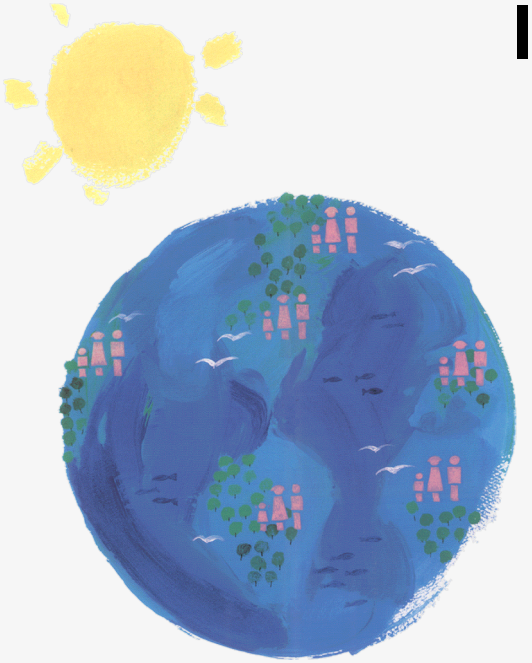
NICEATM

National Toxicology Program
Interagency Center for the Evaluation of Alternative Toxicological Methods

ICCVAM

Interagency Coordinating Committee on the Validation of Alternative Methods



Recommendations from the ICCVAM-NICEATM International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity

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

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Natcher Conference Center
Bethesda, Maryland

National Toxicology Program

NIEHS

National Institutes of Health

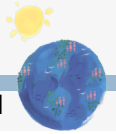
Department of Health & Human Services • USA

International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity

- International Workshop held in Crystal City, Arlington, VA, October 17 - 20, 2000
- Workshop Sponsors: National Institute of Environmental Health Sciences (NIEHS), National Toxicology Program (NTP), U.S. Environmental Protection Agency (EPA)
- Breakout Groups (BG)
  - BG 1 *In Vitro* Screening Methods for Assessing Acute Toxicity
  - BG 2 *In Vitro* Methods for Assessing Acute Toxicity: Biokinetic Determinations
  - BG 3 *In Vitro* Methods for Organ-Specific Toxicity
  - BG 4 Chemical Data Sets for Validation of *In Vitro* Acute Toxicity Test Methods

