Institute for In Vitro Sciences, Inc.

August 14, 2003

Dr. William Stokes, D.V.M.
NICEATM Director, NIEHS
P.O. Box 12233, MD EC-17
Research Triangle Park, NC, 27709

Advancing
Science &
Animal
Welfare
Together

Dear Dr. Stokes:

The members of the NICEATM and ICCVAM DCIWG committee are to be commended for the considerable effort that went into preparing the draft Minimum Performance Standards (MPS) documents for the three in vitro corrosivity assays. In requesting that these documents be prepared, the EPA has helped us all. Conceptually, the MPS documents are a substantial step forward in regulatory toxicology. They link the validation of an assay system (test system, protocol, endpoint determinations, controls, and prediction model) not only to the application of the assay system, but also to the production of data for regulatory review. These documents, and those that follow, will serve several purposes that are discussed in more detail below.

To start with some background, new test methods undergo several stages of maturation. A newly developed test will first be subject to prevalidation where the effectiveness of the technology transfer process and final protocol development occurs. The final protocol will be used to develop the prediction model that will allow the data from the new test to be calibrated against the desired toxicological action. A training set of reference test materials is used to develop the prediction model. Finally, the new test is subjected to formal validation, usually in several laboratories. For the validation study, a new set of reference test materials is employed. From the validation study, the performance characteristics of the new test (test system, protocol, and prediction model) are determined. Part of this process involves the identification of the essential elements of the test and test system; those elements (independent variables) that must be maintained/controlled to make the test reliable and predictive. This analysis should be performed with both proprietary and nonproprietary tests.

The ICCVAM submission guidelines require the use of controls (specifically positive controls). Performance norms for the positive control are established as part of acceptance criteria for a given "run" of the assay. The acceptable result obtained with the positive control helps to assure that the test system and test execution are functioning properly. While the concept of controls is not new to toxicology, the specification that the controls be performed concurrently with each unknown (or group of unknowns in a single batch) is new and tremendously important. The positive control provides a measure of consistency over time and across laboratories. The MPS documents also identify an important selection criterion for the positive control. The positive control must be able to demonstrate both over and under prediction (sensitivity) relative to the historical performance of the test. Using a 9-pound hammer (i.e., concentrated nitric acid) as a positive control is unlikely to effectively measure assay response. The discussion of benchmark controls (either chemical or formulation) is very helpful. While the positive

21 Firstfield Road
Suite 220
Gaithersburg, MD 20878
tel: 301.947.6523
fax: 301.947.6538
www.iivs.org

control selected for a given assay should remain constant over time, benchmark materials tested concurrently with the unknowns would be selected to match the chemical class of the unknowns. The response of the benchmark controls facilitates interpretation of the results for the unknowns.

Validation studies are complex, time consuming and expensive. They serve to validate the complete test (test system [target tissue], protocol, endpoint measures, and prediction models). The successful validation of the test also tends to validate the mode of action measured by that test. For example, the mode of action for many corrosive chemicals is to penetrate the stratum corneum of the skin and rapidly kill the underlying keratinocytes. Conceptually, it is not hard to imagine modeling such a mode of action with an engineered human skin construct. However, modeling the quantitative (kinetic) aspects of the action is much more difficult. How much test material must be applied and for how long? How to measure the viability of the keratinocytes? How to translate the assay endpoint (e.g., percent viability) to a prediction of corrosive action? The test developer produces an assay protocol to address all of these parameters. The protocol may be based on a proprietary test system (e.g., skin construct) or assay endpoint (e.g., company X's ATP assay). Are those proprietary components of the test absolutely essential or could substantial equivalence be established for another test system or endpoint measure? By identifying the essential structural and functional elements of the test, the MPS approach will allow us (collectively) to draw on validation studies where a successful mode of action has been identified.

There are several additional reasons that the MPS approach is important:

1) In the original ECVAM-sponsored validation of in vitro assays for corrosivity, two skin constructs were tested. At the end of the validation program, neither skin construct was available commercially. Therefore, ZEBET conducted a study to show that the EpiDerm (MatTek, Ashland MA) construct was substantially equivalent to the validated tissue. Thus, the effort and expense of the validation study was not lost.

2) The Organization of Economic Cooperation and Development (OECD) prepares test guidelines for review and acceptance by its 30 member nations (including the United States). Their policy precludes specification of a proprietary test in OECD guidelines. As a result, the OECD has begun to specify structural and functional characteristics of a test (or test system) so that the guideline can draw on validation programs that employ proprietary methods or components.

3) Some proprietary test developers may have made substantial investments in the validation programs for their test. Do the MPS guidelines diminish the economic value of that investment? We believe that they do not. The MPS guidelines provide a controlled mechanism for entry of a new test system or endpoint measure so that the field can grow, but they maintain and codify the standards for that assay. The MPS guidelines assume that the new or modified

"component" of the test will show substantial equivalence to that component of the validated test. For example, it is reasonable to expect that the substantially equivalent test will use the same prediction model as the validated test. Otherwise, a new set of training test materials will be needed to develop the model. Clearly, one can not use the chemicals provided in the MPS to develop and then validate a prediction model! At some point in the number or degree of changes, a more complete validation of a modified method could be necessary.

Once a new test is accepted for regulatory use, additional laboratories are likely to begin using the method. The MPS documents provide the guidance needed to help demonstrate that the new test is being conducted properly. Successful execution of the test with the reference chemicals will help show that the equipment and reagents used in the new laboratory are within "normal limits" for the assay as it was validated. It will also help assess proper assay execution. The MPS guidelines are not a barrier to entry for a new laboratory but a means to link its performance with that of the validation laboratories. Data developed on unknown test materials would then be more credible for both the producers and users of such data.

Again, the authors of these documents are to be commended for developing the MPS concept and creating the subsequent documents. The format is well designed. I would ask however, that the authors become less prescriptive in their specifications for the report contents. Not every test substance will fit into the box that they have built. Perhaps more of the bullet points could include "if relevant to the conduct of the study". One item missing from the list is designation of the acceptance criteria (i.e, range acceptable positive control responses). For ease, the report section might have its own number (rather than being part of section 3).

The Minimum Performance Standards guidelines are an important step forward and NICEATM and ICCVAM DCIWG deserve a great deal of credit for their contribution.

Sincerely yours,

John W. Harbell, Ph.D.
Chief Scientific Officer

Rodger D. Curren, Ph.D.
President