ICCVAM/NICEATM/ECVAM/JaCVAM Scientific Workshop on Acute Chemical Safety Testing: Advancing *In Vitro* Approaches and Humane Endpoints for Acute Systemic Toxicity Evaluations

J Strickland[1], M Paris[1], D Allen[1], R Tice[2], H Kojima[3], P Prieto[4], M Wind[5], W Stokes[2]

[1]ILS, Inc., RTP, NC, USA; [2]National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods, RTP, NC, USA; [3]JaCVAM, Tokyo, Japan; [4]*In Vitro* Methods/ECVAM, Ispra, Italy; [5]U.S. CPSC, Bethesda, MD, USA

Abstract

The evaluation and promotion of alternatives for acute systemic toxicity testing is one of ICCVAM's four highest priorities because (1) acute toxicity testing is the most commonly required product safety test worldwide, and (2) it can cause significant pain and distress to test animals. We cosponsored a public workshop in February 2008 to review and consider standardized procedures to collect information pertinent to understanding the mechanisms of lethality that should be included in future rat acute systemic toxicity studies to support further development of predictive mechanism-based *in vitro* test methods. This international workshop also implemented a goal of the NICEATM–ICCVAM Five-Year Plan to identify approaches that would further reduce the potential pain and distress associated with acute toxicity testing by identifying more humane acute toxicity endpoints. The workshop reviewed public health significance and regulatory testing needs; human and animal assessments, biomarkers, and key pathways; humane endpoints; and the state of the science regarding *in vitro* methods that predict acute systemic toxicity. Breakout Groups identified knowledge gaps in understanding key toxicity pathways; recommended earlier humane endpoints for animal testing; suggested ways to obtain, from current *in vivo* testing models, mode of action and mechanistic information needed to develop and validate *in vitro* methods for assessing acute systemic toxicity; and explored avenues that would encourage industry to share information on *in vitro* and *in vivo* studies conducted in-house. This workshop recommended how mechanism-based *in vitro* test systems and earlier, more humane endpoints could be developed to further reduce, refine, and eventually replace animal use for acute systemic toxicity testing while ensuring the protection of human and animal health.

ILS staff supported by NIEHS contract N01-ES-35504.

Introduction

- Poisoning is a more serious public health problem than generally recognized. According to the Institute of Medicine:
  - More than 4 million poisoning episodes occur annually in the United States
  - In 2001, poisoning (30,800 deaths) placed second behind automobile accidents (42,433 deaths) as the leading cause of injury-related death
- Regulatory agencies throughout the world require that certain products and chemicals be tested to determine their potential to cause life-threatening or fatal acute systemic toxicity.
- Concerns about animal use have led to the development and evaluation of alternative *in vivo* test methods that significantly reduce animal use for acute systemic toxicity testing.[1] Attempts to identify *in vitro* alternatives that correctly predict *in vivo* toxicity, however, have made little progress.
- Acute systemic toxicity studies provide little information about the mode or mechanism that causes toxicity or death. Without such information it is difficult to develop mechanism-based *in vitro* test methods that can adequately model and predict *in vivo* toxicity. A greater understanding of critical toxicity pathways is needed to facilitate development of alternative test methods and subsequent replacement of animals in acute systemic toxicity testing.
- Participants of the **Scientific Workshop on Acute Chemical Safety Testing: Advancing *In Vitro* Approaches and Humane Endpoints for Systemic Toxicity Evaluations** developed approaches to identify the key toxicity pathways for acute systemic toxicity. This mechanistic information can then be used to develop predictive *in vitro* alternative test methods. Mechanistic information on acute systemic toxicity might also help identify predictive biomarkers of systemic toxicity for use as earlier, more humane endpoints during *in vivo* tests, thereby further reducing pain and distress.

[1]*A reduction alternative* is a new or modified test method that reduces the number of animals required; *A refinement alternative* is a new or modified test method that refines procedures to lessen or eliminate pain or distress in animals or enhances animal well-being (*ICCVAM Guidelines for the Nomination and Submission of New, Revised and Alternative Test Methods*, NIH Publication 03-4508, 2003).