Major Workshop Objectives

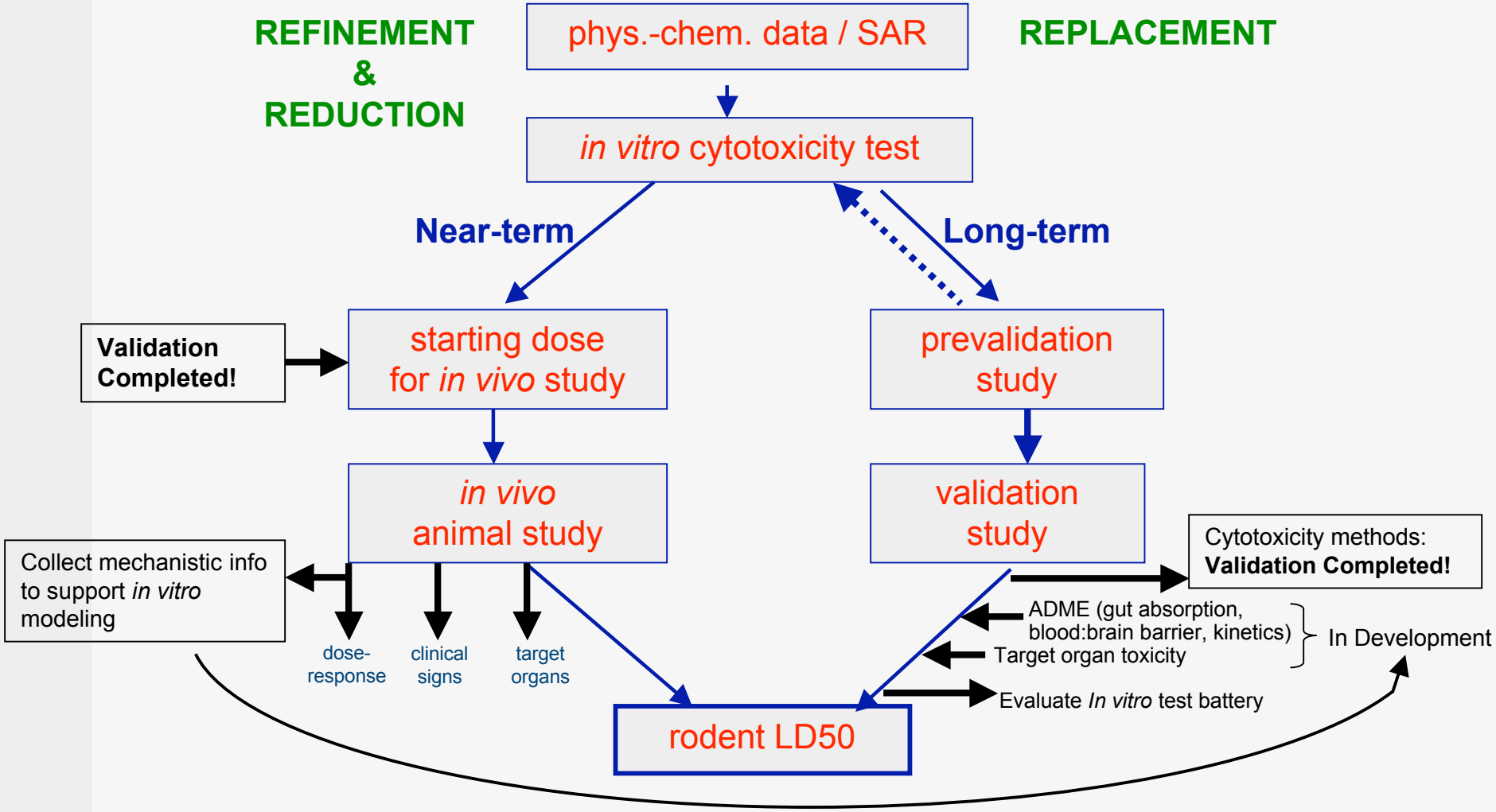
- Review validation status of *in vitro* methods
- Recommend priority *in vitro* methods for further evaluation and appropriate validation studies
- Identify reference chemicals for validation studies
- Identify priority research and development efforts



BG 1: *In Vitro* Screening Methods for Assessing Acute Toxicity

- **Recommended Goals**
  - **Short-term**: Use *in vitro* methods to reduce animal numbers for rodent (oral) "LD_{50}-type" test (OECD TGs 401, 420, 423, 425)
  - **Medium-term**: Replace animals with *in vitro* method(s) that accurately predict the rodent LD_{50}
  - **Long-term**: Predict human acute systemic toxicity directly using human cells and tissues

BG 1: Strategy for the Reduction, Refinement and Replacement of Animals in Acute LD_{50} Testing[1]



