public to state their name and affiliation for the record. Dr. Brown asked all individuals to identify themselves when they spoke and to use the provided microphones. Dr. Brown stated that three public comment sessions were scheduled during the meeting and she reminded individuals who wished to speak to register at the registration table. Dr. Brown emphasized that there was no need to repeat the same comments at each comment session.

**Welcome from the ICCVAM Chair**

Dr. Marilyn Wind, Consumer Product Safety Commission and Chair of ICCVAM, welcomed everyone to the Peer Review Panel meeting and thanked the Panel members for their participation. Dr. Wind stressed the importance of an independent scientific peer review to the ICCVAM test method evaluation process.

### **Welcome from the Director, NICEATM, and Conflict of Interest Statements**

Dr. William Stokes, Director of NICEATM, welcomed everyone and reiterated Dr. Wind's appreciation to the participants for agreeing to serve on the Panel. Dr. Stokes stated that he would be serving as the Designated Federal Official for the public meeting. He stated this meeting was being held in accordance with the Federal Advisory Committee Act regulations and that the Panel was constituted under the NIH Special Emphasis Panel charter. Dr. Stokes read the conflict of interest statement and asked the Panel members to declare if they had any direct or indirect conflicts, and to recuse themselves from discussion and voting on any aspect of the meeting where there might be a conflict. None of the Panel members declared a conflict of interest.

### **Overview of the ICCVAM Test Method Evaluation Process**

Dr. Stokes provided an overview of the ICCVAM test method evaluation process. He stated that the international Panel was made up of 13 scientists from five different countries (Austria, Japan, Turkey, United Kingdom, and United States). Dr. Stokes described that the purpose of the Panel was to assist ICCVAM by carrying out an independent scientific peer review of the information provided in the ICCVAM Background Review Document (BRD) on the validation studies of five *in vitro* test methods proposed for assessing the potential pyrogenicity of pharmaceuticals and other products. He stated that Panel members were experts selected and appointed by the National Institute of Environmental Health Sciences (NIEHS) to ensure sufficient scientific expertise to carry out a comprehensive review of these test methods.

Dr. Stokes listed the 15 ICCVAM member agencies and provided a brief review of ICCVAM's history. He summarized the ICCVAM Authorization Act of 2000 (available at: [http://iccvam.niehs.nih.gov/docs/about\\_docs/PL106545.pdf](http://iccvam.niehs.nih.gov/docs/about_docs/PL106545.pdf)) and detailed the purpose and duties of ICCVAM as mandated in the Act. He noted that one of ICCVAM's duties is to review and evaluate new, revised, and alternative test methods applicable to regulatory testing. He stated that all of the reports produced by NICEATM are available from the ICCVAM/NICEATM website or directly from NICEATM. Dr. Stokes pointed out that ICCVAM does not carry out research, development, or validation studies, but instead, facilitates these processes by convening scientific symposia, workshops, and expert Panel reviews such as this one.

Dr. Stokes then described the ICCVAM test method evaluation process, which begins with a test method nomination or submission. NICEATM conducts a prescreen evaluation to summarize the extent to which the proposed submission or nomination addresses the ICCVAM prioritization criteria. A report of this evaluation is then provided to ICCVAM, which in turn develops recommendations regarding the priority for evaluation. ICCVAM then seeks input on their recommendations from the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) and the public. Given sufficient regulatory applicability, sufficient data, resources, and priority, a method will move forward to a formal evaluation. A draft BRD is prepared by NICEATM in conjunction with an ICCVAM working group for the relevant toxicity testing area (e.g., pyrogenicity), which provides a

comprehensive review of all available data and information. ICCVAM then considers all of the available information and prepares draft recommendations for 1) proposed usefulness and limitations of the test methods, 2) test method protocol, 3) performance standards, and 4) future studies. The draft BRD is then made publicly available for review and comment. An independent peer review Panel is then convened to provide comments and recommendations on the draft BRD, public comments, and ICCVAM draft test method recommendations. A *Peer Review Panel Report* is published and considered by ICCVAM, along with public and SACATM comments, when their final recommendations are forwarded to the appropriate ICCVAM agencies.

Dr. Stokes reviewed the criteria for adequate validation. He stated that validation is defined by ICCVAM as the process by which the reliability and relevance of a procedure are established for a specific purpose, and that adequate validation is a prerequisite for consideration of a test method by U.S. Federal regulatory agencies. Dr. Stokes listed the ICCVAM acceptance criteria for test method validation and acceptance.