ICCVAM and Alternative Methods for Oral Acute Toxicity Testing

2000 International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity
- Identified research, development, and validation efforts that might further enhance the use of *in vitro* methods to predict and assess acute systemic toxicity

2001 Acute Oral Toxicity Testing Guidelines Revised Up-and-Down Procedure
- Reduced animal use by as much as 70%

2002 NICEATM/ECVAM *In Vitro* Cytotoxicity Validation Study

2005
- Evaluated *in vitro* cytotoxicity data (mouse fibroblast [3T3] and human normal human epidermal keratinocyte [NHK] cells) to predict rodent *in vivo* LD_{50} values and starting doses for acute oral toxicity test methods

2007 Test Method Evaluation Report: *In Vitro* Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Tests
- ICCVAM Recommendations:
  - Always consider before using animals for acute toxicity testing, and use where determined appropriate before using animals
  - Use as adjunct tests in a weight-of-evidence approach to determine starting doses for acute oral toxicity test methods to reduce the use of animals
    - Can further reduce animal use up to 50% per test
    - Endorsed in 2008 by all 15 ICCVAM agencies, including EPA, FDA, and CPSC

2008 NICEATM-ICCVAM Five-Year Plan
- Includes several goals in the area of acute systemic toxicity testing, including the organization of a workshop to:
  - Identify standardized procedures for collecting mechanistic information from acute oral toxicity testing to aid in developing batteries of predictive *in vitro* test methods that can further reduce and eventually replace animals for acute toxicity testing
  - Seek more predictive and more humane endpoints that may be used to terminate studies earlier in order to further reduce pain and distress

ICCVAM/NICEATM/ECVAM/JaCVAM Scientific Workshop

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

February 6-7, 2008
Natcher Center, NIH, Bethesda, MD

The workshop was organized by the ICCVAM Acute Toxicity Working Group (ATWG) and cosponsored by ICCVAM, NICEATM, the European Centre for the Validation of Alternative Methods (ECVAM), and the Japanese Center for the Validation of Alternative Methods (JaCVAM).

Workshop Goals

- 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 involved in acute systemic toxicity
- Recommend how these knowledge gaps can be addressed by collecting mechanistic biomarker data during currently required *in vivo* safety testing
- 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 might serve as predictive earlier, more humane endpoints for *in vivo* test methods
- 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

Workshop Objectives

- 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
- 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
- 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
- Discuss how the key toxicity pathways indicated by these *in vivo* measurements and observations might be modeled using alternative *in vitro* test methods
- 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
- 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
- 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
- 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 Sessions

Session 1: Acute Systemic Toxicity: Public Health Significance and Regulatory Testing Needs
- This session reviewed the public health problem of poisoning from acute chemical exposures along with incidence of acute poisonings for various demographic groups, the types of chemicals involved in acute poisonings, likely causes of death, and the methodology for clinical assessments (i.e., diagnosis and treatment) of acute poisoning cases.

Session 2: Acute Systemic Toxicity: Human and Animal Assessments, Biomarkers, and Key Pathways
- This session reviewed the state of the science and understanding of the key pathways of acute systemic toxicity, and covered qualitative and quantitative objective biomarkers (i.e., measurements and observations) that could be considered for inclusion in the current acute systemic toxicity tests to elucidate key toxicity pathways.

Session 3: Humane Endpoints
- This session discussed the humane endpoints used for acute toxicity testing and the potential for information on key toxicity pathways to yield earlier, more humane endpoints. Descriptions of methods to identify and monitor pain and distress in experimental animals were provided.

Session 4: State of the Science: Using In Vitro Methods to Predict Acute Systemic Toxicity
- This session provided a summary of a previous workshop, including major conclusions, recommendations, and initiatives, as well as the status of ongoing activities resulting from the workshop. ICCVAM's current recommendations for the use of *in vitro* methods for assessing acute systemic toxicity were discussed.

Information about all sessions, including all presentations, can be obtained on the NICEATM-ICCVAM website at:
http://iccvam.niehs.nih.gov/methods/acutetox/toxwksp-present.htm

Breakout Group 1: Key Pathways for Acute Systemic Toxicity

Panelists: Drs. Daniel Acosta (Co-Chair), Melvin Andersen, Richard Becker, Daniel Cobaugh, Eugene Elmore, Hajime Kojima, Frank Paloucek (Co-Chair), Amy Rispin, Robert Scala, Raymond Tice, Marilyn Wind, and Mr. Robert Guest

Objectives
- Determine the key toxicity pathways associated with *in vivo* acute systemic toxicity and acute human poisonings.
- Identify *in vivo* test observations/measurements and data that might be the most helpful for diagnosis and treatment of human poisonings and assessing acute systemic toxicity.

