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

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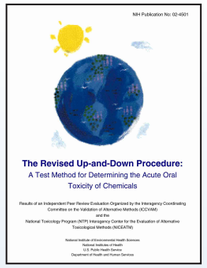
Abstract



In 1999, the U.S. Environmental Protection Agency (EPA) asked ICCVAM to evaluate the validation status of the Revised UDP as a substitute for the conventional acute oral toxicity test (i.e., OECD Test Guideline [TG] 401 and EPA OPPTS 870.1100, 1998). ICCVAM and NICEATM organized an independent scientific peer review evaluation of the Revised UDP by an international panel of expert scientists. On July 25, 2000, the Panel met in a public meeting to evaluate the extent to which the Revised UDP met ICCVAM validation and acceptance criteria and to develop conclusions regarding the usefulness and limitations of the Revised UDP. The Panel agreed that the UDP Primary and Limit Tests would perform as good as or better than the conventional LD50 test, and would also reduce and refine animal use. Based on the Panel's conclusions and recommendations, the EPA UDP Technical Task Force modified the UDP test guideline and added a computational procedure to calculate the LD50 confidence intervals (CI). The EPA also developed a software program to accompany the Revised UDP. A second meeting of the UDP Panel was convened via teleconference on August 21, 2001. The Panel endorsed the modifications to the Revised UDP, the CI calculation procedure, and the software program. Based on these conclusions, ICCVAM forwarded recommendations to Federal agencies supporting the use of the Revised UDP as a substitute test for the conventional LD50 test. Supported by NIEHS Contract N01-ES-85424.

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Background

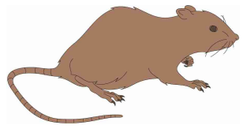
1981 Organisation for Economic Co-operation and Development (OECD) adopted an international test guideline (TG) for acute oral toxicity (TG 401)
- used 30-50 test animals

1987 OECD adopted revised TG 401
- used 20 - 25 test animals

1987 –
1998 OECD adopted three additional test guidelines for acute toxicity:
- Fixed Dose Procedure (FDP; TG 420)
- Acute Toxic Class Method (ATCM; TG 423)
- Up-and-Down Procedure (UDP; TG 425)

1998 OECD proposed deletion of TG 401
- Prior to deletion, OECD requires revision of FDP, ATCM, and UDP to conform to Globally Harmonized Hazard Classification Scheme

1998 U.S. EPA agreed to organize Technical Task Force to revise UDP
- The UDP Technical Task Force was charged with preparing a revised UDP which comprised three procedures:
  1. *Primary Test* – estimates LD50 using average of 7 and maximum of 15 animals
  2. *Limit Test* – for substances anticipated to have minimal toxicity
  3. *Supplemental Test* – determines slope and confidence interval (CI) for the dose-response curve
- Computer simulations used to design and validate the revised test – NO ANIMALS WERE USED FOR THE VALIDATION



ICCVAM Peer Review of the UDP

1999

August U.S. EPA asked ICCVAM to conduct an Independent Scientific Peer Review of the UDP

ICCVAM convened the Acute Toxicity Working Group (ATWG) composed of knowledgeable individuals in ICCVAM agencies

November First ATWG Meeting

ATWG Charge:
- Review the Revised UDP submission for completeness
- Propose expert scientists for the Peer Review Panel
- Provide guidance to the UDP Technical Task Force to assemble adequate information for scientific peer review in accordance with ICCVAM Submission Guidelines
- Prepare evaluation questions to be addressed by the independent scientific Peer Review Panel
- Develop draft ICCVAM Test Recommendations based on Panel's evaluation

ICCVAM Peer Review of the UDP (Cont'd)

2000

February *Federal Register* Notice (Vol. 65, No. 34, 8385-8386)
- Requested nominations for Peer Review Panel
- Requested data and information regarding usefulness and limitations of UDP as a replacement for conventional LD50 test

March Peer Review Panel Finalized by ATWG
- Recommended 19 members with expertise in acute toxicity testing, biostatistics, alternative methods, pharmacology, and toxicokinetics
- Included members from industry, academia, and government from the US, UK, New Zealand and The Netherlands

April UDP Technical Task Force submitted Revised UDP to ICCVAM

June *Federal Register* Notice (Vol. 65, No. 106, 35109-35110)
- Announced availability of UDP review materials
- Requested public comment on materials
- Announced Peer Review Meeting information

All comments received in response to Federal Register notice were provided to the Panel for consideration

July 25, 2000 - UDP Peer Review Meeting

UDP Peer Review Panel Charge:
- Evaluate all of the available information in the Background Review Document (BRD) in accordance with published criteria for validation and acceptance of toxicological test methods (NIEHS, 1997).
- Prepare a written report that summarizes the extent to which each of these criteria have been addressed, and the usefulness and limitations of the UDP for determining the acute oral toxic potential of chemicals and products.

