ICCVAM-NICEATM/ECVAM/JaCVAM Workshop

Acute Chemical Safety Testing: Advancing *In Vitro* Approaches and Humane Endpoints for Systemic Toxicity Evaluations

February 6-7, 2008

Workshop Goals and Objectives

Breakout Groups 1-5 Assigned Objectives and Questions

Workshop Goals

1. Review the state-of-the-science and identify knowledge gaps (at the whole organism, organ system, cellular, and/or molecular levels) regarding the key *in vivo* pathways[1] involved in acute systemic toxicity
2. Recommend how these knowledge gaps can be addressed by collecting mechanistic biomarker data during currently required *in vivo* safety testing
3. Recommend how *in vivo* key pathway information can be used to develop more predictive mechanism-based *in vitro* test systems and to identify biomarkers that may serve as predictive earlier more humane endpoints for *in vivo* test methods
4. Recommend how mechanism-based *in vitro* test systems and earlier more humane endpoints can be used to further reduce, refine, and eventually replace animal use for acute systemic toxicity testing while ensuring the protection of human and animal health.

[1] Cellular response pathways that, when sufficiently perturbed, are expected to result in adverse health effects are termed *toxicity pathways*. **(***Toxicity Testing in the Twenty-first Century: A Vision and a Strategy*. Committee on Toxicity and Assessment of Environmental Agents. National Research Council. 2007. pp. 1-2.)

Workshop Objectives

1. Discuss the current understanding of key pathways for *in vivo* acute systemic toxicity and identify the knowledge gaps that exist, especially for
   (1) *in vivo* pathways, and
   (2) chemicals and products tested for acute systemic toxicity
2. Identify and prioritize future research initiatives that would address these knowledge gaps and that are considered necessary to advance the development and validation of *in vitro* methods for assessing acute systemic toxicity.
3. Review molecular, cellular, tissue, or other physiological, and clinical biomarkers that are or could be measured or observed during *in vivo* acute systemic toxicity testing and discuss their potential usefulness for indicating key pathways of acute systemic toxicity.
4. Discuss how the key toxicity pathways indicated by these *in vivo* measurements and observations might be modeled using alternative *in vitro* test methods.
5. Discuss and identify observations and quantitative, objective measurements that could or should be included in the current *in vivo* acute systemic toxicity tests to elucidate key toxicity pathways that would support the future development and validation of predictive *in vitro* methods.
6. Identify and prioritize research, development, and validation activities for *in vitro* test methods that model the key *in vivo* toxicity pathways and more accurately predict acute systemic toxicity hazard categories.
7. Discuss what *in vivo* data collected to elucidate key toxicity pathways might lead to the identification and validation of more humane endpoints for acute systemic toxicity testing, and what data should be a priority for collection to aid in identifying earlier more humane endpoints.
8. Discuss how to promote the collection and submission of *in vitro* and *in vivo* toxicity test data to ICCVAM in order to advance the development and validation of more predictive *in vitro* test methods and earlier more humane endpoints for acute systemic toxicity testing.

Breakout Group 1

Key Pathways for Acute Systemic Toxicity

Co-chairs: Drs. Daniel Acosta and Frank Paloucek

Objectives

1. Discuss the current understanding of key pathways for *in vivo* acute systemic toxicity and identify the knowledge gaps that exist, especially for
   (1) *in vivo* pathways, and
   (2) chemicals and products tested for acute systemic toxicity (Workshop Objective 1)
2. Identify and prioritize future research initiatives that would address these knowledge gaps and that are considered necessary to advance the development and validation of *in vitro* methods for assessing acute systemic toxicity. (Workshop Objective 2)
3. Review molecular, cellular, tissue, or other physiological, and clinical biomarkers that are or could be measured or observed during *in vivo* acute systemic toxicity testing and discuss their potential usefulness for indicating key pathways of acute systemic toxicity. (Workshop Objective 3)

Questions

1. What are the key toxicity pathways for acute human poisonings?
2. Which *in vivo* test observations/measurements and data have been most helpful for diagnosis and treatment of human poisonings?
3. What are the knowledge gaps associated with diagnosis and/or treatment of human poisoning?
4. What toxicological observations and measurements would address these knowledge gaps and improve the information available for the diagnosis and/or treatment of human poisoning (e.g., how might *in vivo* mechanistic data be helpful for diagnosis and treatment of human poisonings?)?
5. Prioritize research and development activities. Discuss how these activities might best be implemented.