Key Pathways for Better Understanding Toxic Effects of Chemicals and Treatment of Acute Human Poisonings
- General cellular function
- Neuronal transmission, both central and peripheral
- Sodium/potassium ATP-ase pump
- Xenobiotic metabolism
- Cardiac conduction and aerobic metabolism
- Oxidative stress
- Receptor activity
- Immune response and function

Knowledge Gaps to be Addressed to Inform Diagnosis and/or Treatment
- More toxicant serum concentration vs. time of exposure data
- Accuracy of patient history reports
- Laboratory confirmation of known toxicant from reported cases
- Time course of acute life-threatening poisonings
- Chemical interactions (e.g., mixtures, polypharmacy, food additives)

Toxicological Observations and Measurements to Address Knowledge Gaps
- Biomarkers of organ/system damage (e.g., cardiac troponin, acute renal damage [Kim-1]), and other renal biomarkers [beyond proximal tubule damage])
- Cholinesterase measurements could provide substantial clinical information
- Markers of oxidative stress (e.g., glutathione, 8-oxoguanine) could be used to inform when to continue or discontinue anti-oxidant therapies
- Serum/blood determinations are preferred to urine measurements because of the greater temporal association of serum/blood levels with acute toxicities
- Dosimetry in humans during adverse events and consideration of dosage formulations (e.g., sustained release vs. immediate release formulations) would inform clinical assessment

Research and Development
- Mode of action (MOA)-based test methods
- Human cell-based systems as screening models (the human condition is the desired reference)
- Cell models for assessing affected cellular pathways to assess the likelihood of interactions among these pathways
- Methods to evaluate recovery and/or reversibility of an effect
- Methods to address chemicals that are typically physicochemically incompatible with conventional *in vitro* cell systems (e.g., hydrophobic chemicals)
- Tools for determining *in vitro* and *in vivo* toxicokinetics for dose-response assessments and various associated extrapolations (e.g., *in vivo* to *in vitro*, interspecies)

Breakout Group 2: Current Acute Systemic Toxicity Injury and Toxicity Assessments

Panelists: Drs. June Bradlaw, Helen Diggs, Steven Hansen, Thomas Hartung, Gabrielle Hawksworth, A. Wallace Hayes (Co-Chair), Albert Li, Daniel Marsman (Co-Chair), Kathleen Murray, Steven Niemi, Karen Steinmetz, William Stokes, William Stott, and Mr. Gary Winorowski

Objectives
- Review clinical observations and quantitative measurements that should be included in acute systemic toxicity tests to support development of predictive *in vitro* methods.
- Identify toxicity pathways that could be modeled by using *in vitro* test methods.

Key Pathways to be Modeled using Alternative Test Systems

Animal and Human Systems
- ADME
- Components of metabolism that can be mimicked *in vitro*
- Information (bioavailability, Structure-Activity Relationship [SAR]) available before testing
- Human toxicokinetic information (when available)

Whole Organs (priority order)
- Pulmonary
- Renal
- Hepatic
- Cardiovascular
- Neurological (e.g., neurochemical, behavioral, brain swelling
- Gastrointestinal (e.g., production of endotoxin as a marker for sepsis
- Hematopoietic (including hemorrhaging)

Cellular Systems
- Chemical toxicity (key issue is whether it is greater for dividing or non-dividing cells)

Research and Development
- Short-term activities
  - Noninvasive telemetry systems for real-time monitoring of physiological parameters in rodents
  - Automated systems for collecting behavioral information
  - Noninvasive analytical devices for analyzing small blood/urine volumes
  - Bioinformatics tools
- Long-term activities
  - "-omics" technologies to identify biomarkers
  - Noninvasive imaging techniques
  - Nanotechnology development for biomarker measurements

Breakout Group 2: Current Acute Systemic Toxicity Injury and Toxicity Assessments (cont'd)

Biomarkers for Pathophysiological Effects and Modes/Mechanisms of Acute Systemic Toxicity

	Whole Animal Systems	Organ/Cellular Systems
Acute Systemic Toxicity Test Biomarker Observations and Measurements	• Clinical observations • Body weight • Feed consumption • Water consumption	• Gross pathology
Recommended Biomarker Observations and Measurements	• Clinical observations • Body weight • Feed consumption • Water consumption • Functional observations – Heart rate – Electrocardiogram – Respiratory rate – Respiratory volume – Body temperature – Limited observations for neurotoxicity	• Gross pathology • Clinical pathology and urinalysis (early, mid and late time points) • Serum and urine pH (anion gap, etc.) • Histopathology • Gastrointestinal (measure presence of cytokines [tumor necrosis factor; TNF] in body cavity as a measure of endotoxin)
Recommended Research and Development of Biomarkers	• Stress: corticosteroids • "-omics" technologies • Toxicokinetics • Metabolism	• Kidney: creatinine, tubular markers, protein (urine), blood urea nitrogen (BUN) test, glutathione-S-transferase (GST; urine), n-acetyl glucosamine • Liver: glutathione, serum glutamic oxaloacetic transaminase (SGOT) • Heart: physiological measurements, body chemistry, blood pressure, heart rate and rhythm, serum troponin levels • Neurological: neurotransmitter levels (e.g., catecholamines, FOB [optional]) • Lungs: respiratory imbalance • Blood and other tissue concentrations of toxicants (early, mid and late time points)

