

## Summary of Opening Plenary Session and Public Comments

### The International Workshop on *In Vitro* Methods for Assessing Acute Toxicity

October 17-20, 2000

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

The National Toxicology Program (NTP)

Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

National Institute of Environmental Health Sciences (NIEHS)

#### Opening Plenary Session

Speakers:

- Dr. John Frazier, USAF/ICCVAM, Workshop Co-Chair
- Dr. Philip Sayre, EPA/OPPT/ICCVAM, Workshop Co-Chair
- Dr. William Stokes, NIEHS/ICCVAM/NICEATM
- Dr. John Bucher, NIEHS
- Dr. Steve Galson, EPA/OPPT
- Dr. James Cone, California Department of Health Services
- Dr. Manfred Liebsch, ZEBET
- Dr. Bas Blaauboer, Research Institute of Toxicology, Utrecht University
- Dr. Oliver Flint, Bristol-Meyers Squibb

#### Call to Order and Introductions

Dr. William Stokes called the workshop to order at 8:38 a.m. Dr. Stokes explained that the Workshop was organized by ICCVAM and NICEATM and was co-sponsored by the U.S. Environmental Protection Agency (EPA), the National Institute of Environmental Health Sciences (NIEHS) and the National Toxicology Program (NTP). He thanked everyone for their participation and attendance. He discussed the goals of ICCVAM and NICEATM stating that the overall goal is to validate and achieve regulatory acceptance of test methods that will provide improved protection of human health and the environment, while incorporating the three Rs for the use of animals (refinement, reduction and replacement) whenever scientifically feasible. He stated that the purpose of the workshop was to evaluate the validation status of *in vitro* test methods for assessing acute systemic toxicity. He reviewed the functions of ICCVAM, which include the technical evaluation of new methods including independent scientific peer reviews, and organizing expert panel meetings to review test methods at various stages of development and validation. Dr. Stokes concluded by stating that ICCVAM also organizes workshops to identify additional research and validation efforts necessary to develop and further enhance the usefulness of new methods.

#### Welcome from the National Toxicology Program (NTP)

Dr. Stokes introduced Dr. John Bucher of NIEHS as the next speaker. Dr. Bucher thanked Dr. Stokes and welcomed the participants of the workshop. He conveyed the regrets of Dr. Christopher Portier of NIEHS/NTP who was unable to attend the workshop and then thanked the ICCVAM agencies and the

U.S. EPA for the effort provided for the workshop. Dr. Bucher remarked that the purpose of the workshop was to seek scientific advice and opinion concerning alternative test methods. He expressed hope that the scientists would work to advance alternatives for acute toxicity testing and provide information to move *in vitro* alternative tests forward. He concluded by thanking the workshop participants for their knowledge, experience and time.

#### Workshop Objectives

Dr. Sayer reintroduced the objectives of the workshop, provided background remarks and listed points for the participants to consider: 1) determine the hazards of chemicals by alternative methods; 2) find non-lethal acute toxicity testing endpoints; and 3) ascertain which *in vitro* methods might be helpful and could be validated. He challenged the scientists to review *in vitro* screening methods for toxicokinetics and specific organ toxicity and to recommend applicable methods for pre-validation and validation studies. Dr. Sayre asked the scientists to recommend validation study designs, to determine lists of reference chemicals and to prioritize *in vitro* methods.

Dr. Sayre discussed the general structure of the workshop. Four breakout groups would investigate their respective topics and the invited expert scientists would lead the discussions. Time would be made available for public comment at the meetings. The workshop would begin each morning with a short plenary session to discuss the previous day's activities and would end each evening with a meeting of the co-chairs and rapporteurs. A final report from each breakout group would be compiled as a workshop report ready for publishing by January 2001. He also said that a workshop monograph could be published by NIEHS' Environmental Health Perspectives Supplements in April 2001. Dr. Sayre concluded his remarks by naming the organizing committee for the workshop and then thanked everyone for their work.

#### Memoriam for Björn Ekwall

Dr. Stokes thanked Dr. Sayre and continued the session by mentioning the recent untimely death of Dr. Björn Ekwall. He spoke of Dr. Ekwall's extensive contributions and dedication to alternative test method development. Dr. Stokes then introduced Dr. Erik Walum, a close friend and colleague of Dr. Ekwall.