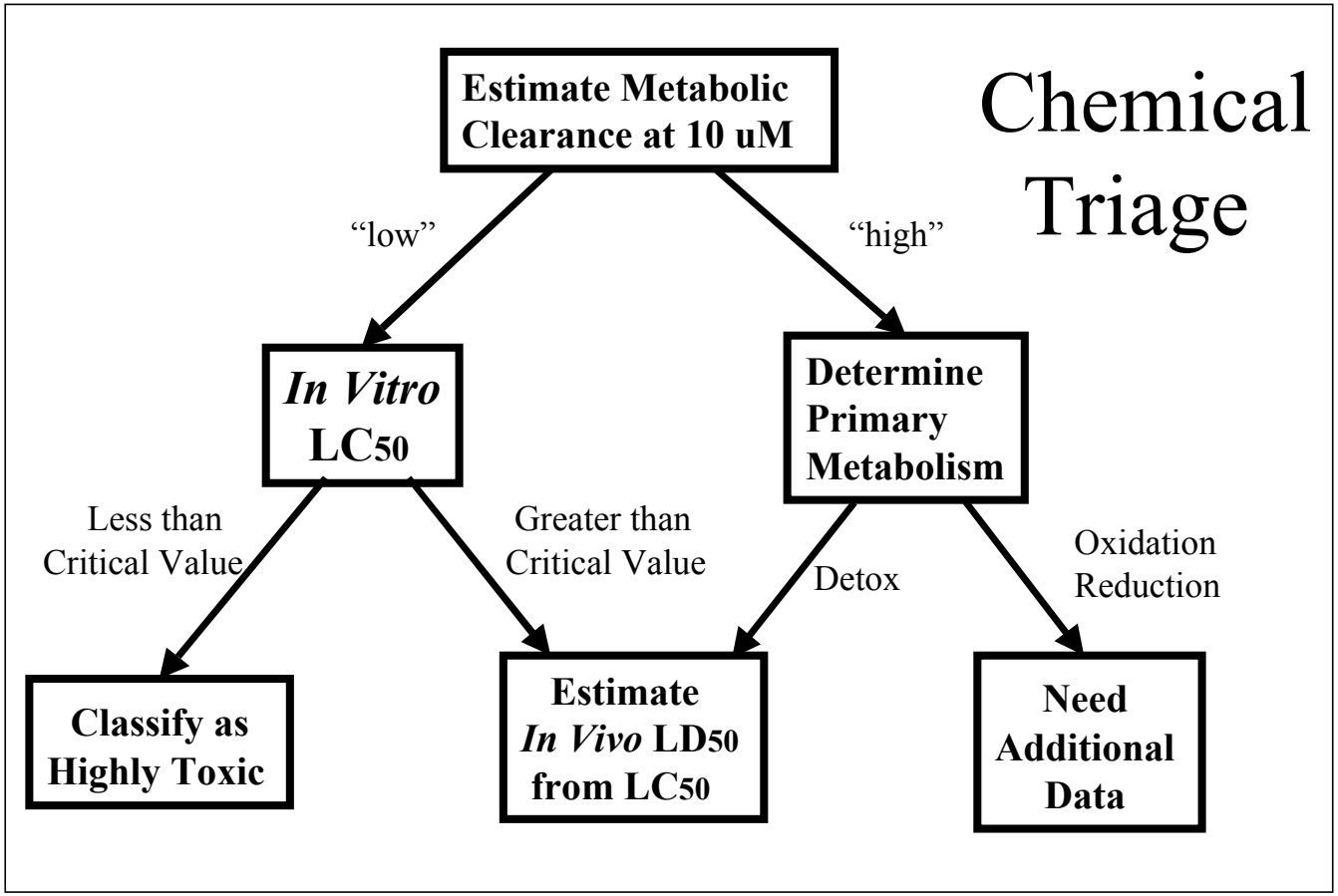
[1]Adapted/updated from ICCVAM. 2001. Report of the International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity. NIH Publication No. 01-4499. Research Triangle Park, NC:National Institute for Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/

BG 2: *In Vitro* Methods for Assessing Acute Toxicity - Biokinetic Determinations

Long Term Issues

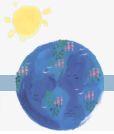
- Convert from animal based toxicity approach to human toxicity approach (e.g., human hepatocyte systems, *in vitro* systems for key transporters [e.g., renal, biliary]).
- Develop PBBK (Physiologically-Based Biokinetic) modeling techniques for various classes of chemicals for humans.
- Develop *in vitro*/Quantitative Structure-Activity Relationship (QSAR) methodology to estimate chemical-specific biokinetic parameters (e.g., absorption, distribution, metabolism, excretion [ADME]).
- Need metabolite-specific data (i.e., biomarkers) to estimate target tissue exposure
- Need validated human hepatocyte systems
- PBBK Model for kinetic extrapolation
  - *In Vitro* target tissues included in physiological structure
  - Mechanistic description of barrier functions (gut, bile, kidney, BBB, skin)
  - QSAR and *in vitro* models to predict kinetic parameters
- Kinetic/Dynamic Interactions (e.g., the effect of toxicity on the metabolism and excretion of a chemical; effect of metabolism or reabsorption on the toxicity of a chemical)