Breakout Group 3: Identifying Earlier Humane Endpoints for Acute Systemic Toxicity Testing

Panelists: Drs. June Bradlaw, Daniel Cobaugh, Helen Diggs (Co-Chair), Steven Hansen, Kathleen Murray, Steven Niemi (Co-Chair), Karen Steinmetz, William Stokes

Objectives
- Determine objective biomarkers that can
  - Elucidate key pathways that are sufficiently predictive of lethality
  - Be validated and used along with clinical signs and observations for pain and distress as routine humane endpoints for acute systemic toxicity testing.

Recommendations of the Breakout Group
- The group noted that evident toxicity is an earlier, more humane endpoint than moribund condition or death. Evident toxicity is the endpoint used in the Fixed Dose Procedure (FDP) for acute oral toxicity testing.
- The majority of the group recommended that the FDP become the preferred acute oral toxicity testing method to be used routinely instead of the Up-and-Down Procedure (UDP) or the Acute Toxic Class Method (ATC) unless adequate scientific justification and rationale can be provided to justify that the UDP or ATC would be more appropriate (for example, derivation of a point estimate of the LD_{50}).[1]
- To effectively implement this recommendation, the group recognized the need for two separate, globally standardized scoring systems that would allow for weighting of observations. One scoring system would describe evident toxicity (a term that has been accepted by regulators with the international adoption of OECD TG 420), and one would describe severe toxicity and lethality.
- The group also recommended using fixed-dose/concentration approaches for acute toxicity testing by the dermal and inhalation routes, respectively, in order to use evident toxicity as an earlier, more humane endpoint for such studies.

Biomarkers Sufficiently Predictive of Evident Toxicity that Should be used Routinely during Acute Toxicity Testing
- Simple behavioral observations for evaluating level of activity
- Body temperature decreases
- Body weight and feed and water consumption, if appropriate (give consideration to the potential impact of social housing versus individual housing on these measurements; hydration status could be used as a surrogate for water consumption)

Recommendations for Measurements and Observations
- Gather clinical pathology data shortly after exposure
- Obtain objective physiological measurements (e.g., heart rate, heart rhythm, respiratory rate, blood pressure, O2 saturation)
- Perform a functional observational battery [FOB] for neurotoxic effects
- Measure toxicant levels in body fluids (with blood levels used for time points)
- Observe fecal occult blood for indications of gastrointestinal hemorrhage
- Obtain fecal measurements of corticosteroids
- Measure catecholamine levels in blood or serum
- Measure cytokines (TNF) in the body cavity or detect proteinuria in mice as a surrogate measurement of endotoxin levels in the body

Research and Development
- Address knowledge gaps currently associated with predictive early humane endpoints
- Develop and publish internationally harmonized guidance on objective criteria to characterize evident toxicity before initiating routine use of the FDP
- Evaluate measurements for their usefulness in defining humane endpoints for acute toxicity testing (e.g., those previously discussed, quantitative measures of activity/behavior, use of saliva and exhaled air instead of blood for detection of potentially useful biomarkers)

[1]Although the FDP is an adopted OECD Test Guideline, some regulatory scientists did not support its use. Because the FDP does not satisfy the regulatory needs for an LD_{50} estimate, some U.S. regulatory agency representatives at the workshop did not agree that the FDP should be the preferred method for any acute systemic toxicity testing, including potential applications to acute dermal toxicity and acute inhalation toxicity. Currently, there are no OECD guidelines for these test methods and therefore the recommendation would not be able to be implemented. Recommendations for using the FDP were made only in the context of identifying humane endpoints; there are scientific and regulatory reasons for using a method other than the FDP. The UDP for acute oral toxicity was developed to provide LD_{50} values to satisfy U.S. regulatory requirements.