Dr. Walum described Dr. Ekwall as a medical doctor and toxicologist who pushed seriously for implementation of *in vitro* test methods. He discussed Dr. Ekwall's life and work in Uppsala, Sweden and related Dr. Ekwall's belief that the United States must accept *in vitro* alternative testing methods in order for the world to embrace the methodology. Dr. Ekwall established the Scandinavian Cell Toxicology Society whose mission is to gather scientists for meetings and show that chemical effects on cells should translate to *in vivo* effects. He initiated the Multicenter Evaluation of *In Vitro* Cytotoxicity (MEIC) to test 50 chemicals and collect the results. Sixty-five different test methods were employed for testing the chemicals. He introduced the concept to test compounds in simple systems such as cell cultures and to extrapolate the results to human toxicity. He felt that one could break down systems to elementary parts then analyze them by *in vitro* methods. Dr. Walum concluded his remarks by relating that Dr. Ekwall knew that if he were not able to continue his work, then someone else would take over. Dr. Stokes thanked Dr. Walum for his remarks.

#### The Role of ICCVAM

Dr. Stokes described the evolution, structure, and function of ICCVAM, and its role in facilitating the development and validation of alternative test methods. The driving forces for the establishment and need for ICCVAM were listed: 1) the opportunity to incorporate new science and technologies into toxicological testing practices; 2) the potential benefits of improved prediction of toxicity, improved efficiency and improved animal welfare; 3) legislation including the NIH Revitalization Act of 1993

(Public Law 103-43); and 4) the need for development and validation of test methods for new endpoints of concern, such as the Endocrine Disruptor Screening and Testing Program at EPA. ICCVAM also fulfills other mandates provided to NIEHS by Public Law 103-43, such as alternative test method development and validation.

Dr. Stokes related that ICCVAM began as an ad hoc committee comprised of representatives from 15 Federal regulatory and research agencies in September 1994. The committee developed a report on criteria and processes for the validation and regulatory acceptance of toxicological test methods that was published in 1997. A standing ICCVAM committee was established in May 1997 to implement the Public Law 103-43 mandate that NIEHS establish a process to achieve the regulatory acceptance of scientifically valid alternative methods. The committee evaluates proposed test methods and provides recommendations to Federal agencies, which in turn decide the regulatory acceptability of the methods. He explained that NICEATM is located at NIEHS and provides operational and technical support for ICCVAM by co-organizing workshops and peer reviews of test methods, disseminating information, and developing partnerships with stakeholders.

Dr. Stokes reviewed the prerequisites for using new methods which include: 1) adequate validation, which involves determining the reliability and relevance of test methods for specific purposes, and 2) acceptance, which involves determination of the acceptability for regulatory risk assessment purposes. The evolution process for new testing includes: the review of existing risk assessment methods, research, development, pre-validation, validation, peer review, regulatory acceptance, and implementation. The current ICCVAM/NICEATM role in test method development and validation is to provide information, to evaluate test methods, and to provide recommendations to agencies. The objectives of ICCVAM Workshops include: to evaluate the adequacy of current test methods; to identify toxicological endpoints; to identify promising methods which need further development and validation; to recommend appropriate validation studies; and to recommend research and model development efforts needed to support improved test methods for specific toxicity endpoints. ICCVAM/NICEATM has completed independent peer review evaluations for the following tests: 1) the murine local lymph node assay (LLNA); 2) Corrositex ; 3) FETAX; and 4) the revised UDP. Dr. Stokes concluded his presentation by acknowledging the contributions of the ICCVAM Agency Representatives, the ICCVAM Workshop Organizing Committee, and the NICEATM staff.

#### Acute Toxicity Testing: Historical and Current Regulatory Perspectives

Dr. Galson began by saying that the workshop represents the working relationship of EPA and NIEHS. He thanked Dr. Richard Hill of the EPA and Dr. Stokes for their work and participation in the workshop. He acknowledged the animal welfare groups for their role in pushing forward the objectives of alternative testing. He also thanked Dr. Amy Rispin of the EPA for her contributions to forwarding alternative testing. Dr. Galson said the EPA committee assures that the 3Rs will be the primary objective of the workshop and the committee will work toward regulatory acceptance with the protection of public health foremost in mind.

