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I. LOCATION OF BACKGROUND MATERIALS/PRESENTATIONS AND FREQUENTLY USED ABBREVIATIONS

Background materials and presentations for the SACATM working group meeting are available on the SACATM meeting page web site (*http://ntp.niehs.nih.gov/go/7441*).

CPSC	Consumer Product Safety Commission
ECVAM	European Centre for the Validation of Alternative Methods
EPA	U.S. Environmental Protection Agency
FDA	Food and Drug Administration
IC50	Inhibitory Concentration 50%
ICCVAM	Interagency Coordinating Committee on Alternative Methods
ILS	Integrated Laboratory Systems, Inc.
LD50	Lethal Dose 50%
NICEATM	The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NCI	National Cancer Institute
NIH	National Institutes of Health
NTP	National Toxicology Program
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
USDA	U.S. Department of Agriculture

II. ATTENDANCE

SACATM met as a working group via teleconference on August 3, 2006. The following individuals were present on the teleconference.

SACATM Members

Daniel Acosta, Ph.D.(Chair, University of Cincinnati)
Frank Barile, Ph.D. (St. John's University)
Marilyn Brown, Ph.D. (Charles River Laboratories)
George DeGeorge, Ph.D. (MB Research Laboratories)
Martin Stephens, Ph.D. (Humane Society of the U.S.)
Calvin Willhite, Ph.D. (California EPA)

ICCVAM Ex Officio Members

Karen Hamernick, Ph.D. (EPA)
Jodie-Kulpa-Eddy, D.V.M. (USDA)
Alan Poland, M.D. (NCI)
Leonard Schechtman, Ph.D. (FDA)
Margaret Snyder, Ph.D. (NIH)
William Stokes, D.V.M, (NIEHS)
Marilyn Wind, Ph.D. (CPSC)

NIEHS/NTP Staff, NTP Contractors, and other Federal Staff

Brad Blackard, M.S.P.H. (ILS)
Meta Bonner, Ph.D. (EPA)
John Bucher, Ph.D. (NIEHS/NIH)
Jeff Charles, Ph.D. (ILS)
Allen Dearry, Ph.D. (NIEHS/NIH)
Abby Jacobs, Ph.D. (FDA)
Debbie McCarley (NIEHS/NIH)
Michael Paris (ILS)
Amy Rispin, Ph.D. (EPA)
Judy Strickland, Ph.D. (ILS)
Kristina Thayer, Ph.D. (NIEHS/NIH)
Raymond Tice, Ph.D. (NIEHS/NIH)
Mary Wolfe, Ph.D. (NIEHS/NIH)

Public

Sara Amundson (Doris Day Animal League)
Karen Cazarin, MSc (Planitox)
Rodger Curren, Ph.D. (Institute for *In Vitro* Sciences)
Paul Jean, Ph.D. (Dow Corning)
Molly Laas (The Rose Sheet)
Damani Parran, Ph.D. (Colgate-Palmolive Company)
Jessica Sandler (People for the Ethical Treatment of Animals)
Katherine Stitzel, D.V.M. (consultant)

Sue Leary (Alternatives Research & Development Foundation)
Daniel Marsman, D.V.M., Ph.D. (Procter & Gamble)
Kristie Stoick, M.P.H. (Physicians Committee for Responsible Medicine)

III. WELCOME

Dr. Daniel Acosta, Chair, called the meeting to order at approximately 1 p.m. on August 3, 2006. The meeting was convened as a SACATM working group because a quorum of SACATM members was not in attendance. Meeting minutes will serve as the working group report and be disseminated to the entire SACATM. SACATM comments on the working group report will be posted on the SACATM website and discussed at the next SACATM meeting, scheduled for November 30, 2006. Dr. Kristina Thayer read the conflict of interest statement. Dr. Allen Dearry, Interim Associate Director of the NTP, and Dr. Leonard Schechtman, ICCVAM Chair, made welcoming remarks.

IV. ICCVAM EVALUATION OF *IN VITRO* METHODS FOR ESTIMATING STARTING DOSES FOR ACUTE ORAL SYSTEMIC TOXICITY

A. Presentations

Dr. William Stokes, NICEATM Director and ICCVAM Executive Director, provided a historical perspective and overview of the evaluation effort. Dr. Kathy Stitzel, consultant and peer review panel chair, summarized the major findings of the peer review panel report.

