4.0 ICCVAM Consideration of Public and SACATM Comments

In response to three *FR* notices that were released between December 2005 and May 2007, eight public comments were received (see **Appendix D**). Comments received in response to or related to the *FR* notices are also available on the NICEATM/ICCVAM website (http://ntp-apps.niehs.nih.gov/iccvampb/searchPubCom.cfm). The following sections, delineated by *FR* notice, provide a brief discussion of the public comments received.

4.1 Public Comments in Response to *FR* Notice (70FR74833, December 16, 2005): Peer Panel Evaluation of *In Vitro* Pyrogenicity Testing Methods: Request for Comments, Nominations of Experts, and Submission of *In Vivo* and *In Vitro* Data

NICEATM, in an *FR* notice (Vol. 70, No. 241, pp. 74833-74834, December 16, 2005), requested (1) public comments on the appropriateness and relative priority of convening an independent peer review panel (Panel) to evaluate the validation status of five *in vitro* pyrogen test methods, (2) the nomination of scientists with relevant knowledge and experience to potentially serve on the Panel should it be convened, and (3) submission of data from the RPT, the BET, and *in vitro* pyrogenicity testing using any of the five *in vitro* pyrogen test methods under consideration by NICEATM.

In response to this *FR* notice, NICEATM received two comments. No additional data or information was submitted in response to this request. One nomination requested consideration of three potential panelists.

One commenter provided a reference for an *in vitro* pyrogen test method that measured TNF-α (Martinez et al. 2004). The comment and article were provided to the Panel. However, the reference was not included in the ICCVAM BRD because the *in vitro* pyrogen methods being evaluated by NICEATM measured only IL-1β and IL-6.

A second commenter requested an expeditious review of the *in vitro* pyrogen test methods and described limitations of the currently used *in vivo* pyrogen test methods (i.e., the RPT and the BET). This commenter also stated that the peer review of the *in vitro* test methods is appropriate, necessary, and should be given extremely high priority.

4.2 Public Comments in Response to *FR* Notice (71FR74533, December 12, 2006): Announcement of an Independent Scientific Peer Review Meeting on the Use of *In Vitro* Pyrogenicity Testing Methods; Request for Comments

NICEATM, in an *FR* notice (Vol. 71, No. 238, pp. 74533-4, December 12, 2006), announced (1) an independent scientific peer review meeting to evaluate the validation status of five *in vitro* pyrogen test methods proposed as replacements for the RPT, and (2) the availability of an ICCVAM draft BRD on five *in vitro* pyrogen test methods, which describes the current validation status of these methods and contains all of the data and analyses supporting their current validation status, and ICCVAM draft recommendations on the proposed use of these test methods, draft test method protocols, and draft performance standards. NICEATM invited the submission of written comments on the ICCVAM draft BRD and on the ICCVAM draft test method recommendations. In response to this *FR* notice, NICEATM received four comments.

One commenter expressed that it was not clear why ICCVAM was neither considering the *in vitro* pyrogen test methods for detection of non-endotoxin pyrogens nor for replacement of both the RPT and the BET. The commenter suggested that exclusion of these broader uses would minimize the impact of these test methods on reduction in animal use and urged ICCVAM "to significantly revise its recommendations and BRD to more accurately reflect the potential use of these methods as full replacements for both the {BET} and RPT." Furthermore, they "strongly encouraged ICCVAM to delete the recommendation regarding the conduct of *de novo* RPTs to further demonstrate *in vivo/in vitro* concordance." ICCVAM appreciates the concern for the proposed limited use of these test methods. However, neither data comparing the *in vitro* test methods to the BET nor data directly comparing non-endotoxin pyrogens to the BET or the RPT were included in the validation studies submitted by ECVAM. Therefore, ICCVAM was unable to consider the *in vitro* test methods as replacements for the BET or to propose the use of these test methods for non-endotoxin pyrogens. However, ICCVAM did identify and recommend future studies that could fill these data gaps and in turn, potentially broaden the applicability of these test methods to that suggested by the commenter.

Several commenters argued that the scope of the test substances was limited and the data provided were inadequate to support the intended use of the *in vitro* test methods (i.e., as a complete replacement for the RPT). These commenters emphasized that additional testing is needed before these test methods can be recommended for this broader application. ICCVAM agreed with these comments, which are reflected in the ICCVAM recommended future studies.