Dr. Galson spoke of alternative methods for determining acute toxicity being used by the regulatory agencies to revise acute toxicity studies. The long-term goal is to develop *in vitro* methods to replace animals and recommendations from the workshop participants will move *in vitro* methods forward. He outlined the current methods used for determining acute toxicity as the “classical” LD50 test and OECD Acute Oral Toxicity Tests 401, 420, 423 and 425. He related that OECD 401 test was to be dropped and that U.S. agencies will accept this decision.

Regulatory uses of acute toxicity data include hazard labeling (only EPA requires), hazard classification (LD50 dose points – required by some EPA offices, e.g., Office of Pesticide Programs), and risk

assessment. Dr. Galson listed the regulatory agencies and illustrated how they use hazard labels, and how they receive data and perform risk assessment. It is important to harmonize test methods between the various federal agencies (CPSC, DOT, OSHA, EPA, FDA, NIOSH, and ATSDR). Dr. Galson concluded by urging the workshop participants to revise methods for determining acute toxicity and to meet the scientific challenges. Recommendations of the workshop would be relevant to the federal regulatory agencies, in particular, the EPA for the HPV chemical program. Dr. Stokes thanked Dr. Galson and then introduced Dr. James Cone who would speak about clinical perspectives in occupational health.

#### Acute Toxicity Data -- A Clinical Perspective

Dr. Cone defined acute toxicity as health effects resulting from exposure over a short period of time. Though no single definition for acute exposure had been agreed upon, he felt that unintended releases of chemicals into the environment and poisonings would constitute a working definition. Many chemicals have acute toxicity human data and he related the clinician's experience with acute toxicity data by listing the available tools: Physicians Desk Reference (PDR), Material Safety Data Sheets (MSDS), poison control centers (PCC), Medline searches, the internet and the telephone. Knowledge is often based on human exposure. The clinician views acute toxicity as an immediate exposure to a substance while chronic toxicity occurs from exposure over a long period of time.

Dr. Cone discussed two incidents of toxic exposure that occurred in California. One incident involved a four-hour release/spill of oleum into the environment and required the evaluation of 20,000 residents at local emergency facilities. A second case study resulted from the release of 19,000 gallons of metam sodium into a river. Problems faced by agencies responding to these incidents included determining: the toxic agent, the acute health effects of the release, medical treatment and whether evacuation of the area was necessary. Exposure assessment was difficult in these cases because of differences in the odor threshold and the irritant threshold. It was important to know whether the substance traveled as a plume or flowed in the waterways. Dr. Cone discussed the examination of personnel close to the spills and the difficulty in detecting acute exposure in the individuals.

Dr. Cone suggested that the clinician's tools for measuring acute toxicity are mostly crude. Data from HSDB may be too old, as are data for threshold limit values (TLV) and legal permissible exposure limits (PELs). The limitations of the existing toxicity data include the lack of acute toxicity data for some chemicals and the lack of toxicity information for exposure to multiple chemicals, which is a common exposure scenario for humans. Dr. Cone also provided sources/websites of acute toxicity data. Dr. Cone stated that the clinician is challenged on how to interpret acute toxicity data on chemicals and on how to keep updated on human data. Dr. Cone ended his presentation by reminding the participants of the Nuremberg Code for Medical Experimentation on Humans. Dr. Stokes thanked Dr. Cone and dismissed the participants for a break.

#### ***In Vitro* Approaches to Estimate the Acute Toxicity Potential of Chemicals**

Dr. John Frazier opened the second phase of the plenary session by introducing Dr. Manfred Liebsch from the Center for Documentation and Evaluation of Alternative Methods to Animal Experiments (ZEBET).