B. Public Comments

Ms. Jessica Sandler from People for the Ethical Treatment of Animals (PETA) and Ms. Sara Amundson from the Doris Day Animal League made oral public comments. Ms. Sandler expressed concern for the slow pace of progress since the October 2000 workshop "*In Vitro* Methods for Assessing Acute Systemic Toxicity." She noted that experts at that workshop agreed that cell-based methods could be used immediately for estimating the starting dose, which would reduce the number of animals used in lethality testing. Also, workshop participants believed it was possible that a replacement test battery might be achieved in 2-3 years if there was a strong commitment to validation studies. Ms. Sandler noted, however, that several pre- and intermediate validation activities were never initiated. She asked why the recommendations agreed upon 6 years ago were not pursued and asked the SACATM working group to consider what could be done now to put in place an *in vitro* replacement for the use of animals in lethality studies. She suggested that the cytotoxicity methods could be used for testing those substances that are suspected to be at the extremes of toxicity (i.e., relatively non-toxic or very toxic) and if the *in vitro* predictions corroborate the expectations, then no animal testing should be conducted for those substances. Ms. Amundson strongly supported these comments. In addition, she noted the United States lags far behind Europe in efforts to find a replacement for lethality testing. She encouraged the SACATM working group to recommend a strategy for advancing the issue.

C. SACATM Working Group Discussion

Dr. Acosta reviewed the charge for the SACATM working group:

> Comment on whether members support the conclusions reached by the panel on the validation status and draft ICCVAM test method recommendations (i.e., test method uses, future studies, test method protocols, and performance standards) for the two evaluated test methods [*in vitro* neutral red uptake (NRU) basal cytotoxicity test methods using 3T3 and NHK cells]? Why or why not?

Dr. Frank Barile approved of the peer panel's evaluation but wondered why other indicator tests for acute and basal cytotoxicity besides NRU were not considered especially since some of the other indicators appear to be more sensitive. Dr. Stokes said this issue was discussed extensively at the 2000 workshop and participants felt the NRU was the most practical indicator with which to move forward. Dr. Raymond Tice added that the major reason the workshop participants recommended the NRU was because it had gone through a validation study for phototoxicity and had shown good performance in different laboratories. Thus, minimal or no assay optimization would be required. Dr. Barile commented that now it appears the NRU tests are not as accurate and reliable as hoped at least for acute or systemic toxicity. Dr. Stokes welcomed suggestions for other endpoints. He noted that both the NTP and ECVAM have high throughput testing initiatives that could be used to generate data for other cytotoxicity endpoints. Dr. Tice said it is unlikely that any single cytotoxicity test method would be very reliable. To improve reliability, other components such as metabolism and target organ specificity would need to be incorporated into a testing battery to better predict *in vivo* acute lethality.

Dr. DeGeorge had several questions on Dr. Stokes' presentation regarding the LD_{50} prediction and estimated amount of animal reduction (slides 11 and 12). He said there appears to be an approximately 10,000-fold difference between the IC_{50} and the LD_{50} values (when mg/kg is considered equal to μg/ml). The factor of 10,000 is very high and suggests that different mechanisms are being measured in the *in vitro* and *in vivo* assays. Dr. DeGeorge believes it is difficult to extrapolate from a measure as sensitive as cytotoxicity to an *in vivo* effect as severe as lethality. He suggested using a less sensitive *in vitro* indicator than cell viability because using more sensitive *in vitro* indicators (e.g., proliferation, ATP, or other biochemical measurements) could make the fold-difference IC_{50} and the LD_{50} values even greater than 10,000. Also, the adjusted R^2 of 0.353 seems to be very low. Dr. DeGeorge expressed more confidence in using basal cytotoxicity data with an $IC_{50} \geq 10$ μg/ml (corresponding to an $LD_{50} \geq 300$ mg/kg). Dr. Stitzel said these issues are part of the reason why the peer panel had concern about the accuracy of the *in vitro* approaches for predicting the LD_{50} although the panel also believed that performance was better for less toxic substances. Dr. DeGeorge questioned why there are few data points for more toxic substances in slide 11. Dr. Judy Strickland said slide 11 is an example dataset showing a subset of chemicals in the Registry of Cytotoxicity database - those that had a mechanism of toxicity expected to be detected in the cytotoxicity assays. The IC_{50} values were obtained from the literature so the selection of which compounds to include was based on data availability rather than consideration of whether the different toxicity classes were equally represented. Dr. DeGeorge commented that the number of animals saved was very low (between ½ and ¾ an animal) and in some cases more animals would be used if cytotoxicity was used to estimate the starting dose. Dr. Tice explained that more animals are saved in the least toxic categories (i.e., higher LD_{50}) because the IC_{50} data can indicate that lethality testing should begin at or near the limit dose. In the absence of this information, dosing begins at the much lower default dose (e.g., 175 mg/kg for the UDP), which would result in almost twice as many animals being used when nontoxic substances are tested. Dr. DeGeorge suggested that using a "weighted" approach in slide 12 would be useful to present a more accurate picture of how many animals can be saved. Dr. Stokes said that his comment that 80% of substances have an $LD_{50} > 2000$ mg/kg is based on the European New Chemicals Database, which contains data on almost 2,000 industrial compounds.[1] Because most compounds have a relatively high LD_{50} using a weight approach would show more animal savings than the current figure. Dr. Stitzel agreed with this statement in principle but said the difficulty is finding a database that represents reality regarding the proportion of chemicals that belong in each toxicity class. Dr. Marilyn Brown recognized that a database showing the true proportion of compounds in each toxicity category does not exist, but thought existing databases could be used for weighting purposes to give a range of possible animal savings. Dr. Stitzel agreed. Dr. Stokes expressed interest in databases that show distributions of LD_{50} values.