Breakout Group 2

Current Acute Systemic Toxicity Injury and Toxicity Assessments

Co-chairs: Drs. A. Wallace Hayes and Daniel Marsman

Objectives

1. Discuss and identify observations and quantitative, objective measurements that could or should be included in the current *in vivo* acute systemic toxicity tests to elucidate key toxicity pathways that would support the future development and validation of predictive *in vitro* methods. (Workshop Objective 5)

Questions

1. What are the pathways involved in acute systemic toxicity that will need to be modeled using *in vitro* test systems?
2. What biomarkers might be used to provide more information on *in vivo* pathophysiological effects and mechanisms of acute systemic toxicity? For example:
   - Histopathology findings
   - Gross pathology findings
   - Clinical biochemistry data
   - Hematology data
   - Body weight and food/water consumption
   - Detailed clinical observations
   - Functional measurements (e.g., heart rate, electrocardiogram, respiratory rate, respiratory volume, body temperature, functional observational battery for neurotoxicity)
3. How might the timing of these measurements/observations impact on their interpretation?
4. Identify which data should be routinely considered for collection and which data should be considered desirable, but optional.
5. What would be the optimal way to measure these suggested biomarkers as part of the current acute systemic toxicity tests?
   a. To what extent should there be a standardized format for reporting biomarker data?
   b. What biomarkers have standardized methods?
   c. What biomarkers are in need of standardized methods?
6. How might the protocols for current acute systemic toxicity tests (i.e., the Up-and-Down Procedure, the Acute Toxic Class method, and the FDP) be modified to collect additional data while minimizing interference with the standard test procedures and interpretation?
7. Suggest and prioritize research and development activities for obtaining more information on key toxicity pathways from the current *in vivo* acute systemic toxicity tests (e.g., should imaging techniques be further explored?). Discuss how to implement these activities.

Breakout Group 3

Identifying Earlier Humane Endpoints for Acute Systemic Toxicity Testing

Co-chairs: Drs. Helen Diggs and Steven Niemi

Objectives

1. Discuss what *in vivo* data collected to elucidate key toxicity pathways might lead to the identification and validation of more humane endpoints for acute systemic toxicity testing, and what data should be a priority for collection to aid in identifying earlier more humane endpoints. (Workshop Objective 7)

Questions

1. Are there other objective biomarkers that are sufficiently predictive of lethality that they should be collected and used as routine humane endpoints (e.g., body temperature measurements)?
2. Should clinical signs and observations for pain and distress be routinely recorded?
3. Would the use of humane endpoints interfere with the collection and interpretation of mechanistic data?
4. Conversely, to what extent might the collection of mechanistic data lead to incorporating more humane endpoints for acute systemic toxicity testing?
5. Are there additional data that are recommended for routine collection during future animal studies that might aid in identifying earlier more humane endpoints (e.g., before an animal reaches moribund condition) for acute toxicity testing?
6. What considerations should be made for data collection for inhalation exposures exposure (e.g., nose only and/or whole body exposure])?
7. What are the knowledge gaps associated with predictive early humane endpoints that should be addressed in research, development, and validation efforts?
8. What are the most effective ways to implement the recommended activities?

Breakout Group 4

Application of *In Vivo* Mode of Action and Mechanistic Information to the Development and Validation of *In Vitro* Methods for Assessing Acute Systemic Toxicity

Co-chairs: Drs. Melvin Andersen and Eugene Elmore

Objectives

1. Discuss how the key toxicity pathways indicated by these *in vivo* measurements (molecular, cellular, tissue, or other physiological, and clinical biomarkers [see Workshop Objective 3]) and observations are currently or could be modeled using alternative *in vitro* test methods. (Workshop Objective 4)
2. Identify and prioritize research, development, and validation activities for *in vitro* test methods that model the key *in vivo* toxicity pathways and more accurately predict acute systemic toxicity hazard categories. (Workshop Objective 6)

Questions

1. To what extent do the current and proposed *in vitro* test methods adequately model the key toxicity pathways associated with acute systemic toxicity?
2. What are the knowledge gaps between the planned activities and the activities necessary to accurately predict acute systemic toxicity using *in vitro* methods?
3. What are the priorities for research, development, and validation activities for *in vitro* systems?
4. How might *in vitro* tests be incorporated into testing currently being conducted to meet regulatory testing requirements?
5. How might *in vivo* mode of action and mechanistic information be used to further improve *in vitro* testing?
6. How might the timing of observations be adjusted to differentiate the initial pathway effects from downstream effects?
7. Discuss how to implement the recommended activities.

Breakout Group 5

Industry Involvement in Test Method Development, Validation, and Use

Co-chairs: Drs. Robert Scala and William Stott

Objectives

1. Discuss how to promote the collection and submission of *in vitro* and *in vivo* toxicity test data to ICCVAM in order to advance the development and validation of more predictive *in vitro* test methods and earlier more humane endpoints for acute systemic toxicity testing. (Workshop Objective 8)

Questions

1. How can industry be effectively encouraged to collect and submit mechanistic observations and measurements from animals used in acute systemic toxicity studies?
2. What can be done to increase the use of adequately validated *in vitro* cytotoxicity test methods for reducing the use of animals in acute systemic toxicity tests?
3. How can test method users be encouraged to submit concurrent *in vitro*/*in vivo* toxicity test data to ICCVAM to advance the development and validation of alternative *in vitro* test methods for acute systemic toxicity?
4. What are the impediments to data collection and how can they be overcome?