One commenter provided data on an alternative *in vitro* pyrogen test method that is based on the measurement of reactive oxygen species from the human HL-60 promyelocytic leukemia cell line (Blatteis 2006; Timm et al. 2006). The comment and articles were provided to the Panel. However, these data were not included in the ICCVAM BRD because the *in vitro* pyrogen methods being evaluated by NICEATM measured only IL-1β and IL-6.

4.3 Public Comments in Response to *FR* Notice (72FR26395, May 9, 2007): Peer Review Panel Report on Five *In Vitro* Pyrogen Test Methods: Availability and Request for Public Comments

NICEATM, in an *FR* notice (Vol. 72, No. 89, pp. 26395-26396, May 9, 2007), announced the availability of the Panel report and invited the submission of written comments on the report. In response to this *FR* notice, NICEATM received two comments.

One commenter indicated that several of the Panel's observations and recommendations were "nonsensical, irrelevant, or inappropriate." This commenter also expressed concern about the “random” selection of Panel members and recommended both simplification of the questions posed to the Panel and an orientation meeting to provide the panelists with background information and focus. It was recommended that "ICCVAM coordinate with the pharmaceutical and medical devices industry to conduct product-specific validation on a set of pre-selected products and devices to serve as further validation work." ICCVAM appreciates comments related to the evaluation process of new alternative test methods. ICCVAM notes that Panel members were selected from nominations received in response to an *FR* notice (Vol. 70, No. 241, pp. 74833-74834, December 16, 2005), in conjunction with recommendations from the ICCVAM PWG, which includes a liaison from ECVAM.

Additionally, orientation sessions are routinely convened for the Panel to provide background information on the ICCVAM test method evaluation process.

A second commenter outlined responses to specific comments and/or recommendations made in the Panel report. These comments provided rationale for the design of the ECVAM validation study and summarized existing data to address many of the Panel's concerns. ICCVAM appreciates these written responses and clarifications to specific Panel comments. ICCVAM considered all comments prior to finalization of the ICCVAM BRD and in preparation of the ICCVAM test method evaluation report.

4.4 Public and SACATM Comments: SACATM Meeting on June 12, 2007

The June 12, 2007 SACATM Meeting included a discussion of the ICCVAM review of the *in vitro* pyrogen test methods. At this meeting, three public comments and four SACATM comments were presented.

One public commenter reiterated the written comments submitted in response to the *FR* notice announcing the availability of the Panel report (see **Section 4.3**, first commenter).

A second public commenter (who was also the Chair of the ICCVAM peer review panel) stated that, "given more time to discuss these methods, the Panel might have been able to provide a stronger recommendation for one or more of the assays." ICCVAM appreciates comments related to the evaluation process and now intends to extend the time allocated for Panel meetings to ensure that sufficient time is allotted.

A third public commenter noted that the long list of future studies recommended by the Panel were impractical and not feasible to complete, particularly considering the expense that had already been invested in the validation effort. This commenter also provided additional comments relevant to the criticisms of these *in vitro* test methods made by the Panel (e.g., the limitations of the *in vitro* methods were not fairly compared to the limitations of the RPT and BET; only endotoxin was included in the validation study because no non-endotoxin reference standard is available; and false positives were recorded because the assays are too sensitive). ICCVAM considered many of these comments in the revisions of the ICCVAM BRD and in the preparation of the ICCVAM test method evaluation report.

One SACATM member expressed concern with the high false negative rates reported for some of the assays, the proprietary issues associated with using the Novartis IL-6 ELISA, the lack of concordance assessment between the RPT and the *in vitro* data, and the range of substances included in the validation studies. A second SACATM member provided comments on the statistical analyses used to assess the *in vitro* data. ICCVAM agrees with many of these concerns, which are reflected in the ICCVAM test method recommendations.

A third SACATM member recommended that multiple test methods not be reviewed simultaneously. As stated above, ICCVAM plans to allocate additional time for deliberation at Panel meetings.

A fourth SACATM member suggested the concept of "core panelists" who are knowledgeable about the ICCVAM evaluation process for ICCVAM reviews with the addition of *ad hoc* experts for specific methods. ICCVAM also appreciates this suggestion and makes every effort to include in each panel individuals with direct experience with the ICCVAM evaluation process as well as experts in the subject matter being evaluated.