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

Panelists: Drs. Daniel Acosta, Melvin Andersen (Co-Chair), Eugene Elmore (Co-Chair), Thomas Hartung, Hajime Kojima, Albert Li, Frank Palloucek, Raymond Tice

Objectives
- Determine the extent of applicability of *in vitro* test methods to adequately model the key toxicity pathways indicated by *in vivo* measurements (molecular, cellular, tissue, or other physiological and clinical biomarkers) and observations associated with acute systemic toxicity, and to subsequently identify any relevant knowledge gaps.

Potential In Vitro Test Methods to Evaluate Toxicity Pathways to Access Both Specific Endpoints and Dose-Response Characteristics
- Basal cytotoxicity
- Blood-brain barrier function
- Cardiac aerobic metabolism
- Cardiac conduction
- Cell arrhythmia
- Cellular glutathione content
- Cellular respiration
- Cytotoxicity (as apoptosis and other non-necrotic pathways)
- Hepatic metabolism
- Hepatocytes (associated pathways that trigger inflammatory response)
- Immunology/inflammation
- Mitochondrial function/damage
- Neuronal cells (in test methods)
- Neuronal transmission
- Overstimulation of receptors
- Oxidative stress
- Renal tubule cell (in test methods) for excretory function
- Sodium/potassium pump
- Specific organ sites (functional test methods)
- Transporter protein function
- Various ion channels

Implementation strategies

Implementation strategies for relating *in vitro* test results with acute toxicity will vary for those approaches attempting to establish correlations between outcome and *in vitro* test results (i.e., correlative approaches) and for those that attempt to mimic the sequential cellular and tissue responses that lead to toxicity (i.e., mechanistic approaches). This implementation requires attention to multiple factors:
- Collect any available standardized data from animal studies to aid in pathway determination
- Identify model cellular systems for assessing chemical activity in the pathway
- Identify agents that relate to toxicity in the model cellular systems
- Develop model systems for testing, including methods and endpoints
- Interpret results using standardized test panels to compare with the rodent LD_{50}
- Use statistical tools, currently being developed and implemented, to facilitate interpretation for association between potency in specific pathway test methods and the rodent LD_{50}
- Examine the effectiveness of each system alone and in combination
- Convene expert panels to address development of cell lines, design and use of appropriate biomarkers, test method implementation, and data analysis procedures
- Consider incorporating individual test methods into the assessment of acute toxicity in parallel with the *in vitro* basal cytotoxicity test method
- Develop appropriate procedures to compare the performance of new test methods in relation to the *in vitro* basal cytotoxicity test methods for predicting the rodent LD_{50}
- Consider how multiple measures of cellular toxicity pathways might be used to predict acute systemic toxicity

Research and Development
- Identify and develop tissue-specific cellular models to assess critical toxicity pathways and to allow for genetic variability
- Use human cord blood to isolate stem cells and direct their differentiation to express biomarkers normally expressed in the tissues
- Develop standardized testing protocols and identify the necessary controls before initial evaluation of each cellular response pathway as a predictor of acute systemic toxicity
- Use test methods to examine chemicals active in the toxic response pathway, as well as negative controls
- Standardize data from animal studies with acute dosing/other forms of dosing; validation of *in vitro* models requires a wider variety of data (e.g., ADME) than simply acute toxicity
- Apply a broad array of *in vitro* test methods to screen for modes of action
- Collect as much data as possible from those animal studies that are conducted to better understand modes of action, and use this information to guide selection of *in vitro* test methods for these modes of action
- Develop databases of "-omic" changes, and assess affected tissue-level pathways in animals being tested for acute systemic toxicity
- Broaden the association between LD_{50} and *in vitro* measures by completing studies with larger numbers of chemicals, assaying more integrated measures of cellular function
- Develop computational systems biology approaches to predict *in vivo* acute toxicity from sequential activation of specific cellular pathways

The Future of Toxicity Testing

"Toxicity Testing in the 21st Century: A Vision and a Strategy", Report by the Committee on Toxicity and Assessment of Environmental Agents, National Research Council (2007)
- Envisions the significant reduction and replacement of animal use with batteries of predictive *in vitro* assays to evaluate alterations to key toxicity pathways that can be elucidated using a systems biology approach.

National Toxicology Program's Vision for the 21st Century
- Supports the evolution of toxicology from a predominantly observational science at the level of disease-specific models to a predominantly predictive science focused upon a broad inclusion of target-specific, mechanism-based, biological observations in cell systems and short-term animal studies.