### **ICCVAM Charge to the Panel**

Dr. Stokes reviewed the charge to the Panel, which was to: 1) review the draft BRD for completeness and identify any errors or omissions; 2) determine the extent to which each of the applicable criteria for validation and regulatory acceptance had been addressed for the proposed use; and 3) consider and provide comment on the extent to which the ICCVAM draft test method recommendations including the proposed use, recommended protocols, performance standards, and recommended additional studies are supported by the information provided in the BRD.

Dr. Stokes thanked the PWG, ICCVAM, and NICEATM for their work on this project, and he acknowledged the NICEATM staff for organizing the Panel meeting and preparing the materials being reviewed.

### **Overview of Pyrogenicity Testing Requirements and Current Pyrogenicity Testing Procedures**

Dr. Richard McFarland, Food and Drug Administration (FDA), Center for Biologics Evaluation and Research (CBER) and Chair of the PWG, thanked the PWG members for their efforts in producing the draft BRD, and thanked the Panel members for their participation in the peer review process. Dr. McFarland discussed the scientific need for pyrogenicity testing and its relationship to the regulatory mandate for protection of public health. He discussed the need for risk management, especially the detection of endotoxin and non-endotoxin pyrogen-contaminated products, and he noted the need for classification and labeling of products as pyrogen-free (i.e., the product does not exceed established endotoxin limits). Dr. McFarland then summarized the U.S. and European legislation and statutory protocol requirements for pyrogen testing.

### **Overview of the Five *In Vitro* Pyrogen Test Method Protocols**

Dr. Thomas Hartung, Head of ECVAM and invited test method expert, remarked that he has been closely involved in the ECVAM validation studies and as such recognized his considerable conflict of interest. Dr. Hartung summarized the disadvantages of the rabbit pyrogen test (RPT) and the bacterial endotoxins test (BET), and related these limitations to the development of the *in vitro* pyrogen test methods.

Dr. Hartung indicated that a typical *in vitro* pyrogen test method consists of two parts: 1) incubation of the test sample in a cellular cytokine release system (i.e., whole blood [WB], Mono Mac 6 [MM6] cells, Peripheral blood mononuclear cells [PBMC]); and 2) cytokine detection using a specific enzyme-linked immunosorbent assay (ELISA) (e.g., Interleukin [IL]-1 $\beta$  or IL-6). He stated that the European Commission granted \$2.5 million for the validation of these novel test methods, but that this sum was only sufficient to cover "the basics". Dr. Hartung then made the following comments regarding the design of the ECVAM validation study:

- For the validation study, the endotoxin threshold was set at 0.5 Endotoxin Units (EU)/mL, based on the positive response of 50% of the most sensitive rabbit strain to 50 pg of endotoxin. A substance was considered pyrogen-free if the endotoxin level in an *in vitro* test method corresponded to less than 0.5 EU/mL. A positive product control (PPC) was used in a pretest to insure that there is no interference. Specific criteria were used to minimize assay variability (e.g., blood donors, coefficient of variation).
- In 1988, Dr. Stephen Poole described an IL-6 cytokine assay using isolated leukocytes. The PBMC test method evolved from this study and has subsequently been used by Novartis for U.S. Food and Drug Administration (FDA) release of one product (i.e., after product-specific validation and in conjunction with the rabbit pyrogen test).
- Two of the assays included in the validation exercise, WB/IL-1 $\beta$  and WB/IL-6, utilize human WB. Many research studies have described using these test systems for routine pyrogen testing of up to 80 pharmaceutical products against a variety of pyrogens. A commercial kit has been developed using the WB/IL-1 $\beta$  test system.
- A catch-up validation study was performed using the Cryo-WB/IL-1 $\beta$  test method, which was not available during the original validation study. This assay utilizes cryopreserved WB pooled from several donors. Although the cells remain in diluted dimethyl sulfoxide, an effect on cell morphology or viability is not observed.

### **Overview of the Draft *In Vitro* Pyrogen Test Method Background Review Document (BRD)**

Dr. David Allen, Integrated Laboratory Systems, Inc. (the NICEATM support contractor), presented an overview of the ICCVAM draft BRD. Dr. Allen indicated that five BRDs were submitted by ECVAM in June 2005. A *Federal Register* notice was used to request data from over 100 interested stakeholders, but no additional data were submitted. Following this request, a comprehensive ICCVAM draft BRD, which describes the current validation status of the five *in vitro* test methods based on U.S. Federal regulatory standards, was compiled and made available to the public on December 1, 2006.

