ICCVAM Test Recommendation for the Up-and-Down Procedure:
A Test Method for Determining the Acute Oral Toxicity of Chemicals

I. Introduction

In August 1999, the U.S. Environmental Protection Agency (EPA) requested the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) to conduct an independent scientific peer review evaluation of the validation status of a revised Up-and-Down Procedure (UDP) for determining the acute oral toxicity of chemicals. The revised UDP was proposed as an alternative to the existing conventional LD50 test [OECD Test Guideline (TG) 401, 1987; U.S. EPA 870.1100, 1998]. An earlier version of the UDP test method had been adopted by the Organisation for Economic Co-operation and Development (OECD) Test Guidelines Program in 1998 (TG 425; OECD 1998a). The U.S. EPA subsequently determined it was necessary to revise the UDP. The revisions were needed to 1) conform to a newly harmonised global hazard classification scheme for acute toxicity (OECD, 1998b); and 2) to incorporate changes to ensure the regulatory and testing needs would be met using the revised UDP prior to the OECD's proposed deletion of the TG 401 (OECD, 1987).

The revised UDP test method submitted to ICCVAM included three components:

- A Primary Test, which provided an improved estimate of acute oral toxicity with an accompanying reduction in the number of animals used, when compared to TG 401 and the existing TG 425;
- A Limit Test for substances anticipated to have minimal toxicity; and
- A Supplemental Test to determine the slope and confidence interval (CI) for the dose-response curve.

II. ICCVAM Independent Scientific Peer Review

July 25, 2000 Peer Review Meeting

In a public session on July 25, 2000, ICCVAM convened an international independent scientific peer review panel (Panel) to evaluate the validation status of the revised UDP (Federal Register, NIEHS, 2000a, 2000b). The Panel evaluated the extent to which established validation and acceptance criteria (ICCVAM, 1997) had been addressed, and developed conclusions regarding the usefulness and limitations of the revised UDP. The Panel also responded to the following questions:

- Has the revised UDP been evaluated sufficiently, and is its performance satisfactory to support its adoption as a substitute for the currently accepted UDP (OECD, 1998a), and as a substitute for the conventional LD50 test for acute oral toxicity (U.S. EPA OPPTS 870.1100, 1998; OECD, 1987)?

- With respect to animal welfare, does the revised UDP adequately consider and incorporate where scientifically feasible, procedures to refine, reduce, and/or replace animal use?

The Panel's report is included in the publication: "The Revised Up-and-Down Procedure: A Test Method for Determining the Acute Oral Toxicity of Chemicals," NIH Publication 02-4501 (ICCVAM, 2001b). The Panel's conclusions were as follows:

- **UDP Primary Test**
  "The performance of the revised UDP Primary Test is satisfactory and exceeds the performance of OECD TG 401 in providing, with fewer animals, both an improved estimate of the LD50 for the purpose of hazard classification and more accurate information on acute toxicity. In particular, the use of 0.5 log units for dose spacing is reasonable and appropriate based on experience and the results of computer simulations. Three disadvantages of the revised UDP Primary Test recognized by the Panel are: a) the increased length of time needed to conduct a study; b) the increased costs per test material evaluated; and c) the increased complexity of the protocol."

- **UDP Limit Test**
  "The revised UDP Limit Test at 2000 or 5000 mg/kg is expected to perform as well as or better than the Limit Test in OECD TG 401, with a reduction in the number of animals needed to conduct a test."

- **UDP Supplemental Test**
  "The UDP Supplemental Test for slope and CI is not recommended for adoption. The Panel was unable to evaluate the utility of the test because sufficient information regarding the use of the resulting data was not provided. As a consequence, any impact on animal use was not assessed."

- **Animal Welfare Considerations**
  "The revised UDP Primary Test and the revised UDP Limit Test will reduce the number of animals used, but will not replace the use of animals. The Panel could not reach a consensus on the overall issue of refinement. However, the OECD Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluations (OECD, 2000), referenced in the revised UDP Guideline, provides an element of refinement."

Revisions to the UDP in response to the July 25, 2000 Panel Report

Based on the Panel's conclusions and recommendations from July 25, 2000, the UDP Technical Task Force further revised the UDP test method guideline as follows:

- Revisions recommended by the Panel were incorporated into the proposed UDP Primary and Limit Tests;
- The UDP Supplemental Test to determine the slope of the dose-response curve was deleted;
- A procedure was added (for use with the Primary Test) to calculate the CI for the estimated LD50. This procedure is a statistical method that does not require the use of additional

animals. The CI helps to place the estimated LD50 in a statistical context for hazard and risk assessment purposes.
- The U.S. EPA developed a publicly available software program for use in establishing test doses, determining when to stop the test, estimating the LD50, and providing a CI for the LD50.

The Technical Task Force also responded with the following clarifications regarding animal welfare:

- The revised UDP significantly reduces the number of animals used in comparison to OECD TG 401 by the incorporation of the following: 1) a stopping rule which limits the maximum number of animals in a test; and 2) a sequential dosing method which introduces greater efficiencies in animal use.
- The revised UDP provision that the initial starting dose should be below the LD50 will result in fewer animals receiving a lethal dose, thereby potentially providing further reduction in pain and distress.
- Adherence to the OECD Guidance Document on Humane Endpoints (2000) should provide additional reduction or minimization of pain and distress in animals used in this procedure.

