

**Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)
Independent Peer Review of the Revised Up-and-Down Procedure (UDP)
for Acute Oral Toxicity**

Tuesday, August 21, 2001, 10:00 a.m. – 12:00 p.m. EDT

**UDP Peer Review Panel
Teleconference Call Minutes**

**National Institutes of Environmental Health Science (NIEHS)
79 T.W. Alexander Drive
Building 4401, Room 3162
Research Triangle Park, North Carolina**

Teleconference Attendees:

UDP Peer Review Panel

- Dr. Diane Gerken (Co-Chair)
Battelle Memorial Institute
- Dr. Curtis Klaassen (Co-Chair)
University of Kansas Medical Center
- Dr. George Alexeeff
California Environmental Protection
Agency
- Dr. Phil Botham
Syngenta, Ltd.
- Dr. Robert Condon
Consulting Biostatistician
- Dr. Robert Copeland
Howard University
- Dr. Nancy Flournoy
American University
- Dr. A. Wallace Hayes
The Gillette Company
- Dr. Janice Kuhn
Stillmeadow, Inc.
- Mr. John Reeve
New Zealand Ministry of Agriculture
and Forestry
- Dr. Robert Scala
Toxicology Consultant
- Mr. Gary Wnorowski
Product Safety Labs

UDP Technical Task Force Representatives

- Mr. David Farrar
U.S. Environmental Protection Agency
- Dr. Michael Green
Consumer Product Safety Commission
- Dr. Kailash Gupta
Consumer Product Safety Commission
- Dr. Elizabeth Margosches
U.S. Environmental Protection Agency
- Mr. William Meyer
U.S. Environmental Protection Agency
- Dr. Amy Rispin
U.S. Environmental Protection Agency
- Dr. Kathy Stitzel
Proctor and Gamble Co.

ICCVAM Agency Representatives

- Dr. Suzanne McMaster
U.S. Environmental Protection Agency
- Dr. Marilyn Wind
Consumer Product Safety Commission

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

- Dr. William Stokes (ICCVAM Co-Chair)
National Institute of Environmental Health Sciences
- Mr. Bradley Blackard
Integrated Laboratory Systems, Inc.
- Ms. Ashlee Duncan
Integrated Laboratory Systems, Inc.
- Ms. Loretta Frye
National Institute of Environmental Health Sciences
- Dr. Raymond Tice
Integrated Laboratory Systems, Inc.

General Public

- Dr. David Bombick
R.J. Reynolds Tobacco Company
- Dr. Ian Pate
Syngenta, Ltd.
- Mr. Andrew Ballard
Reporter, Bureau of National Affairs

Call to Order and Introductions

Dr. Klaassen welcomed all participants and called the meeting to order at 10:20 a.m. He asked everyone to state his or her name for the record and requested that discussions be limited to Panel members only.

ICCVAM Test Method Review Process for the UDP

Dr. Stokes, co-chair of ICCVAM, thanked the Panel for their participation in the teleconference and provided background information and timelines pertaining to the UDP. He explained that the conclusions and recommendations of the Panel from the July 2000 Peer Review meeting were considered by the UDP Technical Task Force and incorporated into a revised UDP Test Guideline. The Task Force also developed a proposed procedure for calculating confidence intervals and a software program for use with the UDP. These are the items that the Panel has been asked to review during this teleconference meeting. Dr. Stokes then read the Conflict of Interest Statement; no conflicts were stated among the participants. He explained that the Panel will prepare a written report following the teleconference for publication in the UDP Peer Panel Final report, scheduled to be printed in November 2001. In accordance with Public Law 106-545, this report and accompanying ICCVAM recommendations will be forwarded to Federal agencies for consideration and action.

Peer Review Panel Discussion

Dr. Klaassen began the meeting by discussing the Panel's position on Evaluation Guidance Question #1 – *The revised draft UDP Test Guideline (June 20, 2001) incorporates modifications in accordance with the Panel's recommendations at the July 25, 2000 Peer Review Panel meeting.*

- a) *Are the changes consistent with the Panel's recommendations?*
- b) *Do you concur with the revisions that have been made?*

The Panel concluded that many of the requested changes had been appropriately considered and that they agreed with the changes made. However, several recommendations appeared to have not been adequately addressed in the revised UDP Test Guideline and these were considered during the teleconference on a case-by-case basis.

Recommendation: to increase flexibility and adaptability in animal use, the use of either sex or the more sensitive sex (if information is available indicating that one sex is more sensitive) should be permitted. The Panel unanimously re-affirmed this recommendation

Recommendation: the body weight of an animal on day 1 of dosing should be within 20% of the mean body weight of all previous animals used. The Panel recognized the confusion in wording in this recommendation (day 1 and previous animals) and, based on the revised language included in paragraph 14 of the revised draft Guideline, decide to withdraw this recommendation.

Recommendation: to include additional guidance for use of pre-start data (data available before the acute toxicity test is conducted) that may be helpful in determining the starting dose. The revised draft UDP Test Guideline addresses this recommendation in paragraph 4 as follows: *All available information on the test substance should be considered by the testing laboratory prior to conducting the study. Such information will include the identity and chemical structure of the substance; its physical chemical properties; the results of any other in vitro or in vivo toxicity tests on the substance or mixtures; toxicological data on structurally related substances or similar mixtures; and the anticipated use(s) of the substance. This information is useful to determine the relevance of the test for the protection of human health and the environment, and will help in the selection of an appropriate starting dose.*

Several Panel members expressed an opinion that this type of information was more appropriate for inclusion in a training session or guidance document, rather than in a guideline. Dr. Flournoy stated that the concept of this recommendation was to provide a better idea of the types of information or data to consider when selecting a starting dose level and to provide an alternative for the default starting dose level. The Panel unanimously recommended the following modification to the guideline “*All available information on the test substance should be considered by the testing laboratory prior to conducting the study. Such information may include the identity and chemical structure of the substance; its physical chemical properties; the results of any other in vitro or in vivo toxicity tests on the substance or mixtures; toxicological data on structurally related substances or similar mixtures; and the anticipated use(s) of the substance. This information is useful to determine the relevance of the test for the protection of human health and the environment. This information may be valuable in selecting a dose other than the default starting dose.*”

Recommendation: that a practicability evaluation be conducted of the revised UDP Test Guideline. The Panel unanimously re-affirmed this recommendation.