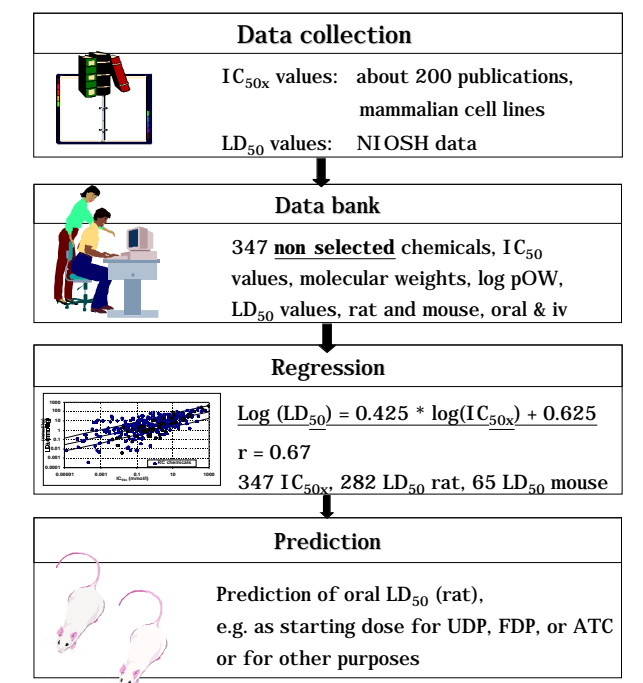
#### Estimating Starting Doses for *In Vivo* Studies using *In Vitro* Data

Dr. Liebsch began his presentation with an overview of ZEBET, which is part of the Federal Institute for Health Protection of Consumers and Veterinary Medicine of Germany. The three divisions of ZEBET are for documentation, evaluation and research. ZEBET uses *in vitro* data for prediction of *in vivo* toxicity. One hundred ten chemicals were evaluated in 1954 and another 15 chemicals were evaluated in 1956

using data from Dr. Willi Halle (Registry of Cytotoxicity) and Dr. Björn Ekwall (MEIC). Dr. Halle produced a monograph, which include a registry of 347 chemicals, in 1998. Dr. Liebsch provided the scheme used for predicting starting doses for acute toxicity tests for these chemicals: NIOSH data → concentration response curve → databank → regression → prediction of starting dose.

The Registry of Cytotoxicity (RC) acceptance criteria includes: 1) *in vitro* IC50 data gathered from the literature; 2) data from mammalian primary cells or cell lines (no hepatocytes); 3) chemical incubation time 16 hours; and 4) data from two different laboratories or two different cell types or two cytotoxicity endpoints. *In vitro* cytotoxicity endpoints include cell profiles, viability (MTT, Neutral Red, Trypan Blue data) and markers for differentiation. *In vivo* LD50 data includes only values found in NIOSH databases. If more than one LD50 value is available, then the largest value is used. LD50 data from rats and mice (oral and iv route) were collected; rat data are preferred. The ZEBET chemical list was shown and IC50x (i.e., geometric mean of IC50s for each chemical) values were discussed.

## RC: Summary



ICCVAM / NICEATM: Arlington, October 17-20, 2000  
 Liebsch, Genschow, Halle & Spielmann:  
 The use of *in vitro* data to estimate starting doses....



Dr. Liebsch presented the RC method of validation:  $\text{LD}_{50} = a + b \times \log \text{IC}_{50x}$  ( $a =$  intercept,  $b =$  regression coefficient,  $r =$  correlation coefficient). Changes in the estimates of  $a$ ,  $b$ , and  $r$  were small for the four regression analyses of the RC using 102, 117, 230, and 347 chemicals. The regression analysis provides a better prediction of LD<sub>50</sub> for less toxic chemicals. Dr. Liebsch continued by discussing ECVAM Workshop 16 (1994) that produced 10 recommendations for determining starting doses. He discussed the UDP test, which uses sequential dosing starting close to the LD<sub>50</sub> value, and said that the RC data could predict acute oral LD<sub>50</sub>s. One would determine the IC<sub>50</sub> in a cytotoxicity test, predict the LD<sub>50</sub> using the RC, and then determine the LD<sub>50</sub> in the animal. A tiered approach to the LD<sub>50</sub>, as shown in Dr. Liebsch's slide on the left, would use a cytotoxicity test to determine the starting dose for non-toxic chemicals where only the highest dose is applied (Limit Test). In a classification of 1115 industrial chemicals for acute toxicity in Europe, the majority were found to be non-toxic. Dr. Liebsch concluded his presentation with the following points: 1) the use of basal cytotoxicity to predict the oral