Dr. Allen briefly summarized the performance characteristics of the *in vitro* test methods, which are detailed in the ICCVAM draft BRD (available at: <http://iccvam.niehs.nih.gov/methods/pyrogen/pyrodocs/pyroBRDUdocs/PyroBRD01Dec06.pdf>).

Dr. Allen noted that Dr. Marlies Halder, ECVAM liaison to the PWG, provided additional information requested by the Panel, including data audits, evidence of Good Laboratory Practice (GLP) compliance of testing laboratories, information on the protocol used for the historical RPT studies, and lot numbers of the test substances. He also stated that a request was made for the ECVAM Science Advisory Committee (ESAC) peer-review documents, but that these documents are not available to the public.

**Peer Review Panel Evaluation:**

Dr. Brown introduced the relevant Panel Group Leaders for each BRD Section: (Dr. Melvyn Lynn - Sections 1, 2, and 11; Dr. Jack Levin - Sections 3, 5, and 6; Dr. Anthony Mire-Sluis - Sections 7 and 8; Dr. Jon Richmond - Sections 4, 9, and 10). The Group Leaders presented the draft responses to the Evaluation Guidance Questions for consideration by the entire Panel. The Panel discussion and their recommended revisions to each section of the ICCVAM BRD are reflected in the *Independent Peer Review Panel Report: Five In Vitro Test Methods Proposed for Assessing Potential Pyrogenicity of Pharmaceuticals and Other Products*, published in April 2007 (hereafter, the Panel report, available at: <http://iccvam.niehs.nih.gov/docs/pyrogen/PrRevPanFinRpt.pdf>).

**Public Comments (Session 1)**

**Ms. Mary Lou Chapek - President and Chief Executive Officer of MBP Laboratories, Inc.**

Dr. David Allen read the written comments submitted by Ms. Chapek to ICCVAM/NICEATM. Her comments are summarized as follows:

- Ms. Chapek expressed disappointment in the number of test methods reviewed by ICCVAM and accepted by federal agencies over the past 15 years. She commented that the pyrogenicity BRD and recommendations currently under discussion indicate a lack of focus. Ms. Chapek noted that substantial work remains to be done for validation of these test methods and she suggested the phased strategy outlined below.
- Phase I should concentrate on replacement of the BET, not the RPT. A large array of test substances compatible with the BET could be spiked with endotoxin to determine their accuracy and sensitivity and to determine the level of interference, if any, with each of these test systems.
- Phase II should consist of validation of one or two *in vitro* test methods for replacement of the RPT. Cell-based assays that do not depend on blood, which has an impractical limited time domain, would be preferable and could be compared directly to RPT data. The task would still be complex, but with a smaller focus. Phase II would also require evaluation and validation of all materials currently tested in the RPT, as well as the pyrogens detected in the RPT. Some of these standards would have to be developed. Although these studies may take years for completion, replacement of the RPT by one or two of the *in vitro* pyrogen tests in Phase II would constitute an achieved goal by ICCVAM.

**Dr. Thomas Montag - Paul Ehrlich Institute (PEI) - Germany**

Dr. Montag provided the following comments:



- He stated that the PEI is responsible for the quality and safety of biological drugs in general and that his laboratory has been involved in alternative pyrogen testing with Dr. Hartung for over 12 years. While the data is proprietary, he confirmed that he has used the WB/IL-1 $\beta$  assay for approximately two years.
- Dr. Montag commented that donors are now pooled (up to 10 at a time) to minimize variability, especially for detection of non-endotoxin pyrogens. For the Cryo WB/IL-1 $\beta$  pyrogen test, the blood is typically pretested for reactivity after pooling. In response to the PPC issue mentioned previously, he remarked that this was a design flaw that had been corrected in the ECVAM Standard Operating Procedure. He also stated that an expert Panel from the EDQM is now in the process of creating a draft of this alternative test method for publication.

### **Final Review of the BRD for Errors and Omissions**

Dr. Brown asked the Panel to review the recommended revisions for each BRD section, taking into account the public comments, and to decide if additional changes are necessary. No changes were made to the draft recommendations based on the public comments.

### **Validation Status of the *In Vitro* Test Methods**

Dr. Brown asked the Panel if they agreed that the applicable validation criteria had been adequately addressed in the ICCVAM BRD in order to determine usefulness and limitations of these *in vitro* test methods, to serve as a substitute for the RPT, for the identification of Gram-negative endotoxin on a case-by-case basis subject to product-specific validation.