[1] Spielmann H, Genschow E, Liebsch M, Halle W. 1999. Determination of the starting dose for acute oral toxicity (LD50) testing in the up and down procedure (UDP) from cytotoxicity data. Altern Lab Anim. 27:957-966]

Dr. Stephens endorsed the main conclusions of the peer review report and noted several positive aspects of the report (e.g., use of simulations, agreement that additional animal data do not have to be obtained for validation efforts). However, he is also frustrated at the limited progress since the October 2000 workshop, given the modest scope of the current evaluation (i.e., use of *in vitro* to predict a starting dose rather than a proposal for replacing the LD_{50} test). Dr. Stephens was critical of accepting rodent LD_{50} as the gold standard for predicting human lethality. He suggested terms such as concordance be used instead of accuracy. He thought there is more *in vitro* data available than was used in the analysis although he understood that obtaining this data could be difficult. While disappointed that the projected animal savings are not higher, he thought a potential 20% savings is not trivial. He did not agree with a statement in the peer review panel report that said requiring the use of *in vitro* to predict starting doses could lead to greater animal usage, especially when toxicity category prevalence is taken into account. Dr. Stitzel explained that the panel did not have access to prevalence data and that companies may have other, high quality information data that would suggest a more appropriate starting dose for the LD_{50} test than would an *in vitro* basal cytotoxicity test. In this case, using the *in vitro* cytotoxicity test results might result in the use of more animals. Dr. Stephens noted that since the proposal is to use a weight of evidence approach other informative data might carry more weight than cytotoxicity data in certain circumstances.

Dr. Barile praised the quality of the peer panel evaluation. He was sympathetic to the points raised by members of the animal welfare community on the pace of the process, but thought the progress is relatively fast from the regulatory and scientific acceptance perspectives. He suggested that animal welfare organizations continue, and perhaps increase, their support for research on alternative methods in academia. Dr. Stephens noted that animal welfare organizations do support research efforts, but could perhaps do more.

Dr. DeGeorge asked how much funding is being devoted to *in vitro* toxicological methods research. Dr. Stokes said it is impossible to give a single dollar amount. Funding for the basal cytotoxicity validation effort is complete and there are no plans for additional work. Approximately 12-15 million dollars is devoted to the ECVAM AcuTox project. Between 1994 and 1997, NIEHS funded a 4.5 million dollar Request for Applications (RFA) for alternative method development. Eleven academic-based investigators were supported from this effort.

Dr. Acosta agreed with many of the previous comments. He said many of the suggested future activities (i.e., development of a testing battery) are not new ideas and have been discussed for decades. He emphasized the long history of neutral red release and uptake and thought the background review document should recognize early pioneers in the field. He supported the use of *in vitro* data to generate information on mechanism in general, but did not believe this is as essential for acute toxicity. He also understood the perception that the scientific process for evaluating these methods appears slow. He thought SACATM might be more effective if they met more frequently.

Dr. Stephens reiterated Ms. Sandler's proposal as a possible first target for replacement of the animal test with an *in vitro* method(s), namely, that the cytotoxicity methods could be used for testing those substances that are suspected to be at the extremes of toxicity, and if the *in vitro* predictions corroborate the expectations from preliminary evidence, then no animal testing should be conducted for those substances. He noted especially the good correlation between the *in vitro* and *in vivo* data at the high dose/low toxicity end of the toxicity spectrum, which Dr. DeGeorge had commented on earlier. Dr. Stitzel said the problem is determining accuracy for unknowns. Dr. Stokes added that while the approach of relying on the cytotoxicity assays to label the most toxic compounds works from a public health protection perspective (compounds would not be "under-labeled"), it presents a potential problem of false

over-prediction (and subsequent "over-labelling"). Dr. Stokes added that predicting systemic toxicity from *in vitro* methods is much more complex than for local toxicities (i.e., skin, eye irritation).

Dr. Acosta summarized major points of discussion and said the SACATM working group supports the conclusions of the peer panel review report on the validation status and draft ICCVAM test method recommendations.

The meeting adjourned at 3:40 p.m.