Preface

In the past the testing of chemicals for acute toxicity focused on determining the dose level which killed half the animals (the median lethal dose, or LD50). The "classical" LD50 used up to 100 animals to determine a median lethal dose within certain statistical bounds. More recently, several methods, which use far fewer animals, have been proposed and adopted. Attention has also broadened to include careful observation related to the onset, nature, severity, and reversibility of toxicity as well as lethality following single chemical exposures. Such information is crucial to properly identify, classify, and label human health hazards that may result from acute exposures in the workplace and home, and to make judgments pertaining to acute chemical hazards.

In 1981, the Organisation for Economic Co-operation and Development (OECD) adopted an international test guideline for acute oral toxicity testing which used as few as 30 animals. This guideline was revised in 1987 to reduce the number of test animals to as few as 20. In a continuing attempt to improve the estimate of acute toxicity while further reducing the number of animals used per test, three alternative test methods were subsequently developed and adopted as additional OECD Guidelines for acute toxicity. These were the Fixed Dose Procedure (FDP), the Acute Toxic Class Method (ATCM), and the Up-and-Down Procedure (UDP). Each of these methods used fewer animals when compared to the OECD 1987 conventional LD50 procedure.

In 1998, the OECD proposed deletion of the conventional LD50 test in light of the adoption of the three alternative methods (FDP, ATCM, UDP). Prior to formal deletion, the OECD determined it was necessary to revise the three methods to conform to a new globally harmonized hazard classification scheme. The U.S. EPA agreed to organize a Technical Task Force to revise the UDP. The Task Force was charged with preparing a revised UDP which comprised three procedures: a Primary Test to estimate the LD50, which would use an average of seven animals; a Limit Test for substances anticipated to have minimal toxicity; and a Supplemental Test to determine the slope and confidence interval for the dose-response curve. The Task Force used computer simulations to help revise the test. The revised UDP was proposed as a substitute for the existing conventional LD50 test (OECD Test Guideline 401, 1987; EPA OPPTS 870.1100, 1998) used to evaluate the acute oral toxicity potential of chemicals.

In August of 1999, the U.S. EPA asked the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) to conduct an independent scientific peer review evaluation of the revised UDP. Upon agreement, the ICCVAM requested knowledgeable individuals from participating Federal agencies to serve on an ICCVAM Acute Toxicity Working Group (ATWG); subsequently, ICCVAM would organize the peer review in collaboration with the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Methods (NICEATM). The ATWG held its first meeting in November 1999, and was charged with reviewing the revised UDP submission for completeness, proposing expert scientists for the peer review panel, developing questions for the peer review panel, and developing draft ICCVAM test recommendations based on the peer review panel's evaluation. During the next six months the ATWG provided guidance and interacted with the UDP Technical Task Force and ICCVAM to assemble adequate information for scientific peer review of the method in accordance with the *ICCVAM Test Method Submission Guidelines* (ICCVAM, 1999). A final revised UDP was submitted to ICCVAM in April 2000.

A *Federal Register* notice (February 18, 2000, Vol. 65, No. 34, 8385-8386) requested nominations of experts for the peer review panel (Panel). Nominations were also solicited from Federal agencies and national and international professional societies and organizations. The ATWG recommended a Panel composition with a broad range of experience and expertise, including

acute toxicity testing, biostatistics, alternative methods, pharmacology, and toxicokinetics. The Panel was composed of 19 experts from industry, academia, and government, and included scientists from the US, UK, New Zealand, and The Netherlands.

The Panel was charged with evaluating the usefulness and limitations of the three tests described in the UDP (Primary Test, Limit Test, and Supplemental Test) as a substitute for the conventional LD50. In reaching this determination, the Panel was asked to evaluate all available information and data on the UDP and to assess the extent to which each of the ICCVAM validation and regulatory acceptance criteria were addressed. These criteria are described in the document *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 (ICCVAM, 1997). A series of questions were posed to the Panel to facilitate ICCVAM and agency decisions on the UDP.

