**ICCVAM Recommendations on *In Vitro* Methods for Assessing Acute Systemic Toxicity**

An International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity was convened in Arlington, VA, on October 17-20, 2000. The Workshop was organized by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), and was co-sponsored by the U.S. Environmental Protection Agency (EPA), the National Institute of Environmental Health Sciences (NIEHS), and the National Toxicology Program (NTP). The Workshop focused on reviewing the validation status and possible current uses of *in vitro* methods to assess acute oral lethality potential of chemicals. Workshop participants also recommended research, development, and validation efforts that would further advance the usefulness of *in vitro* methods. For a complete account of Workshop discussions and recommendations, please refer to the *Report of the International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity* (NIH Publication 01-4499). Based on a review of the Workshop Report, ICCVAM developed the following recommendations to forward to Federal agencies with the Report and Guidance Document.

**Current Uses for *In Vitro* Methods**

Workshop participants considered the merit of using *in vitro* cytotoxicity tests for predicting the acute oral lethality of chemicals in humans and animals, as suggested by previous studies (e.g., Clemedson and Ekwall, 1999; Halle and Goeres, 1988). They concluded that the available *in vitro* assays would require further development to accurately predict acute lethality (i.e., LD50). Workshop participants recommended that *in vitro* cytotoxicity data be included as one of the factors used to identify appropriate starting doses for *in vivo* acute lethality studies as described by Spielmann et al. (1999). In the approach developed by Spielmann, *in vitro* cytotoxicity tests are used to predict starting doses for acute *in vivo* lethality assays.

ICCVAM agrees with the Workshop Report that data from *in vitro* cytotoxicity assays can be useful as one of the tools (e.g., SAR or bridging from similar compounds or mixtures) in setting a starting dose for the *in vivo* assessment of acute oral toxicity. The attached *Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity* (NIH Publication 01-4500) describes one method, the murine BALB/c 3T3 neutral red uptake assay, for which data for a number of chemicals supports its potential utility for estimating the starting dose. Starting doses are calculated using a regression formula based on an *in vitro-in vivo* correlation for 347 chemicals. Preliminary information suggests that use of this *in vitro* approach could reduce the number of animals currently used in *in vivo* acute toxicity tests. Additionally, new OECD Guidelines for *in vivo* acute toxicity testing recommend a starting dose below the estimated LD50 to minimize the number of animals that receive lethal doses and to avoid underestimating the hazard. ICCVAM recommends that Federal agencies consider making information about this *in vitro* approach available as one of the tools that can be used to select an appropriate starting dose for acute oral toxicity tests.

**Research Directions**

Workshop participants identified several areas for research and development activities to advance the use of *in vitro* methods for predicting acute oral toxicity in animals and humans. ICCVAM recognizes that there are many directions that such future research and testing might take. These include both near-term and long-term research activities.

- **Near-Term Research**

ICCVAM concurs with the Workshop recommendation that near-term validation studies should focus on two standard cytotoxicity assays: one using a human cell system and one using a rodent cell system. Since the murine BALB/c 3T3 cytotoxicity assay has been evaluated for only a limited number of chemical classes, there is

merit in determining its usefulness with a broader array of chemical classes. Cell lines established from the rat rather than the mouse might also be considered, as most acute oral toxicity testing is conducted in this species. Human cell lines should also be considered since one of the aims of toxicity testing is to make predictions of potential toxicity in humans. Future validation studies should therefore compare rodent and human *in vitro* data with one another, with rodent *in vivo* data, and with human *in vivo* data. Correlations between *in vitro* and *in vivo* data might help in selecting cytotoxicity assays for further evaluation.

The U.S. EPA and NIEHS are collaborating to further characterize the usefulness of *in vitro* methods for acute toxicity testing. ICCVAM recognizes that these activities may yield important information on the near-term and long-term application of *in vitro* tests. ICCVAM recommends the establishment of an interagency expert group under ICCVAM to advise on near-term activities such as assay selection, study design, and chemical selection.

➢ **Long-Term Research**

Longer-term research activities should be directed at improving *in vitro* systems that provide information on biokinetics, metabolism, and organ-specific toxicity. *In vitro* methodologies for gathering biokinetic and target organ specific effects data are needed to facilitate reasonably accurate predictions of LD50s, signs and symptoms associated with toxicity, and pathophysiological effects. Research efforts that might increase the predictive capability of *in vitro* assays include:

- Developing the use of quantitative structure-activity relationship (QSAR)/quantitative structure-property relationship (QSPR) models that predict kinetic parameters such as gut absorption and passage across the brain, kidney, and skin barrier systems.

- Developing efficient *in vitro* systems that provide accurate metabolic and biokinetic data.

- Developing accurate physiologically-based biokinetic models.

- Developing *in vitro* systems that accurately predict organ-specific toxicity.

- Investigating the mechanistic basis for "outlier" chemicals in *in vitro-in vivo* correlations and developing "exclusion" rules for identifying chemicals that cannot be accurately evaluated using *in vitro* methods.

- Investigating the utility of toxicogenomics/proteomics for the assessment of acute toxicity, especially the prediction of NOAELs/LOAELs for acute exposure.

ICCVAM appreciates that most of these long-term research activities will yield further improvements in the usefulness of *in vitro* methods for predicting acute systemic toxicity, but that significant resources would be required. ICCVAM concludes that such activities will warrant consideration along with other potential research efforts in establishing priorities.

Adopted by ICCVAM
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