The revised version of the UDP and the UDP software program were then provided to the Panel and made available for public comment in July 2001.

August 21, 2001 Peer Review Panel Meeting

The UDP Panel met, via public teleconference, on August 21, 2001 (Federal Register, NIEHS, 2001) to evaluate the appropriateness and suitability of the further revised UDP, the approach for obtaining the CI, and the suitability of the software program. Their conclusions and recommendations were as follows:

- **Further Revisions to the Revised UDP**
  The Panel concluded the changes made in the revised UDP Test Guideline were acceptable, but also recommended further clarifications to the UDP as follows:

  - Either sex of animal can be used, or if information is available indicating that one sex is more sensitive, the more sensitive sex should be used.
  - A practicability evaluation of the usability of the *in vivo* test should be conducted to supplement the computational analyses.
  - A separate section on how the revised UDP Primary Test addresses reduction, refinement, and replacement of animals when compared to the previous tests should be included to the UDP guideline.
  - Constant concentration in dosing should be used unless there is a clear scientific or regulatory justification for using constant volume. In the event that constant volume is used, information on the actual concentrations utilized should be provided.

- **CI Procedure**
  The Panel endorsed the proposed procedure for calculating the CI for the estimated LD50. However, the Panel recommended the inclusion of language in the UDP guideline and

software to fully describe the limitations and uncertainties of the proposed method, and to provide appropriate cautions for interpretation of test results. The Panel noted that statistical techniques are evolving and recommended the future development of alternative approaches, such as nonparametric methods, be encouraged.

· **Software Program**
The Panel concluded the software program was appropriate and suitable for establishing test doses, determining when to stop the test, estimating the LD50, and providing a CI for the LD50.

Revisions to the UDP in response to the August 21, 2001 Panel Report
Following the August 21, 2001 Panel meeting, the UDP Technical Task Force revised the UDP Guideline in response to the Panel's recommendations. This revised version is included in the Final Peer Review Report (ICCVAM, 2001b) as Appendix B.

III. ICCVAM Test Method Recommendations

The ICCVAM agrees with the UDP Peer Review Panel that the revised UDP test guideline, with incorporation of the Panel's recommendations from the August 21st Panel Meeting, is acceptable as a substitute for the conventional LD50 test for acute oral toxicity (U.S. EPA OPPTS 870.1100, 1998; OECD, 1987) for the purpose of hazard classification and for obtaining certain information on acute toxicity. The ICCVAM also agrees with the Panel that the revised UDP Test Guideline will reduce and refine animal use. The ICCVAM concurs also with the other conclusions and recommendations of the Panel.

ICCVAM therefore recommends that the final revised UDP test guideline should: (1) replace the current OECD UDP test guideline (TG 425; OECD, 1998a) and (2) be used instead of the conventional LD50 test to determine the acute oral toxicity hazard of chemicals.

The ICCVAM also concludes:

1. The revised UDP performs appropriately and will result in a reduction in animal usage compared to the conventional LD50 test. The recommendation to use a starting dose level below the anticipated LD50 and to follow the OECD Guidance Document on Humane Endpoints (2000) will refine animal use by decreasing pain and distress.

2. The revised UDP is an appropriate method for generating a point estimate for the LD50 for use in hazard classification and in estimating a CI for the LD50 under specified circumstances. The revised UDP does not provide information about the slope of the dose-response curve for lethality. If other human health or ecological risk assessment information is desired, including hazard dose-response and slope information, a different test should be conducted.

3. Compared to the conventional LD50 procedure, the UDP will require additional time. However, it provides potential improvements in animal welfare and is the only alternative

to OECD TG 401 that will generate a point estimate for the LD50 and an accompanying CI.

4. Compared to the conventional LD50 procedure, the UDP is computationally more complex. However, the UDP does provide increased statistical power with the use of sequential dosing. The publicly available statistical software will greatly simplify and facilitate efficient conduct of the UDP. The software calculates subsequent test dose levels, determines when testing is complete, estimates the LD50, and provides an appropriate and useful CI for the LD50.

5. Due to the reduction in the number of animals required when compared to the conventional LD50 test, the amount of test material needed will also be decreased.

6. The UDP may not be appropriate for chemicals causing delayed deaths (especially after five days).

7. Limit dose testing may be conducted at 2000 or 5000 mg/kg, depending on regulatory program needs.

8. For scientific purposes, the testing of three to five animals in the Limit Test is adequate. However, it is recognized that OECD stipulates utilizing five animals at 2000 mg/kg for all alternative acute toxicity methods as a way of harmonizing procedures.

9. Either sex can be used for the UDP. However, in the absence of information indicating males may be more sensitive, it is recommended that females be used based on available data showing females to be generally more sensitive.

10. Statistical methods are evolving rapidly, thereby providing reason to consider revisiting the UDP test design in the future.

11. A practicability assessment of the revised UDP should be considered.

12. *In vitro* data may be helpful in estimating an appropriate starting dose level for UDP studies. This approach may further reduce the number of animals needed, especially if the results indicate a Limit Test may be appropriate. Further guidance can be found in the "Guidance Document on Using *In Vitro* Data To Estimate *In Vivo* Starting Doses for Acute Toxicity", NIH Publication 01-4500 (ICCVAM, 2001a).

Adopted by ICCVAM:
October 10, 2001

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