1.0 INTRODUCTION

This report summarizes the proceedings and outcome of the International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity, October 17-20, 2000, in Arlington, VA, U.S. This Workshop, the first convened by ICCVAM and NICEATM, evaluated the status of available *in vitro* methods for assessing acute toxicity. These included screening methods such as those that may be used to predict the starting dose for *in vivo* animal studies, and *in vitro* methods for generating information on toxicokinetics, target organ toxicity, and mechanisms of toxicity. The Workshop also developed recommendations for validation efforts necessary to further characterize the usefulness and limitations of these methods and for research and development efforts that might further improve *in vitro* assessments of acute systemic toxicity. Notice of the Workshop and requests for nomination of scientific experts and submission of information on relevant past, current, or future studies were announced in two Federal Register notices (See **Appendix H**).

This introduction briefly summarizes the purpose and history of acute toxicity testing and the purpose and conduct of the Workshop. The final reports from the Breakout Groups are presented in **Sections 2** through **5**. **Section 6** provides a glossary, while **Section 7** contains the Registry of Cytotoxicity (RC) Data, a database of LD50 values and *in vitro* cytotoxicity IC50 values, and a regression analysis between the two values. **Section 8** contains all references cited in the Breakout Group reports and appendices. The Appendices provide supplementary materials, including the Workshop agenda, a summary of the plenary sessions, guidance for the Breakout Groups, the background document provided to Workshop participants, the NICEATM summary of the Multicenter Evaluation of *In Vitro* Cytotoxicity (MEIC), regulatory requirements for acute toxicity information, a bibliography, the list of Workshop participants, Federal Register notices regarding the Workshop, and ICCVAM test method recommendations forwarded to Federal agencies.

1.1 History and Purpose of Acute Toxicity Testing

Acute oral systemic toxicity testing is conducted to determine the hazard potential of a single oral exposure to various chemicals and products. Four regulatory agencies in the United States, the Department of Transportation (DOT), the Consumer Product Safety Commission (CPSC), the Occupational Safety and Health Administration (OSHA), and the U.S. Environmental Protection Agency (EPA) require industry to label chemicals and products with hazard information based on LD50 estimates. DOT requires oral lethality data to determine the transportation requirements for hazardous substances (49 CFR 173). CPSC requires such information for labeling hazardous substances so as to protect consumers when such products are used in the home, the school, and recreational facilities (16 CFR 1500). OSHA requires the use of acute lethality data to implement labeling requirements for the hazard communication program to protect employees (29 CFR 1910). Certain EPA regulatory programs also require the submission or generation of acute toxicity data for hazard classification purposes (40 CFR 156). During acute toxicity testing, non-lethal endpoints may also be evaluated to identify potential target organ toxicity, toxicokinetic parameters, and/or dose-response relationships.

As shown in Table 1, the international community also uses acute oral toxicity data as the basis for hazard classification and the labeling of chemicals for their manufacture, transport, and use (OECD, 1998a). Other potential uses for acute toxicity testing data include:

- Establishing dosing levels for repeated-dose toxicity studies;
- Generating information on the specific organs affected;
- Providing information related to the mode of toxic action;
- Aiding in the diagnosis and treatment of toxic reactions;
- Providing information for comparison of toxicity and dose response among

- substances in a specific chemical or product class;
- Aiding in the standardization of biological products;
- Aiding in judging the consequences of single, high accidental exposures in the workplace, home, or from accidental release;
- Serving as a standard for evaluating alternatives to animal tests.

Table 1.1 OECD Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances—Oral Toxicity (OECD, 1998a)

Acute Toxicity Route	Toxicity Class 1	Toxicity Class 2	Toxicity Class 3	Toxicity Class 4	Toxicity Class 5
Oral LD50 Values (mg/kg) [approximate]	5	50	300	2000	5000