LD<sub>50</sub> for use as a starting dose will save 30-40% of animals used; 2) basal cytotoxicity tests can be used to determine whether a Limit Test should be performed; 3) the increased number of toxicity classes in OECD-HCL guidelines will increase the animal saving effect of the tiered *in vitro*/*in vivo* approach; and 4) lower animal use is predicted and validation of animal reduction is needed. His final point was that all of the effort is worth it to reduce animal testing. Dr. Frazier thanked Dr. Liebsch and then introduced Dr. Bas Blaauboer as the next speaker.

An Integrated Approach for Predicting Systemic Toxicity

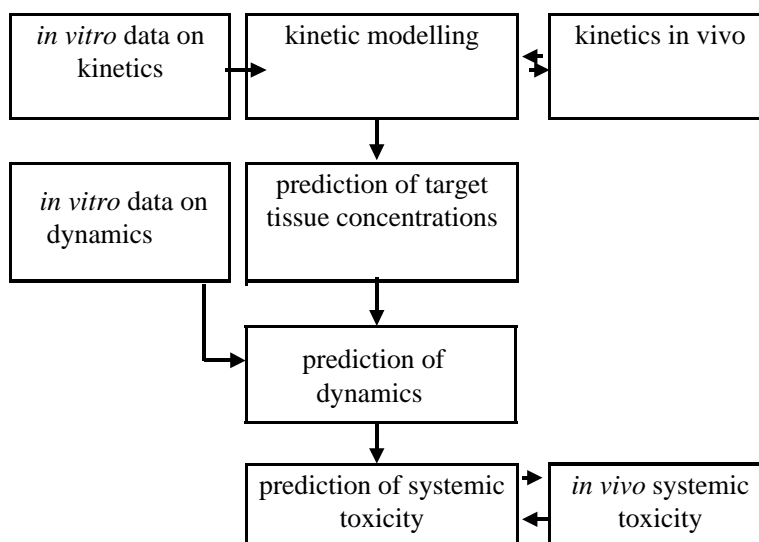
Dr. Blaauboer introduced his presentation on how to integrate *in vitro* data in predictive toxicology. He challenged the workshop participants to eliminate animal use and discussed the Institute of Risk Assessment Sciences, the development of computer based biokinetic models, and *in vitro* tests. He provided a brief discussion of the ECITTS (ERGATT/CFN Integrated Toxicity Testing Scheme) project.

Dr. Blaauboer explained that the aim of “classical” toxicological risk assessment is to establish safety factors for human exposure. Classical *in vitro* toxicology methods are limited because they find concentration for effect instead of determining dose and it is difficult to extrapolate the data to an intact organism. There is also a lack of biotransformation/kinetics data and the tests concentrate on cytotoxicity rather than on mechanisms of importance *in vivo*. He presented the necessary building blocks to produce integrated models: 1) biokinetic modeling; 2) prediction of tissue concentration; 3) knowledge of effective concentration for relative targets; 4) prediction of these effective concentrations; and 5) calculation of doses relevant for risk assessment. He briefly

discussed the European Research Group for Alternatives in Toxicity Testing (ERGATT) and the Swedish National Board for Laboratory Animals (CFN).

The ECITTS project building blocks are: 1) experimental – QSAR and *in vitro* data for biokinetics model; 2) modeling – *in vitro* data for PBBK models, determination of target tissue concentration; and 3) validation – validate against *in vivo* kinetics. The stepwise approach is: 1) determine the relevant parameters for biokinetic model, building

model using non-animal data – physiochemical properties (e.g. tissue partition, air/blood partition) and data from cell culture systems (e.g., biotransformation, passage of cellular layers with barrier functions); 2) validate with *in vitro/in vivo* comparisons; 3) use *in vivo* data to construct or improve biokinetic model; extrapolate data from non-toxic doses; 4) estimate tissue concentration especially in target tissues; 5) use *in vitro* assays to get response surrogates; 6) integrate kinetic and dynamic data, as shown in Dr. Blaauboer’s slide above; and 7) predict surrogate dose.