The Panel agreed that the information was adequate with which to make an informed decision.

Dr. Brown asked the Panel if they agreed that the performance of these test methods in terms of their relevance and reliability support the proposed use for the detection of Gram-negative endotoxin in materials that are currently tested in the RPT, subject to product-specific validation to demonstrate equivalency to the RPT.

The Panel did not agree with this statement based on the reasons indicated in the responses to the questions related to Sections 1.0 to 12.0 of the ICCVAM BRD. Two minority opinions were expressed. Responses to these questions, and the associated minority opinions are detailed in the Panel Report.

### **Public Comments (Session 2)**

#### **Dr. David Hussong - FDA, Center for Drug Evaluation and Research (CDER)**

Dr. Hussong commented that the Code of Federal Regulations (CFR), Section 211.167, states that if a drug is to be labeled as pyrogen-free, an appropriate test is required. The U.S. Pharmacopeia (USP) provides guidelines for the RPT and the BET. While the BET is not considered equivalent to the RPT, data from the BET is accepted. The USP states that use of alternative tests is permitted and that they may be used *in lieu* of the BET, provided that the alternative test uses a reference standard for comparison. It should be noted that the FDA CDER approves drugs, not test methods, but welcomes the use of alternative test methods.

#### **Dr. Thomas Hartung - Head, ECVAM - Italy**

Dr. Hartung stated that the *in vitro* pyrogen tests were designed to determine the threshold level of endotoxin in the most sensitive rabbit strain. This design was ambitious and consequently, resulted in the low sensitivity (58%) and specificity (83%) observed. It should be noted that some assays had values of 80% or 90% at this critical concentration and performed better than the RPT.

### **ICCVAM Draft Recommendations for *In Vitro* Pyrogen Test Methods Presentation of Draft ICCVAM Recommendations**

Dr. Brown asked the Panel to evaluate the extent to which the ICCVAM draft recommendations are supported by the information and data provided in the ICCVAM draft BRD. Dr. Brown reminded the Panel that the purpose is not to approve or disapprove of the ICCVAM draft recommendations, but rather to comment on the extent to which they are supported by the information contained in the ICCVAM BRD. The Panel discussion and associated conclusions relevant to each of the ICCVAM recommendations are reflected in the Panel Report.

### **Public Comments (Session 3)**

#### **Ms. Kristie Stoick - Physicians Committee for Responsible Medicine**

Ms. Stoick reviewed written comments that she previously submitted to ICCVAM/NICEATM. She stated that the pace of acceptance of alternative methods, such as these *in vitro* pyrogen tests, in the opinion of the animal protection community, is unacceptably slow. She continued to state that too much time is spent debating every scientific detail and that the ultimate goal is lost. She closed by asking ICCVAM to take into account her comments when considering the Panel's recommendations for the validation of these assays.

### **Final Review of the ICCVAM Draft Recommendations**

Dr. Brown asked the Panel to review the ICCVAM draft recommendations, taking into account the public comments, and to decide if additional changes are necessary. No changes were made to the draft recommendations based on the public comments.

### **Concluding Remarks**

Dr. Brown thanked the Panel and ICCVAM/NICEATM for their help. She expressed hope that this peer review process helped to establish a focus for ICCVAM and that the reduction in animal use would be the ultimate outcome. Dr. Stokes thanked the Panel for their hard work, thoughtful and objective deliberations, and advice. Dr. Stokes stated that the ICCVAM PWG and ICCVAM would consider these recommendations as they move forward with this process and the results of this meeting would culminate in a Peer Review Panel Report that would be released to the public toward the end of March for additional comment.

### **Adjournment**

The meeting was adjourned at 5:47 p.m.

William S. Stokes, D.V.M.  
NIEHS  
P.O. Box 12233  
MD-EC17  
Research Triangle Park, NC 27709

Dear Dr. Stokes:

The Meeting Summary, Peer Review Panel Public Meeting, Five *In Vitro* Test Methods Proposed for Assessing Potential Pyrogenicity of Pharmaceuticals and Other Products, dated February 6, 2007, accurately summarizes the Peer Review Panel meeting of February 6, 2007, in Bethesda, MD.

Sincerely,

/s/

KAREN K. BROWN

3/4/08

Signature

Printed Name

Date