Recommendation: that a separate section describing how the revised UDP Primary Test addresses reduction, refinement and replacement of animals compared to the previous tests be included in the Guideline. The Technical Task force formed the following response to this recommendation: *The Guideline significantly reduces the number of animals used in comparison to Guideline 401, which often required at least 20 animals in a test: 1) the stopping rule limits the number of animals in a test; 2) sequential dosing introduces further efficiencies in animal use; 3) initial dosing is now set to be below the LD50, increasing the percentage of animals in which dosing levels will be sub lethal and thereby providing some reduction in pain and distress; and 4) the use of a single sex reduces the number of animals needed and minimizes the variability in the test population. Theoretically using females only could lead to an oversupply of males. However, the use of male rats in animal research greatly exceeds that of females and, thus, the preference for females in acute toxicity testing may well result in a better overall balance of the use of both genders. Importantly, the guideline contains a requirement to follow the OECD Guidance Document on Humane Endpoints that should reduce the overall suffering of animals used in this type of toxicity test.*

Dr. Klaassen suggested the removal of gender specific references or the addition of the acceptability to use either gender (as per the preceding recommendation). The Panel decided to

recommend removing the gender reference (see the underlined sentences in the above paragraph) and unanimously recommended that the statement be added to the Guideline.

Recommendation: in paragraph 17a of the revised UDP Test Guideline, constant concentration should be used unless there is scientific or regulatory need for using constant volume. If constant volume is used in the performance of the UDP, concentrations used should also be supplied. The Panel unanimously recommended that this statement be added to the Guideline.

The Panel decided that all editorial recommendations for the revised UDP Test Guideline would be summarized by the Panel's co-chairs and added to the Panel's report for consideration.

Dr. Klaassen continued the deliberations by considering the Panel's position on Evaluation Guidance Question #2 - *Is the proposed procedure for calculating a confidence interval for the LD50 appropriate and adequate for use with the revised draft UDP Test Guideline?*

Dr. Klaassen explained that the biostatisticians on the UDP Panel (Drs. Condon, Flournoy, and Stallard) had been charged with developing the Panel's position for this question. Dr. Flournoy stated that the proposed approach was interesting but, because of limitations and uncertainties with the method, the Panel statisticians felt that language should be added to the UDP Test Guideline that specifically indicates the shortcomings and limitations of the procedure. She continued by stating that as more is learned about the use of these types of statistical methods, the procedure should be modified accordingly.

Many Panel members felt that the wording in the procedure was too technical for non-statisticians to understand and the procedure was asking too much from data from so few animals. Drs. Hayes and Botham suggested that the procedure be rewritten using non-statistical language and outlining specific situations where the procedure does not perform well. Dr. Scala stated that the UDP Technical Task Force had failed to justify the need for confidence intervals and that the analysis was based on too few animals. He presented a motion to not recommend the procedure on these grounds. Dr. Hayes seconded the motion. Dr. Flournoy stated that the proposed procedure moves the field of statistics forward and, if the limitations are clearly described, should be approved by the Panel. She went on by explaining that such a procedure would always work poorly with shallow slopes. The Panel determined that situations where the procedure works poorly were not that common and as long as the limitations are described in detail, it would be appropriate to recommend.

Dr. Scala stated that he would withdraw his previous motion if the UDP Technical Task Force would rewrite the procedure to include details of its limitations. Dr. Condon added that people using the software program would not be cognizant of the limitations of the procedure and might conclude, incorrectly, that the data obtained were inadequate in situations where an infinite confidence limit was calculated by the program. He suggested that specific language be added to the software program also explaining the limitations of the confidence interval procedure.

Dr. Botham reiterated the need for an explanation of the procedure's limitations written in language that study directors would understand. The representatives of the UDP Technical Task Force agreed to work with the Panel's biostatisticians to develop these explanations.

The Panel unanimously accepted the proposed procedure for calculating confidence intervals for the LD50 as appropriate and adequate for use with the revised draft UDP Test Guideline, as long as a description of the applicability, utility, and limitations of the procedure was included in the Guideline and in the software program. The Panel biostatisticians agreed to work with the UDP Technical Task Force biostatisticians on the development of these statements, which would be circulated to the Panel for concurrence.

Dr. Klaassen continued by discussing the Panel's position on Evaluation Guidance Question #3 – *Is the software program adequate and consistent with the procedures in the revised draft UDP Test Guideline?*

The Panel unanimously agreed that the software program to accompany the UDP is adequate and consistent with the procedures in the revised draft UDP Test Guideline. Dr. Condon stated that the program may need some minor revision as related to the Panel's concerns expressed in the Question #2 discussion.

Public Comment

No public comments were made.

Peer Review Panel Conclusions and Recommendations

Dr. Klaassen briefly reviewed the conclusions and recommendations of the Panel that were voted on during the meeting.

Adjourn

Dr. Stokes again thanked the Panel members for participating in the teleconference. Dr. Klaassen adjourned the meeting at 12:30 p.m.