BG 2: Tiered Approach for Evaluating Acute Toxicity

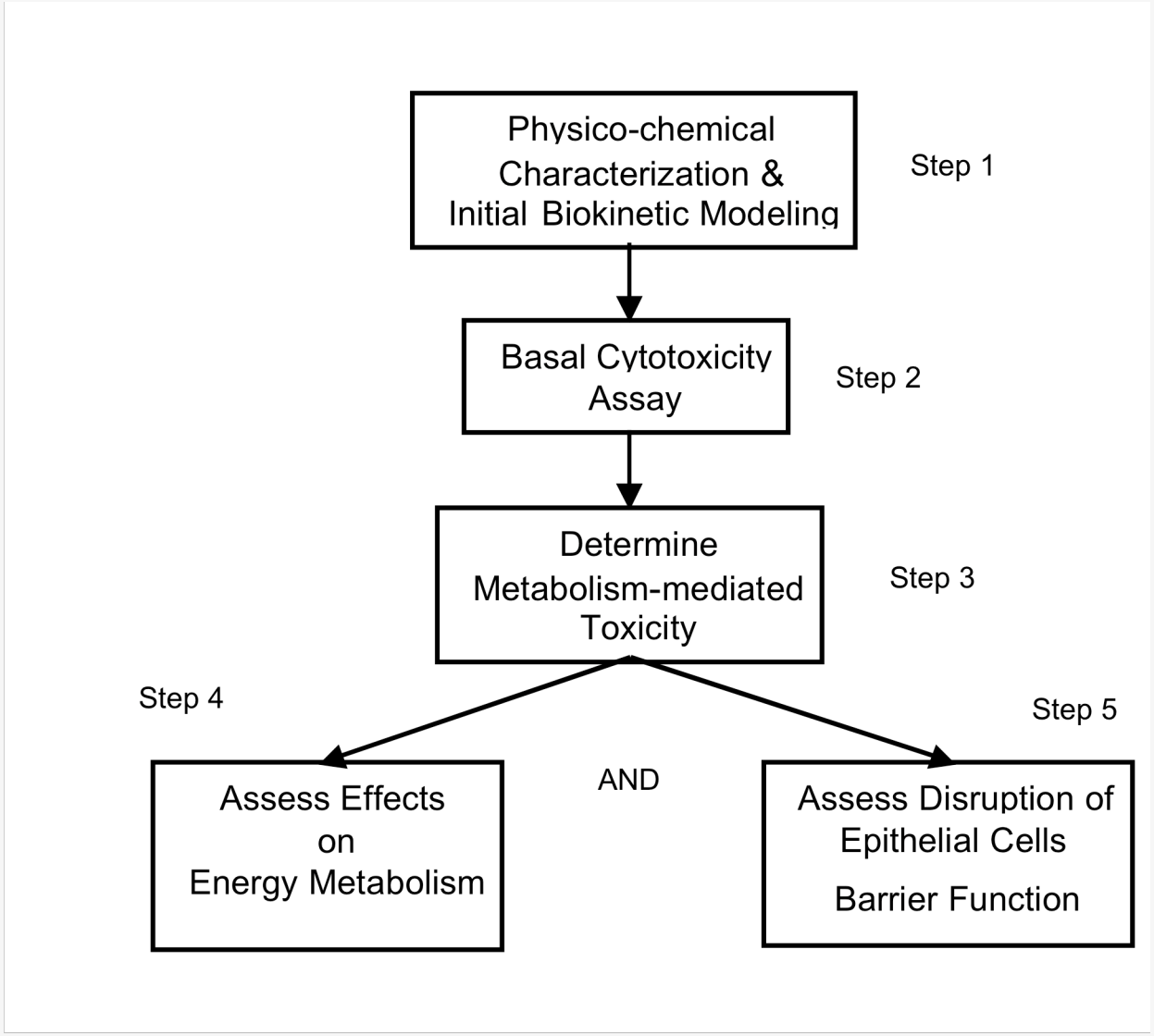


BG 3: *In Vitro* Methods for Organ-Specific Toxicity

- Goal is to identify the organs which are likely to be affected by single dose or short term exposure ≤ 24 hours.
- Strategy is to look for effects on processes specific to the various target organs and to determine a dose effect relationship to use for maximum tolerated dose, lowest-observed-adverse effect-level (LOAEL), etc.
- Major Organ Systems of Interest
  - Liver
  - Kidney
  - CNS
  - Heart
  - Lung
  - Hematopoetic