Historically, lethality has been the primary toxicological endpoint in acute toxicity tests. Trevan (1927) was the first to attempt to standardize a method for assessing the toxicity of potent biological toxicants, the progenitor of the "lethal dose, 50% (LD50) test". The classical LD50 test procedure that evolved from this innovation in the 1970s and early 1980s used from 100 to 200 animals per test substance (Galson, 2000). Although other information, such as the slope of the dose-response curve, confidence interval for the LD50, and toxic signs, could also be obtained from this test, the procedure was severely criticized for both scientific and animal welfare reasons (Zbinden and Flury-Roversi, 1981). These criticisms eventually resulted in the proposal and adoption of a new guideline (OECD TG 401; OECD, 1987) that reduced the required number of animals to 20. This has become the most widely used method for defining the acute toxicity of a chemical and a mandatory-testing requirement for new chemicals. More recently, the acute toxicity test procedure has been modified in various ways to refine and further reduce the number of animals used to a maximum of 16 (OECD, 1992; 1996; 1998b). The Globally Harmonized Scheme for Hazard Classification prompted a re-assessment of all of the OECD *in vivo* test guidelines for acute toxicity (i.e., fixed dose, up and down procedure, acute toxic class method) to ensure that regulatory needs are met while minimizing animal usage and maximizing data quality.

Recent studies suggest that *in vitro* methods may be helpful in predicting acute toxicity and reducing the number of animals necessary to assess acute toxicity. Studies by Spielmann et al. (1999) suggest that *in vitro* cytotoxicity data may be useful in identifying an appropriate starting dose for *in vivo* studies, and thus may potentially reduce the number of animals necessary for such determinations. Other studies (e.g., Ekwall et al., 2000) have indicated an association between chemical concentrations leading to *in vitro* basal cytotoxicity and human lethal blood concentrations. A program to estimate toxicokinetic parameters and target organ toxicity utilizing *in vitro* methods has been proposed that may provide enhanced predictions of toxicity, and potentially reduce or replace animal use for some tests (Ekwall et al., 1999). However, many of the necessary *in vitro* methods for this program have not yet been developed. Other methods have not been evaluated in validation studies to determine their reliability and relevance for generating information to meet regulatory requirements for acute toxicity testing. Development and

validation of *in vitro* methods that can establish accurate dose-response relationships will be necessary before such methods can be considered for the reduction or replacement of animal use for acute toxicity determinations.

1.2 Purpose and Objectives of the Workshop

The International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity examined the status of available *in vitro* methods for predicting acute toxicity, including screening methods for acute toxicity, and other methods that might be suitable to predict the starting dose for *in vivo* animal studies, and methods for generating information on toxicokinetics, target metabolism organ toxicity, and mechanisms of toxicity. The Workshop developed recommendations for validation efforts necessary to further characterize the usefulness and limitations of these methods. Recommendations were also developed for future mechanism-based research and development efforts that might further improve *in vitro* assessments of acute systemic lethal and non-lethal toxicity.

Specific objectives of the Workshop were to:

- Review the status of *in vitro* methods for predicting acute systemic toxicity:
  - — Review the validation status of available *in vitro* screening methods for their usefulness in estimating *in vivo* acute systemic toxicity;
  - — Review *in vitro* methods for predicting toxicokinetic parameters relevant to acute toxicity (i.e., absorption, distribution, metabolism, elimination);
  - — Review *in vitro* methods for predicting specific target organ toxicity;
- Recommend candidate methods for further evaluation in prevalidation and validation studies;
- Recommend validation study designs to adequately characterize the usefulness and limitations of proposed *in vitro* methods;
- Identify reference chemicals for development and validation of *in vitro* methods for assessing *in vivo* acute toxicity;
- Identify priority research efforts necessary to support the development of *in vitro* methods to assess acute systemic toxicity adequately. Such efforts might include incorporation and evaluation of new technologies such as gene microarrays, and development of methods necessary to generate dose response information.

1.3 Conduct of the Workshop

The International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity, which was open to the public, was conducted over three and a half days. The final agenda for the meeting is provided in **Appendix A**. As the agenda shows, the Workshop began with a plenary session to frame the purpose and objectives of the Workshop and formulate the problem of using *in vitro* tests to predict *in vivo* acute toxicity. A summary of the opening plenary session is provided in **Appendix B.** The opening plenary session was followed by Breakout Group discussions for two and a half days. Each of the four Breakout Groups was comprised of 12 to 18 individuals who were invited scientific experts or ICCVAM agency participants. Breakout Groups addressed their assigned objectives for the Workshop by developing responses to questions provided in the background materials for the Workshop (See **Appendix C**). Breakout 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. Written reports of each Breakout Group's findings, conclusions and recommendations are provided in **Sections 2** through **5**. Public observers were invited to provide comments in both plenary and breakout sessions of the Workshop. A summary of public comments during plenary sessions is provided in **Appendix B.** After the Workshop, ICCVAM reviewed the Breakout Group reports and developed test method recommendations for Federal agencies (see **Appendix I**).