Arlington, Oct 2000

Dr. Blaauboer produced a list of compounds tested with a neural aspect (e.g., pesticides) and explained that the test strategy included: determination of basal cytotoxicity and morphological changes; determination of changes in cell physiology and neurochemistry; and determination of neurotoxic concentration (EC20). He illustrated this strategy using acrylamide as an example.

The following schematic would be used for the integrated use of alternative methods in toxicological risk assessment: structure of compound → chemical functionalities → QSAR → *in vitro* testing → classification of compound. This approach would lead to an *in vitro* test battery that could produce EC50 ratios, ultimately leading to limited *in vivo* testing. Dr. Blaauboer concluded that integrating *in vitro* data

in risk evaluation is valid provided biokinetics are taken into account and that the integration of all available data in a stepwise manner will improve risk assessment. Dr. Frazier thanked Dr. Blaauboer and introduced Dr. Oliver Flint.

Opportunities for Future Progress - *In Vitro* Approaches to Predicting Acute Toxicity

Dr. Flint opened his presentation by stating that *in vitro* tests used in a focused way could predict acute toxicity. He provided a test example: Taxol® Neuropathy – Successful *In Vitro* Prediction of Acute Toxicity. The objective was to characterize the neurotoxic effect of Taxol®. The *in vitro* model uses dorsal root ganglia cells and examines cytotoxicity, mitochondrial transport, morphology, and LDH leakage as endpoints. Dr. Flint discussed prediction of lethality as described by the MEIC project. He listed MEIC websites and suggested that mirror sites for the data be established. The basal cytotoxicity hypothesis for lethality using the 50 MEIC compounds correlates with human lethal plasma concentration. Problems with the basal cytotoxicity hypothesis are confounding factors such as interspecies differences in liver toxicity and specific toxicity for cell types; not all cell lines are alike.

He presented lessons in lethality predictions: 1) *in vitro* systems can make general predictions of *in vivo* toxicity; 2) human toxicity is best predicted by human cells; 3) variability is an unavoidable confounding factor; and 4) choosing the right cell is of critical importance. Future directions for predicting acute and other toxicities include computational predictions, molecular biology and *in vitro* systems targeting specific toxicological areas. *In silico* predictive toxicity is good for mutagenicity and carcinogenicity

predictions, but weak for acute and reproductive toxicology. Dr. Flint presented the table, on the left, for the changing paradigm illustrating the great reduction of testing time using *in silico* predictions. He also discussed emerging technologies such as transcriptome, proteome, and metabonome and stated the usefulness and limitations of the techniques. Dr. Flint concluded by stating the need to develop new technologies to characterize predictive biomarkers and to investigate transcriptome

## The Changing Paradigm

	MUTA-GENICITY	CARCINO-GENICITY	TERATO-GENICITY
TRADITIONAL	1-Month Ames	2-Year Rodent Bioassay	4-Month Segment II Rodent Assay
PARADIGM SHIFT - <i>In silico</i> followed by:	1-Day DNA Damage Assay	2-6 Week Cell Transformation Assay	5-Day Cell Differentiation Assay

and proteome for *in vitro* and metabonomics for *in vivo*.

**Public Comments:**

**Ms. Mary Beth Sweetland (PETA)**

Ms. Sweetland spoke of the January 1997 Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC) conference on alternatives and the focus on the need to increase the rate of development of alternatives for toxicology. She expressed concern for the EPA

endocrine disruptor screening program's use of numerous animals. She appreciated Dr. Galson's assurance that the EPA supported dropping OECD's TG 401 but feels that the ICCVAM validation principles are being applied arbitrarily resulting in a double standard. Ms. Sweetland stated that the non-standardized developmental neurotoxicity test uses up to two-thousand animals and is required by the EPA in the pesticide testing program even though testers can't agree on many points of the test. She believes that the EPA should support and practice full validation of all tests, animal and non-animal. Additionally, she feels that transgenics are not a true reduction method. She expressed frustration at the EPA, FDA and DOT for the agencies' continued use of animals in testing and dismay that *in vitro* cytotoxicity testing was being viewed as a novel concept instead of a time tested one. She again expressed appreciation for Dr. Galson's recommendation that *in vitro* cytotoxicity be used for dose setting as an interim step to total replacement. She urged regulatory agencies and companies to not wait for others to solve the problem and move forward on enhancing the cell tests.