Focus of the Review for UDP Primary, Limit, and Supplemental Tests:
- Has the revised UDP been evaluated sufficiently and is its performance satisfactory to support its adoption as a substitute for the traditional LD50 test for acute oral toxicity (U.S. EPA Health Effects Guideline OPPTS 870.1100, 1996; OECD, 1987)?
- With respect to animal welfare, does the revised UDP adequately consider and incorporate where scientifically feasible, procedures that refine, reduce, and/or replace animal use?

UDP Panel Conclusions/Recommendations:
- 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 were: 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.
- 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.
- The UDP Supplemental Test for slope and CI was 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.
- 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 extent that the UDP provided for refinement. However, the OECD Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals used in Safety Evaluation, referenced in the revised UDP Guideline, provides an element of refinement.
- Numerous recommendations were made for the revision to the UDP Test Guideline.

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

In response to the Panel's conclusions and recommendations, the UDP Technical Task Force revised the UDP test method guideline as follows:
- Incorporated recommended Panel revisions 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 calculation 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 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 publicly available software was developed to mitigate complexity for the user and to facilitate correct performance of the UDP.

The UDP Technical Task Force also provided the following clarifications regarding animal welfare:
- The UDP guideline 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 further efficiencies in animal use.
- The UDP guideline provision that the initial starting dose should be below the LD50 will result in fewer animals receiving lethal doses, thereby providing further potential reduction in pain and distress.
- Adherence to the OECD Guidance Document on Humane Endpoints should provide additional reduction or minimization of pain and distress in animals used in this procedure.

ICCVAM Peer Review of the UDP (Cont'd)

2001

June UDP Technical Task Force completed revision of the UDP and the development of the UDP software program. Revised materials submitted to ICCVAM for follow-up UDP Peer Panel review.

June *Federal Register* Notice (Vol. 66, No. 121, 33550-33552)
- Announced availability of revised draft UDP test guideline
- Announced availability of a procedure to calculate the CI for the estimated LD50
- Announced availability of a software program for use in establishing test doses, determining when to stop the UDP test, and estimating the LD50 and the CI for the estimated LD50
- Requested Public comment on all available materials

All comments received in response to the *Federal Register* notice were provided to the Panel for consideration

July *Federal Register* Notice (Vol. 66, No. 133, 36294-36295)
- Announced August 21, 2001 UDP Peer Panel Review Teleconference information

ALL UDP DOCUMENTS AVAILABLE ON THE *ICCVAM/NICEATM* WEBSITE:

http://iccvam.niehs.nih.gov/

August 21, 2001 - UDP Peer Review Meeting

UDP Peer Review Panel Charge:
- Evaluate the extent to which the revised draft UDP test guideline (July 12, 2001) incorporates modifications in accordance with the recommendations of the July 25, 2000 Peer Review Panel meeting;
- Evaluate the appropriateness and adequacy of the proposed procedure for calculating a CI for the LD50; and
- Evaluate the adequacy and consistency of the software program for use in the revised draft UDP test guideline.

UDP Panel Conclusions/Recommendations:

Revised UDP Test Guideline

The Panel concluded that many of the recommended and requested changes had been appropriately considered and all members concurred with the current modifications. However, several previous recommendations appeared to have not been adequately addressed in the revised UDP Test Guideline, and the Panel recommended adding the following:
- 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.
- Additional guidance pertaining to the use of pre-start data (data available before the acute toxicity test is conducted) should be provided, which may be helpful in determining the starting dose level (e.g., using *in vitro* data to estimate starting doses).

CI Procedure
- Endorsed the proposed procedure for calculating the CI for the estimated LD50.
- 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.
- Noted that statistical techniques are evolving and recommended the future development of alternative approaches, such as nonparametric methods, be encouraged.

UDP Software Program
- 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.

Conclusion of ICCVAM Review of the UDP

2001

September UDP Technical Task Force revised the UDP test guideline in response to the Panel's recommendations.

October ICCVAM endorsed the revised UDP test guideline.
- In accordance with the ICCVAM Authorization Act of 2000 (P.L. 106-545), ICCVAM developed and adopted ICCVAM test recommendations for the UDP to be forwarded to Federal agencies for their consideration and appropriate action.

December The ICCVAM Final Report, "The Revised Up-and-Down Procedure: A Test Method for Determining the Acute Oral Toxicity of Chemicals," is published.

2002

February Federal Register Notice (Vol. 67, No. 26, pp. 5842-5844)
- Announced availability of Final Report
- Requested public comment

June ICCVAM requests Director of NIEHS to transmit UDP test recommendations through the Secretary, DHHS, to Federal agencies in accordance with P.L. 106-545.

July Director of NIEHS transmits ICCVAM recommendations through NIH to Secretary, DHHS.