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Via e-mail to: *niceatm@niehs.nih.gov*

Dr. William Stokes
Director, NICETAM
National Institute of Environmental Health Sciences
PO Box 12233, MD ED-17
Research Triangle Park, NC 27709

Dear Dr. Stokes:

These comments are intended to be a follow-up to the recent NICETAM-sponsored Independent Scientific Peer Review: Five *In Vitro* Test Methods Proposed for Assessing Potential Pyrogenicity of Pharmaceuticals and Other Products (February 6, 2007). They are supported by the larger Animal protection community, including the more than 10 million members of the Physicians Committee for Responsible Medicine, People for the Ethical Treatment of Animals, the Humane Society of the United States, and the Doris Day Animal League.

Based on information communicated to me at this meeting, I understand that these additional comments are accepted because of limited time available during my oral public comment. I appreciate the opportunity to submit these additional comments and I urge NICETAM to take them into account when considering the Peer Review Panel's (PRP) recommendations and conclusions.

Panel Recommendations

As you know, the ICCVAM recommendations were written with the intention that the *in vitro* pyrogenicity tests (IVPTs) would replace a small subset of rabbits used in the rabbit pyrogen test (RPT), but not the Limulus amebocyte lysate (LAL), nor rabbits used for non-endotoxin-mediated pyrogenicity testing. As you know, the PRP did not even agree with these limited recommendations. The PRP did, however, make several of their own recommendations. I was pleased to hear of some of them and hope that ICCVAM will consider putting them into place as quickly as possible (abridged below):

1. Human data on pyrogens is extensive and should be analyzed, presented, and consulted.
2. More discussions on the financial and ethical costs (including monetary values and animal numbers) associated with the RPT are needed.
3. Individual product-specific validation studies are required and may negate the need for a large validation study.

However, several of the panel's observations and recommendations seemed nonsensical, irrelevant, or inappropriate (abridged below):

1. The methods should not be called "*in vitro* pyrogen tests" because only bacterial endotoxin was evaluated.
2. The *in vivo* reference data is not adequate and/or of unknown quality.
3. The calculated "theoretical sensitivity" of the RPT data used in the validation study does not reflect current practice and regulatory use.
4. The IVPT validation data should be quantitative

5. Concordance between the IVPTs and the RPT is not demonstrated.

Considerations as to the realities of a validation study are clearly not recognized by the PRP, as reflected in its deliberations and recommendations. The validation study conducted by ECVAM used scientifically-justified *in vivo* reference data, a scientifically-justified method to calculate the theoretical sensitivity of the RPT data (as admitted by the panel), and generated data with theoretical concordance values presented. Indeed, the RPT itself does not give quantitative data, but a decision of "pyrogenic" or "not pyrogenic," while the *in vitro* methods do have this potential.

What went wrong?

Panel Selection: This meeting reflects a growing concern of the animal protection community that ICCVAM is more interested in picking new non-animal methods apart than in seriously considering them for adoption. Accepted peer review process guidelines state: "Peer reviewers should include individuals who will not be affected by the outcome of the results, but who are well-versed in the relevant experimental techniques and the specific method under review."[1] However, many of the panel members were either demonstrably biased against the IVPTs, silent, or ignorant of the validation and acceptance procedures, the PRP's role, or the ICCVAM process. Too often it seems that panelists have unreasonable expectations regarding every minute detail of the alternative methods, without a clear understanding of the limitations of the current animal-based tests. This was especially true in this meeting. Random selection of panel members from the scientific topic of interest biases every single panel towards the null hypothesis, leaving an unreasonably high barrier over which the new alternative methods cannot cross.

Charge/Question Wording: It was clear from the deliberation among the PRP that the panel members had no clear idea of their task, and were unnecessarily confused by the questions posed to them by ICCVAM. One question elicited an hour's debate over what the question actually meant. Simplification of the questions posed to the panel, as well as a pre-meeting orientation, is in order. For example, there was clearly little or no background information provided on the limitations of the animal tests. An orientation process could also help the panelists stay focused. The panel deviated too often from the task at hand into both broad and detailed scientific questions that had no bearing on the validation of the IVPTs. For example, one panel recommendation stated that an explanation should be given as to why *in vitro* responses are a better reflection of *in vivo* human responses than *in vivo* rabbit responses. While biological relevance is important, it has already been demonstrated; this recommendation has no bearing on the validation status of the assays as presented, whether it is true or not.

Validation Study Considerations

Despite public testimony given at the time of the meeting, the PRP did not take the realities of validation studies, nor this particular one, into account. First, the validation study selected a small set of pharmaceuticals and spiked them with endotoxin, because endotoxin standard is the only standard available, and the majority of febrile reactions are due to endotoxin. As is often done with animal tests, practical experience over the past couple of decades led the validation study directors to surmise that the methods would also work with non-endotoxin pyrogens, and with medical devices and blood products. Pages of data were provided to the PRP to support this conclusion. Given that the methods would require product-specific validation in the future, and limited resources for the validation study, a large, complicated validation study was not called for. Further, parallel rabbit testing, for animal welfare reasons, could not be conducted. So, the study directors designed an approach that would allow the use of historical RPT data of a comparable nature. It was determined that in order to "pass," the IVPTs would

[1] NIEHS (National Institute of Environmental Health Sciences), Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods, NIH Publication No. 97-3981, NIEHS, Research Triangle Park , North Carolina, U.S.A., 1997, section 2.4.2.6.

be validated with spiked products at a level of detection comparable with the most sensitive rabbit species. Even with these "unacceptable" sensitivity and specificity values, the IVPTs still surpassed the performance of the RPT. Comments to this effect from the study directors themselves were ignored by the PRP.

The PRP showed no tolerance for adopted tenets of the validation process that call for flexibility: "…the test validation process should be highly flexible and adapted to the specific test and its proposed use."[2] These procedures do not require direct comparison of *in vivo*/*in vitro* methods, and indeed, the BRD and other documentation submitted to the PRP contain all of the Validation Criteria listed in the above-referenced ICCVAM document.

The Way Forward

I would like to reiterate the animal protection community's initial comments, sent before the meeting:

> "We therefore strongly urge ICCVAM to significantly revise its Recommendations and B[ackground] R[eview] D[ocument] to more accurately reflect the potential use of these methods as full replacements for both the LAL and the RPT. The available evidence shows that the IVPTs are fully valid for the detection of all pyrogens. We also strongly encourage ICCVAM to delete the recommendation regarding the conduct of *de novo* RPTs to further demonstrate *in vivo/in vitro* concordance."[3]

Our organizations stand by these initial recommendations. **However, given the PRP's final recommendations, we request 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.** Since this work will need to be conducted anyway, and would be acceptable to the Food and Drug Administration, this would be an appropriate way forward. Further delays or *de novo* validation work would result in the deaths of thousands of additional animals is not recommended.

Thank you for your attention to these comments. I can be reached at *kstoick@pcrm.org* or 510.834.8320 with any questions.

Sincerely,

/s/

Kristie Stoick, MPH
Physicians Committee for Responsible Medicine

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[2] NIEHS (National Institute of Environmental Health Sciences), Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods, NIH Publication No. 97-3981, NIEHS, Research Triangle Park , North Carolina, U.S.A., 1997, sections 2.4.7 and 2.5.

[3] Letter submitted to NICEATM January 26, 2007.