**Dr. Andrew Rowan (U.S. Humane Society)**

Dr. Rowan explained that the Helsinki Declaration has been significantly revised in terms of animal welfare and appropriate animal testing and thus has been significantly modified from the old Nuremberg Code.

**Dr. Giles Klopman (Case Western Reserve University; Multicase, Inc.)**

Dr. Klopman stated that computer models wouldn't come into play if the validation is as lax as validation of short-term assays. He predicted that computer models will replace short-term assays and said that the FDA has a database for short-term assays. He was confident that the scientific community would solve the testing problems in the long run.

**Adjournment**

Dr. Frazier concluded the morning plenary session by restating the charge for the breakout groups and workshop participants. He stated the workshop objectives and described the nature of the four breakout groups. He explained that the workshop was to have the breakout groups answer the prepared questions provided by the Organizing Committee and to produce reports that will eventually be published. The morning session ended at 12:18 p.m.

**Closing Plenary Session**

Dr. Stokes opened the closing plenary session at 8:04 a.m. and introduced the Co-Chairs of the breakout groups. Co-Chairs presented their workshop reports (See **Sections 2-5**) and an opportunity for public comments was permitted.

**Public Comments:**

**Ms. Jessica Sandler (PETA)**

Ms. Sandler spoke of money available for development of non-animal tests: NIEHS committed \$1.5 million for fiscal year 2000 and \$3.0 million for fiscal year 2001; the EPA committed \$0.5 million over two years, and stated that the MEIC study would receive high priority. She expressed concern that the EPA had no single project in development for developing non-animal tests, yet continued requiring massive animal testing programs, in particular the HPV program. Ms. Sandler urged the ICCVAM to take a more aggressive role in developing alternative testing methods. She praised the



workshop for bringing together international and American scientists to persuade government regulators to seriously consider alternative testing methods.

**Dr. Martin Stevens (Humane Society of the U.S.)**

Dr. Stevens complimented ICCVAM for its role in organizing the workshop and hoped to be involved with ICCVAM in moving forward with the recommendations put forth by the workshop. He spoke of three hurdles in the evolution of replacing the LD50 test: 1) use of cytotoxicity data to accurately predict starting doses to reduce animal use; 2) use of limit tests to confirm non-toxicity; and 3) total replacement of the LD50 test.

**Ms. Mary Beth Sweetland (PETA)**

Ms. Sweetland made comments directly to ICCVAM concerning European Union acceptance of four validated test methods (three for corrosion and one for phototoxicity): Episkin , EpiDerm , rat skin TER, and 3T3 Neutral Red Uptake. She stated that the United States should accept the ECVAM validations and present these methods to the OECD as accepted methods. She concluded by thanking those who put the effort forth for the workshop.

In response to Ms. Sweetland's comments, Dr. Stokes stated that ICCVAM has an interagency Corrosivity Working Group that has provided extensive comments on the OECD proposals for the corrosivity methods mentioned, and U.S. government scientists also provided comments on the phototoxicity method. ICCVAM is currently developing an expedited process by which methods reviewed, validated, and accepted in Europe could be reviewed and considered by U.S. agencies.

**Conclusion and Adjournment**

Dr. Stokes presented the closing comments for the workshop, stating that the Breakout Groups had made remarkable progress. He thanked the co-chairs of the breakout groups, the agency representatives and the scientists attending the workshop. He stated that the objectives of the workshop had been met or exceeded in all areas, and that the Workshop's advice will lead to refinement in the near term and contribute to progress toward replacement. He stated that a report of the workshop would be published in 2001 and made available to the public. Dr. Stokes also recognized and thanked the ICCVAM Organizing Committee, Dr. Philip Sayre, Dr. John Frazier, and the NICEATM staff. The meeting was adjourned at 12:00 noon.

