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

National Institutes of Health (NIH) Natcher Conference Center

February 6 – 7, 2008

Workshop Overview

Poisoning is a more serious public health problem than generally recognized. The Institute of Medicine (IOM) estimates that more than 4 million poisoning episodes occur annually in the United States (IOM 2004). In 2001, poisoning (30,800 deaths) placed second behind automobile accidents (42,433 deaths) as the leading cause of injury-related death (IOM 2004). To ensure accurate labeling of hazards and to reduce the risk of accidental poisonings, regulatory agencies in the United States (e.g., the Environmental Protection Agency [EPA] and the Consumer Products Safety Commission [CPSC], Department of Transportation [DOT]) require that certain products and chemicals are tested to determine their potential to cause life-threatening or fatal acute systemic toxicity. This testing currently involves exposure of a small number of rats by applicable routes (oral, dermal, and/or inhalation), and monitoring whether animals die or exhibit any clinical signs of toxicity.

Increasing societal 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]. Additionally, *in vitro* methods have been developed and recommended that can help further reduce the number of animals needed for each *in vivo* test (ICCVAM 2006a). Nevertheless, despite decades of research, attempts to identify *in vitro* alternatives that correctly predict *in vivo* toxicity have made little progress. Since an important goal of acute toxicity testing for regulatory purposes is to determine hazard classification and labeling, it produces information about the relative toxicity/lethality of a substance. Currently, the primary purpose of these studies is not to provide information about the mode or mechanism that causes toxicity or death. Current studies may generate some relevant data, but such data varies from study to study and is generally limited. 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 therefore needed to facilitate alternative test method development and achieve the replacement of animals in acute oral toxicity testing. Both ICCVAM and an independent expert peer review panel recently recommended that standardized procedures to collect information pertinent to an understanding of the mechanisms of lethality should be included in future *in vivo* rat acute oral toxicity studies (ICCVAM 2006a, b). Such information is considered necessary to support the further development of predictive mechanism-based *in vitro* methods. The National Toxicology Program's (NTP) recent Vision for the 21st Century[2] 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

[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 2003).

[2] http://ntp.niehs.nih.gov/index.cfm?objectid=EE4AED80-F1F6-975E-7317D7CB17625A15

systems and short-term animal studies. The EPA has a similar initiative within its ToxCast Program[3]. Likewise, the National Research Council's (NRC) recently published *Toxicity Testing in the Twenty-first Century: A Vision and a Strategy*[4] envisions the significant reduction and replacement of animal use with batteries of predictive *in vitro* assays to evaluate alterations to key toxicity pathways[5] that can be elucidated using a systems biology approach.

Acute systemic toxicity testing provides an excellent opportunity for assessing the feasibility (i.e., a proof-of-concept) of the NTP/EPA/NRC approaches. Acute systemic toxicity testing is an ideal candidate to determine if these proposed non-animal approaches can be sufficiently predictive to totally replace animals. This is because these studies typically evaluate the adverse effects of a single dose of test substance followed by a short observation period (up to 14 days), compared to other systemic toxicity testing that involves repeated daily dosing and observation for 14 days to 2 years. This workshop will contribute to this proof-of-concept by developing approaches to identify the key toxicity pathways for acute systemic toxicity so this mechanistic information can be used to target the development of predictive *in vitro* alternative test methods. Another benefit to collecting this information is that it may identify predictive biomarkers of systemic toxicity that could be used as earlier, more humane endpoints during *in vivo* tests to further reduce pain and distress.

The evaluation and promotion of alternatives for acute systemic toxicity testing[6,7] is one of ICCVAM's four highest priorities because (1) worldwide, it is the most commonly required product safety test and thus large numbers of animals are used, and (2) it can result in significant pain and distress to test animals. This international workshop also implements one aspect of the NICEATM - ICCVAM *Five-Year Plan (2008-2012)*[8] to identify approaches that would further reduce the potential pain and distress associated with acute toxicity testing[9] by seeking to identify more humane acute toxicity endpoints.

