Preface

Acute systemic toxicity testing is conducted to determine the relative health hazard of chemicals and various products. Substances found to cause lethality in animals at or below prescribed doses are labeled to identify their hazard potential. While acute toxicity testing is currently conducted using animals, studies published in recent years have shown a correlation between *in vitro* and *in vivo* acute toxicity. These studies suggest that *in vitro* methods may be helpful in predicting *in vivo* acute toxicity.

An extensive evaluation of *in vitro* methods for acute toxicity, known as the Multicenter Evaluation of *In Vitro* Toxicity (MEIC) Program, was initiated by the Scandinavian Society for Cell Toxicology in 1989 under the direction of Dr. Bjorn Ekwall, Director of the Cytotoxicity Laboratory at the University of Uppsula. Fifty reference chemicals were selected for which there was acute oral toxicity data from animal testing and blood concentrations from fatal human poisonings. Ninety-six laboratories evaluated 30 of the chemicals in 82 different *in vitro* cytotoxicity assays, and all 50 chemicals were evaluated in 61 assays. Detailed analysis of the results identified a battery of three human cell line basal cytotoxicity assays that were highly correlative with peak human lethal blood concentrations.

In 1998, Dr. Willi Halle from Germany published a Register of Cytotoxicity consisting of *in vivo* acute toxicity data and *in vitro* cytotoxicity data for 347 chemicals. These data were used to construct a regression model that could be used to predict estimated LD50 values based on cytotoxicity data. Dr. Horst Spielmann and his colleagues at the German Centre for the Documentation and Evaluation of Alternatives to Testing in Animals subsequently proposed that cytotoxicity methods could be useful for predicting starting doses for *in vivo* acute oral toxicity studies, thereby reducing the number of animals necessary for such determinations.

In 1999, amidst growing awareness of the MEIC and other studies, the National Institute of Environmental Health Sciences (NIEHS) received over 800 letters requesting that the MEIC program results be evaluated by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). Also in 1999, the U.S. Environmental Protection Agency (EPA) Office of Pesticides, Prevention, and Toxic Substances asked ICCVAM to review the validation status of the MEIC proposals.

ICCVAM discussed these requests at its August 1999 meeting and asked the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) to prepare a technical summary of the extensive publications resulting from the MEIC studies. ICCVAM reviewed the MEIC results at its October 1999 meeting and recommended that an expert workshop should be convened to: a) evaluate the current validation status of the proposed MEIC test battery and other available *in vitro* tests that might be useful for predicting acute toxicity; and b) identify research, development, and validation efforts that might further enhance the use of *in vitro* methods to assess acute systemic toxicity.

Names of appropriate scientists to serve on an ICCVAM Workshop Organizing Committee were requested from participating ICCVAM Agencies. The Committee was charged with working with NICEATM to develop the Workshop objectives and program and to identify appropriate expert scientists to participate. The Committee held its first of several meetings in February 2000. Dr. Philip Sayre of the EPA and Dr. John Frazier of the U.S. Air Force co-chaired the Organizing Committee and guided the development of the scope and breadth of the Workshop.

In June of 2000, the International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity was announced in a *Federal Register* notice. Relevant data and nominations of

scientists that should be invited to participate in the Workshop were also requested in the notice. The Organizing Committee invited 33 expert scientists from academia, industry, and Federal agencies to participate in the Workshop. NICEATM assembled relevant background materials for distribution to the invited expert scientists, other workshop participants, and the public. The Organizing Committee also identified knowledgeable agency scientists to participate in the workshop, and developed a series of questions for four breakout groups to address during the three and a half-day meeting. In September 2000, a second *Federal Register* notice announced the availability of the Workshop agenda and background materials, and requested public comments.

Invited scientific experts and ICCVAM agency scientists were assigned to one of the following four Breakout Groups:

- *In Vitro* Screening Methods for Assessing Acute Toxicity;
- *In Vitro* Methods for Toxicokinetic Determinations;
- *In Vitro* Methods for Predicting Organ Specific Toxicity; and
- Chemical Data Sets for Validation of *In Vitro* Acute Toxicity Test Methods.

The Workshop was convened in Arlington, VA on October 17-20, 2000. The NTP, the NIEHS and the EPA sponsored the Workshop, and NICEATM provided logistical, technical, and administrative support. The Workshop was open to the public and was attended by 110 participants from nine countries. In the opening plenary session, speakers provided an overview of *in vitro* acute toxicity methods and described the regulatory use of acute toxicity data. Breakout Groups were then charged with their assigned objectives and asked to develop responses to questions provided by the Organizing Committee.

The Groups reported on their progress each morning of the second and third days and gave a final report on the last day of the meeting. Opportunity for public comment was provided in all plenary and breakout sessions. Following the Workshop, each of the Breakout Groups prepared reports that represented the consensus of the invited scientists assigned to that Group.

The NICEATM subsequently assembled the Breakout Group reports and other relevant information into this Workshop Report. A separate *Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity*, based on contributions from Drs. Rodger Curren, Julia Fentem, and Manfred Liebsch, was also prepared after the workshop. The Organizing Committee and ICCVAM reviewed the report and guidance document, and developed test recommendations to forward with these publications to Federal agencies for their consideration in accordance with Public Law 106-545. The ICCVAM recommendations are included in this report as Appendix I. Both publications are available on the Internet at the ICCVAM/NICEATM website (http://iccvam.niehs.nih.gov), and copies may be requested from NICEATM through email at: NICEATM@niehs.nih.gov.

On behalf of the ICCVAM, we gratefully acknowledge the unselfish contributions of all of the Workshop participants. We extend a special thanks to the Breakout Group co-chairs who worked diligently to ensure the timely completion and accuracy of their Group reports. The efforts of the Organizing Committee members and especially the co-chairs, Drs. John Frazier and Philip Sayre, were instrumental in assuring a productive and useful Workshop. The efforts of the NICEATM staff in coordinating local arrangements, providing timely distribution of information, and preparing the final report are acknowledged and appreciated. We especially acknowledge Dr. Ray Tice for preparation of the comprehensive background materials, Brad Blackard for coordinating communications and logistics throughout the entire project, and Michael Paris and Judy Strickland for their efforts in compiling the final workshop report.

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