International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity
October 17-20, 2000
Arlington, VA. U.S.A.

Guidance for Breakout Groups

Breakout Groups will address the applicable Workshop objectives and develop responses to the questions provided for each Breakout Group.

A. Workshop Objectives:

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

B. Breakout Group Questions

Breakout Group 1: *In Vitro* Screening Methods for Assessing Acute Toxicity

This Breakout Group is asked to evaluate the validation status of available in vitro methods for estimating in vivo acute toxicity. The Group will identify methods and appropriate validation studies that might be completed within the next 1-2 years. The potential uses of QSAR as part of an in vitro strategy will also be evaluated.

Session 1-1: Identifying Needs

1. What are the near-term (< 2 years) goals and potentially attainable objectives for validation and use of *in vitro* methods that might reduce animal use for assessing acute toxicity?
2. What types of in vitro endpoints would be most effective for assessing in vivo acute toxicity; those that relate to general toxicity (e.g., cell death, growth inhibition) or those that are more cell or function specific (e.g., DNA damage/repair/synthesis; mitochondrial functionality; inhibition of other metabolic pathways)?
3. What other issues need to be considered for selecting protocols, e.g., robustness of protocol, reproducibility, stability of cell line?
4. What is the role of QSAR (and other prediction models) in predicting acute toxicity?

Session 1-2: Current Status

1. What are the available in vitro methods that might be useful in estimating acute in vivo toxicity.? Are standardized and/or optimized protocols available?
2. What are the strengths and limitations of available in vitro cytotoxicity assays (e.g., MEIC; ZEBET's validation efforts to extend cytotoxicity data to obtain better starting dose estimations; other mechanism-based cytotoxicity assays)?
3. What is the validation status of available in vitro screening methods (see Validation Criteria)?
4. Have any of these available in vitro methods been adequately evaluated for their usefulness for a specific purpose? If so, is their performance sufficient to recommend their use at this time?
5. What are the relative advantages and disadvantages for the use of human cells/tissues versus human cell lines versus animal cells/tissues versus animal cell lines?

6. To what extent do available methods take into consideration metabolic activation/inactivation of chemicals?
7. How have QSAR and other prediction models been used to estimate acute toxicity? What commercially available software exists? What are their advantages and disadvantages?
8. Are the available toxicity databases adequate to develop useful QSARs for industrial chemicals, consumer products, drugs? If not, what are the data needs?

Session 1-3: Future Directions

1. What are the most promising *in vitro* methods that should be further evaluated for their usefulness in reducing and/or refining animal use for acute toxicity?
   a.. What validation studies would be necessary to adequately evaluate the usefulness and limitations of these proposed methods for their proposed use?
   b. What research and/or developmental needs are required for candidate *in vitro* tests?
   c. What other mechanism-based *in vitro* methods or endpoints should be evaluated in future validation studies (e.g., microarray evaluation of altered gene expression patterns)? If so, which *in vitro* methods or endpoints should be given priority?
2. Which are the most promising *in vitro* methods for further evaluation or validation as replacements for *in vivo* acute toxicity test methods?
   a. What additional validation studies would be necessary to adequately evaluate the usefulness and limitations of these methods as replacements?
   b. What research and/or developmental needs are required for candidate *in vitro* tests?
   c. What other mechanism-based *in vitro* methods or endpoints should be evaluated in future validation studies (e.g., microarray evaluation of altered gene expression patterns)? If so, which *in vitro* methods or endpoints should be given priority?
3. How should individual tests be evaluated to determine their usefulness for integration into an overall acute toxicity testing strategy?
4. What criteria should be used to evaluate QSAR methods? To what extent could QSAR's be improved by an improved understanding of the molecular and cellular mechanisms of action of toxicity? What knowledge gaps exist that should be addressed by future research?

Breakout Group 2: *In Vitro* Methods for Assessing Acute Toxicity –Toxicokinetic Determinations

This Breakout Group will evaluate the capabilities of *in vitro* methods for providing toxicokinetic information (absorption, distribution, metabolism, and elimination) that can be used to estimate target organ dosimetry for acute toxicity testing and to provide recommendations for future research needs to accomplish this goal. The role of QSAR in toxicokinetic determinations will also be explored.

Session 2-1: Identify Needs

1. How can *in vitro* methods for evaluating chemical kinetics in biological systems contribute to the hazard and risk assessment process?
2. What is the role of toxicokinetics in the overall mechanisms by which chemicals illicit acute toxicity?
3. What toxicokinetic techniques should be considered as *in vitro* assays to improve predictivity and increase understanding of toxicity mechanisms? What is the role of QSAR in predicting chemical kinetics?

Session 2-2: Current Status

1. What *in vitro* methods are available for *in vitro* estimations of chemical-specific toxicokinetic parameters in animals and humans?
2. What are the strengths, limitations, and validation status of these available methods?
3. What mathematical approaches are available to predict or model toxicokinetics of

chemicals in mammalian systems based on data from *in vitro* systems?
4. What are the potential strengths and limitations of these approaches?
5. How would the approaches have to be modified/improved to meet acute toxicity testing needs?
6. How effective are the available QSAR systems for predicting *in vivo* toxicokinetic parameters?

