February 5, 2007

Via email to: niceatm@niehs.nih.gov

Dr. William Stokes
Director, NICEATM
National Institute of Environmental Health Sciences
P.O. Box 12233, MD EC-17
Research Triangle Park, NC 27709

Re: Federal Register Vol. 70, No. 238, pp 74533-4, December 12, 2006: NTP Interagency Center for the Evaluation of Alternative Toxicological Methods; Announcement of an Independent Scientific Peer Review Meeting on the Use of *In Vitro* Pyrogenicity Testing Methods; Request for Comments

Dear Dr. Stokes:

I have taken the opportunity to review ICCVAM's recommendations for five *in vitro* pyrogenicity tests (IVPTs) and to provide comments regarding ICCVAM's "Draft Test Method Recommendations" (Recommendations) and "Draft Background Review Document" (BRD) on these methods.

I have always regarded ICCVAM and its member agencies as federal partners who share my commitment to the 3 R's, reducing, refining, and ultimately replacing the use of animals in regulatory testing. I have been greatly disappointed at the minimal number of methods reviewed by ICCVAM and accepted by federal agencies over the past 15 years and would like to see progress in this area, not just stagnation. The pyrogenicity BRD and Recommendations currently under discussion indicate to me that there is a lack of logical focus. I propose a two phase approach whereby ICCVAM can demonstrate success.

The summaries and data provided in the BRD indicate that the five proposed *in vitro* pyrogenicity tests are only being evaluated and validated for their ability to measure the

pyrogenic response produced by endotoxin. Even then, only a few pharmaceutical products were tested by spiking with known amounts of endotoxin. Replacing the RPT fully with the *in vitro* pyrogenicity tests is a noble and worthwhile project. I support it fully. However, the testing still to be conducted is extraordinary. Numerous types of products need to be evaluated (some of which have been reported by ECVAM) and non-endotoxin pyrogens must be tested. I would strongly suggest that the ICCVAM proceed with a phased project in order to demonstrate that something can be accomplished rather quickly and animals' lives can be saved.

I propose that Phase I would concentrate on replacing the BET with one or more of the *in vitro* pyrogenicity tests, a task that appears less daunting than replacement of the RPT. The Phase I testing is important because use of the *in vitro* pyrogenicity tests instead of the BET would eliminate the need for horseshoe crabs to die during or after the process of removing the hemolymph. Additionally, the *in vitro* pyrogenicity tests use human components instead of non-human horseshoe crab hemolymph that could be argued to be less relevant to the human fever response.

There is already a significant amount of work reported in the BRD indicating that the five *in vitro* pyrogenicity tests can detect endotoxin pyrogens with accuracy and sensitivity. Thus, Phase I would only require validation against the BET for those products that can currently be tested in the BET.

It appears from the ECVAM information that the *in vitro* pyrogenicity tests can actually test more varied products since there is no interference with these test systems. Such lack of interference could also be demonstrated during Phase I by spiking an array of test products with known endotoxin levels and demonstrating accuracy, specificity and lack of interference.

As for Phase II, I would strongly suggest that the ICCVAM select one or two of the *in vitro* tests based on the results obtained so far, and use them in validation studies against the RPT in order to replace that test completely. The reason for selecting only one or two of the *in vitro* tests is based on the fact that three of the five proposed *in vitro* pyrogenicity tests require fresh human blood that must be collected within 4 hours of running the test. In today's world, such a task is difficult to say the least. The cell culture assay appears much more adaptable to ease of use. That would certainly be one of my choices.

Phase II would still be complex, as now the focus would be on total replacement of the RPT with one or two of the *in vitro* pyrogenicity tests. However, evaluation and initial validation of one or two tests is less of a challenge than trying to evaluate and validate five tests.

Phase II evaluation would require evaluation and validation of all materials currently tested in the RTP as well as all of the types of pyrogens currently quantified in the RPT.

Because standards are not available for all of the types of pyrogens, such standards would have to be developed. Another possibility would be to find products that failed the RPT and use those for validation purposes (less difficult but less scientific). As you already know, this could require years. At least, if Phase I was complete, there could be a demonstration that ICCVAM had accomplished some of its goal of replacement of animal tests with *in vitro* tests.

I hope that ICCVAM will consider my recommendations.

Best Regards,

Mary Lou Chapek, President and CEO
MVP Laboratories, Inc.