A request for data and information regarding the usefulness of the UDP, including information pertaining to completed, ongoing, or planned studies, was made via a *Federal Register* notice (February 18, 2000, Vol. 65, No. 34, 8385-8386). The availability of the UDP test method submission materials, a request for public comments, and announcement of the planned public peer review meeting were announced in a subsequent *Federal Register* notice (June 1, 2000, Vol. 65, No. 106, 35109-35110). All comments and information submitted in response to these notices were provided to the Panel in advance of the peer review meeting.

The Panel met in public session on July 25, 2000, in Arlington, Virginia. Panel members presented their evaluations and proposed conclusions and recommendations on each of the major sections and the Panel subsequently reached a consensus for each section. The opportunity for public comment was provided during the meeting. Following the meeting, the Panel's written evaluations, conclusions, and recommendations were consolidated as the July 2000 Peer Review Panel Report, which follows as Section I. The Panel agreed the Primary and Limit tests would perform as good or better than the existing conventional LD50 and limit tests, respectively. They also agreed the revised tests would reduce animal use compared to the current test methods. The Panel made several recommendations for revision of the UDP test guideline. The Panel did not recommend the UDP Supplemental Test.

After the July meeting, the UDP Technical Task Force prepared a revised draft test guideline (**Appendix C-1**) which incorporated and addressed the Panel's recommendations. A user friendly software program was added to aid in sequential dose selection, test-stopping decisions, calculation of an estimated LD50, and calculation of a confidence interval around the LD50. Availability of the revised draft UDP guideline and software program, and request for public comment were announced in a June 22, 2001, *Federal Register* notice (Vol. 66, No. 121, 33551-33552). A subsequent *Federal Register* notice (Vol. 66, No. 133, 36294-36295, July 11, 2001) announced the Panel meeting and requested public comment.

The UDP Panel met on August 21, 2001, via teleconference, with public meeting access made available in Research Triangle Park, North Carolina. Opportunity for public comment was provided during the meeting. The Panel reviewed and endorsed the revised UDP guideline, confidence interval procedure, and software program, with the provision that some additional clarifications should be incorporated. The Panel's evaluations, conclusions, and recommendations were consolidated as the August 2001 Peer Review Panel Report, which follows as Section II.

Following the August 2001 peer review panel meeting, the UDP Technical Task Force revised the UDP Guideline in response to the Panel's recommendations. This revised Guideline was reviewed and endorsed by the ATWG and the ICCVAM, and is provided as **Appendix B** in this report. In accordance with the ICCVAM Authorization Act of 2000, Public Law 106-545, the ICCVAM developed and adopted an ICCVAM test recommendation for the UDP, which is included in this report as **Appendix A**.

As required by P. L. 106-545, the ICCVAM test recommendation will be forwarded to Federal agencies for their consideration and appropriate actions. This publication and many of the supporting documents are available on the Internet at the ICCVAM/NICEATM website (http://iccvam.niehs.nih.gov). Agency responses to ICCVAM test recommendations will also be made available at this website.

We gratefully acknowledge all of the individuals who served as Peer Review Panel members for their thoughtful evaluations and unselfish contributions of their time. We extend a special thanks to Drs. Diane Gerken and Curtis Klaassen for their service as Panel Co-chairs, and to Drs. George Alexeeff, Wallace Hayes, Janice Kuhn, and Robert Scala for their service as Section Leaders. The efforts of the ATWG were instrumental in assuring a meaningful and comprehensive review which addressed regulatory needs. The UDP Technical Task Force was responsive to the requests and suggestions for revisions and supporting documentation over the duration of this project. The efforts of the NICEATM staff in coordinating local arrangements, providing timely distribution of information, and preparing this final report are acknowledged and appreciated. We especially thank Mr. Brad Blackard for coordinating communications and logistics throughout the entire project. On behalf of the ICCVAM, we extend our thanks to the many individuals who contributed to the evaluation of the UDP.

William S. Stokes, D.V.M.
Co-Chair, ICCVAM, NIEHS

Richard N. Hill, M.D., Ph.D.
Co-Chair, ICCVAM, U.S. EPA