Session 2-3: Future Directions

1. Which *in vitro*, QSAR or PBBK methods are the most promising for future use or development?
2. How should candidate methods be further developed/validated?
3. What are the more important issues to focus on in the long run (e.g., GI absorption, blood-brain barrier penetration)?
4. What research and development efforts are needed to achieve the ability to predict chemical kinetics in animals and humans?

Breakout Group 3: *In Vitro* Methods for Assessing Acute Toxicity - Specific Organ Toxicity and Mechanisms

This Breakout Group will review *in vitro* methods that can be used to predict specific organ toxicity or toxicity associated with alteration of specific cellular or organ functions, and develop recommendations for priority research efforts necessary to support the development of methods that can accurately assess target organ toxicity.

Session 3-1: Identify Needs

1. How can *in vitro* methods for assessing target organ toxicity contribute to hazard identification and dose-response assessment processes?
2. What is the relationship between *in vitro* mechanisms of toxicity and mechanisms by which chemicals are acutely toxic to animals and humans?
3. How can *in vitro* toxicity assays be used to predict acute organ-specific toxicity?
4. Can mechanism-based *in vitro* methods be developed to evaluate the range of *in vivo* toxicity processes and estimate those which may lead to injury or lethality?
5. What *in vitro* procedures and endpoints should be considered to improve predictability of *in vivo* effects and increase understanding of toxicity mechanisms?

Session 3-2: Current Status

1. What *in vitro* methods are available for target tissue-based estimations of animal and human responses to chemicals?
2. What is the validation status of these available methods?
3. What are their potential strengths and limitations?
4. How would they have to be modified/improved to enhance their usefulness?
5. Are techniques available to extrapolate *in vitro* cell toxicity data to predict acute systemic responses and ultimately system failure?

Session 3-3: Future Directions

1. Which are the most promising assays or methodologies to evaluate further?
2. How should each one be further developed/validated?
3. What are the research needs to attain the ability to predict acute toxicity in animals and humans?
4. What new methods or approaches are available that might improve mechanism-based in vitro estimations of animal and human responses to chemicals? How should they be developed for acute toxicity testing purposes?
5. How might the potential usefulness of microarray technology/differential gene expression for predicting systemic toxicity be further evaluated?
6. What research needs must be supported to improve QSAR methods for predicting target organ toxicity?

Breakout Group 4: Chemical Data Sets for Validation of *In Vitro* Toxicity Tests

This Breakout Group will have the responsibility of defining what chemical data sets are required for validation studies, identifying existing resources, and recommending approaches for using existing data sets and/or compiling or developing new data sets.

Session 4-1: Identify Needs

1. What are the characteristics of chemical [sets] that should be used in the validation of *in vitro* test methods for acute toxicity? For predicting organ-specific toxicity or toxicity based on specific mechanisms?
2. What criteria should be used for selecting chemical classes and chemicals to validate *in vitro* methods for assessing acute toxicity? Considering the different purposes of various *in vitro* methods, which sets of chemicals should be used to evaluate these different purposes?
3. To what extent and how should product classes/chemical classes (as used by regulatory agencies) be used to guide chemical selection?
4. To what extent and how should mode of action and biological target data be used to identify chemicals for use in validation studies?
5. How can QSAR methods help in the selection of validation chemicals?

Session 4-2: Current Status

1. What chemical data sets are available (e.g., EPA-HPV industrial chemicals, pesticides, drugs, food additives, NTP chemicals) that could be used for the validation of acute toxicity testing methods?
2. Are sufficient toxicity data available on existing chemicals or will additional data need to be obtained.
3. Do the available chemical data sets adequately represent the range of regulatory classifications for toxicity?
4. What QSAR models are currently available for such an effort?

Session 4-3: Future Directions

1. What are the characteristics of chemical data sets that could be used for validation of in vitro tests for in vivo toxicity (e.g., estimation of acute toxicity; identification of organ-specific toxic effects; determination of ADME parameters)?
2. To the extent possible, identify reference chemicals for which sufficient information is available that they should be considered for validation of assays/methodologies for predicting starting doses for in vivo studies, assays, or other assays that can be implemented in the near term? Are existing chemical sets adequate? Are additional chemicals needed, and if yes, are additional in vivo acute toxicity data needed?
3. To the extent possible, which reference chemicals should be used in the development/validation of assays/methods developed to predict in vivo acute toxicity in the longer term? Are different sets of chemicals needed to evaluate methods to predict target organ toxicity?
4. Should there be established chemical data sets for use in validation studies, or should they be selected or developed according to the specific test to be evaluated?
5. What additional chemical data sets need to be compiled or developed?
6. How should these chemical data sets be developed, and by whom?