Breakout Group 5: Industry Involvement in Test Method Development, Validation, and Use

Panelists: Drs. Richard Becker, A. Wallace Hayes, Gabrielle Hawksworth, Daniel Marsman, Amy Rispin, Robert Scala (Co-Chair), William Stott (Co-Chair), Marilyn Wind, and Messrs. Robert Guest and Gary Whorowski

Objectives
- Determine the most effective way to encourage industry to collect and submit to ICCVAM (1) mechanistic observations and measurements from animals used in acute systemic toxicity studies and (2) concurrent *in vitro*/*in vivo* toxicity test data to be used in the development and validation of alternative *in vitro* test methods.

Use of In Vitro Cytotoxicity Testing by Industry
- In 2001, the EPA High Production Volume (HPV) Challenge Program sent a letter to 1200 organizations requesting data submitters to consider and use *in vitro* basal cytotoxicity test methods to set the starting doses when testing HPV chemicals for acute systemic toxicity. There has been only one submission of *in vitro* basal cytotoxicity and *in vivo* rodent LD_{50} data. Potential reasons cited by workshop participants included:
  - Alternative *in vivo* protocols have already reduced and refined animal use in acute systemic toxicity testing. Longer-term *in vivo* test methods use far more animals and have far greater opportunities for reduction and refinement of animal usage.
  - At present, because of poor accuracy, *in vitro* cytotoxicity predictions of acute oral toxicity are useful only when there is a complete lack of information (a rare occurrence) for a particular chemical.
  - Collecting data from parallel *in vitro* and *in vivo* toxicity testing would require significant resources, while the impact of *in vitro* test methods on further animal reduction would be limited at best.
  - Although regulatory agencies will accept for consideration data from a well-developed and well-thought-out alternative method, industry is concerned about how *in vitro* cytotoxicity test data might be interpreted by regulators (i.e., *in vitro* data indicating toxicity may have more weight than *in vivo* data that does not indicate toxicity). **Additionally, a more sensitive endpoint might give rise to more stringent regulation.**
- However, participants also recognized that certain factors could result in increased use of *in vitro* test methods
  - *In vitro* test methods could replace the *in vivo* acute toxicity test methods if a full battery of *in vitro* tests were available that accounted for the many mechanisms and modes of action of acute toxicity.
  - Larger organizations might voluntarily use *in vitro* test methods in their acute toxicity testing program for the public relations value. Contract laboratories will implement the procedures for their competitive value in approaching clients.
  - The testing programs of industry tend to follow the most efficient testing track, such as using the standard *in vivo* test methods that regulatory agencies assuredly accept.
  - The availability of a validated *in vitro* test method for acute toxicity and the inclusion of such a test in a formal testing guideline would facilitate its widespread use.

Submission of In Vitro and In Vivo Data to ICCVAM
- Industry would provide parallel testing data from *in vivo* and *in vitro* methods to ICCVAM in order to advance the development and validation of more predictive *in vitro* test methods if adequate assurances (e.g., assurance that unfavorable *in vitro* data in the presence of favorable *in vivo* data would not be used in any regulatory action) and incentives (e.g., grants for development of methods, tax incentives, expedited regulatory review) are available.
- Companies are likely to consider any mechanistic information to be proprietary.
- Data "call-ins" are not often helpful because the findings are based on protocols that are not completely comparable.
- Acute toxicity data constitute valuable proprietary information that companies are not likely to share.
- Creation of a public/private consortium that would facilitate data collection and submission could set priorities, define the level of detail necessary for data submissions, work to standardize protocols, emphasize the value of better science in providing more confident regulatory decisions, and possibly do some cost sharing in the process.

Conclusions

- Objective data to help identify the mechanisms of toxicity and death should be routinely collected during animal studies required for regulatory testing ,
- *In vivo* measurements should also be collected to aid in the identification of predictive earlier endpoints of severe toxicity, and to establish objective parameters for evident toxicity.
- *In vivo* mechanistic data should be used to guide the selection of *in vitro* tests for High Throughput Screening (HTS) and other research initiatives attempting to identify *in vivo* toxicity pathways.
- **Significant R&D efforts will be needed to develop sufficiently predictive *in vitro* models of acute systemic toxicity.**

Acknowledgements

This poster was supported by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences. ILS staff are supported by NIEHS contract N01-ES 35504. The views expressed on this poster do not necessarily represent the official position of any U.S. Federal agency. This presentation reflects the views of the author (Marilyn Wind) and has not been reviewed or approved by, and may not necessarily reflect the view of, the U.S. Consumer Product Safety Commission

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

NIEHS National Institute of Environmental Health Sciences

NTP