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[3] http://www.epa.gov/ncct/practice_community/category_priority.html.

[4] http://books.nap.edu/catalog/11970.html

[5] 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.)

[6] EPA Health Effects Test Guidelines OPPTS 870.1100 Acute Oral Toxicity http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Revised/870r-1100.pdf (EPA 2002)

[7] OECD Series on Testing and Assessment Number 24: Guidance Document on Acute Oral Toxicity Testing http://www.olis.oecd.org/olis/2001doc.nsf/43bb6130e5e86e5fc12569fa005d004c/c1256985004c66e3c1256a92005087fe/$FILE/JT00111082.PDF (OECD 2001)

[8] http://iccvam.niehs.nih.gov/docs/5yearplan.htm

[9] 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 2003).

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Workshop Goals and Objectives

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[10] 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.

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

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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.

References

EPA. 2007. EPA Categorization and Prioritization Community of Practice. Available: http://www.epa.gov/ncct/practice_community/category_priority.html [accessed 01 August 2007].

EPA. 2002. Health Effects Test Guidelines OPPTS 870.1100 Acute Oral Toxicity. EPA 712–C–02–190. Washington, DC: U.S. Environmental Protection Agency. Available: http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Revised/870r-1100.pdf [accessed 01 August 2007].

Gennari A, van den Berghe C, Casati S, Castell J, Clemedson C, Coecke S, et al. 2004. Strategies to replace *in vivo* acute systemic toxicity testing. The report and recommendations of ECVAM Workshop 50. Altern Lab Anim 32:437-459.

ICCVAM. 2006a. ICCVAM Test Method Evaluation Report: *In Vitro* Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Toxicity Tests. NIH Publication No. 07-4519. Research Triangle Park, NC:National Institute for Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/acutetox/inv_nru_tmer.htm [accessed 01 August 2007].

ICCVAM. 2006b. Peer Panel Report: The Use of *In Vitro* Basal Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Testing. Research Triangle Park, NC:National Institute for Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/methods/acutetox/inv_nru_scpeerrev.htm [accessed 01 August 2007].

ICCVAM. 2007. Draft NICEATM-ICCVAM Five-Year Plan. Research Triangle Park, NC:National Institute for Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/5yearplan.htm [accessed 01 August 2007].

IOM. 2004. Forging a Poison Prevention and Control System. Washington, DC: National Academies Press. Available: http://www.nap.edu/catalog/10971.html [accessed 01 August 2007].

Lai MW, Klein-Schwartz W, Rodgers GC, Abrams JY, Haber DA, Bronstein AC, Wruk KM. 2006. 2005 Annual Report of the American Association of Poison Control Centers' National Poisoning and Exposure Database. Clin Toxicol 44:803–932.

National Research Council. 2007. Toxicity Testing in the Twenty-first Century: A Vision and a Strategy. Washington, DC: National Academies Press. Available: http://books.nap.edu/catalog/11970.html [accessed 01 August 2007].

NTP. 2004. Toxicology in the 21st century: The role of the National Toxicology Program. Research Triangle Park, NC: National Institute for Environmental Health Sciences. Available: http://ntp-server.niehs.nih.gov/ntp/main_pages/NTPVision.pdf [accessed 01 August 2007].

OECD. 2001. Guidance Document on Acute Oral Toxicity Testing. 24. Paris, France:Organisation for Economic Co-operation and Development. Available: http://www.olis.oecd.org/olis/2001doc.nsf/43bb6130e5e86e5fc12569fa005d004c/c1256985004c66e3c1256a92005087fe/$FILE/JT00111082.PDF [accessed 01 August 2007].

Solecki R, Davies L, Dellarco V, Dewhurst I, van Raaij M, Tritscher A. 2005. Guidance on setting of acute reference dose (ARfD) for pesticides. Food Chem Toxicol 43:1569–1593.