BG 3: Proposed Scheme for Assessing Acute Toxicity using Non-animal Methods



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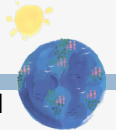
BG 4: Chemical Data Sets for Validation of *In Vitro* Acute Toxicity Test Methods

- **Recommended Actions - Study Rodent Toxicity Databases:**
  - to determine the variation in the rodent oral LD_{50} introduced by differences in protocols
  - to determine the within- and between-laboratory reproducibility of the rodent oral LD_{50} test and other acute toxicity tests that will be used as reference tests.
  - to convene an expert committee to recommend a reference set of test chemicals
    - Available: A list of 72 substances developed for the NICEATM/ECVAM *in vitro* basal cytotoxicity validation study



BG 4: Chemical Data Sets for Validation of *In Vitro* Acute Toxicity Test Methods

- **Recommended Actions - Study Human Toxicity Databases:**
  - to build on the MEIC (Multicentre Evaluation of *In Vitro* Cytotoxicity) and MEMO (MEIC Monograph) exercises
  - to review the MEIC/MEMO approach for measuring acute toxicity parameters in humans
  - to develop a standardized approach for measuring acute toxicity parameters
  - to search existing information to obtain all human data
  - to establish a mechanism to:
    a) Gather human toxicity data from hospital/Poison Control Center (PCC) sources
    b) Retrieve existing human toxicity data
    c) Collect and organize human toxicity data as accidents occur.
    d) Biomonitoring data should also be collected. Such information could define sub- or non-toxic levels, and be used to see if they overlap with the range of reported toxic levels.

ICCVAM Post-Workshop Recommendations - 1

Current Uses for *In Vitro* Methods

- ICCVAM agrees with the Workshop Report that data from *in vitro* cytotoxicity assays can be useful as one of the tools in setting a starting dose for the *in vivo* assessment of acute oral toxicity
- 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
- **ACTION**
  - ICCVAM published a Guidance Document on how to use the *in vitro* cytotoxicity methods to estimate starting doses.
  - EPA sent letters to 1200 companies in 2001 recommending they consider using *in vitro* cytotoxicity methods and provide the data to ICCVAM (Note: No data received yet)

ICCVAM Post-Workshop Recommendations - 2

Near Term Research

- Near-term validation studies should focus on two standard cytotoxicity assays: one using a human cell system and one using a rodent cell system.
- **ACTION**
  - Validation study completed by NICEATM and NIEHS with EPA support
- Future validation studies should compare rodent and human *in vitro* data with one another, with rodent *in vivo* data, and with human *in vivo* data
- Establish an interagency expert group under ICCVAM to advise on near-term activities such as assay selection, study design, and chemical selection
- **ACTION**
  - Established the ICCVAM Acute Toxicity Working Group

ICCVAM Post-Workshop Recommendations - 3

Long Term Research

- Develop and improve efficient and accurate *in vitro* systems that provide information on
  - Biokinetics
  - Metabolism
  - Organ-specific toxicity
- *In vitro* methods to gather biokinetic and target organ specific effects data needed for accurate LD_{50} predictions, signs and symptoms associated with toxicity, and pathophysiological effects
- Develop 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
- **ACTION**
  - ICCVAM and NICEATM collaborating with the European Commission/ECVAM ACuteTox Initiative to address these recommendations

ICCVAM Post-Workshop Recommendations - 4

Long Term Research (cont.)

- Investigate 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.
- Investigate the utility of toxicogenomics/proteomics for the assessment of acute toxicity, especially the prediction of no-observed-adverse-effect-levels (NOAEL)/lowest-observed-adverse-effect-levels (LOAEL) for acute exposure.
- **ACTION**
  - ACuteTox program also addressing these research needs

Summary - 2000 Acute Toxicity Workshop Recommendations

- Validate and implement the use of *in vitro* cytotoxicity methods to estimate starting doses to reduce animal use

  Status: Completed

- Develop and validate a battery of *in vitro* methods to accurately predict *in vivo* hazard categories
  - Metabolism data
  - Organ-specific toxicity
  - Biokinetics

  Status: In progress (ACuteTox)