DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Agency for Toxic Substances
and Disease Registry
Atlanta GA 30333

Parties / Orig

August 25, 2003

Kenneth Olden, Ph.D.
Director
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, North Carolina 27709

cc
Olden
Stokes
Wolfe
ICCVAM
ES 8/28

Dear Dr. Olden:

This is a follow-up to our correspondence of May 5, 2003 regarding testing recommendations for 1) *In Vitro* methods that can be used to estimate starting doses for acute oral toxicity studies, and 2) a revised test method (Up-and-Down Procedure, [UDP]) for determining acute oral toxicity, that were prepared by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and sent to the Agency for Toxic Substances and Disease Registry (ATSDR) pursuant to the ICCVAM Authorization Act of 2000. Specifically, ATSDR (and other agencies) is required to review the test recommendations and notify the ICCVAM in writing of their findings, including identification of relevant test methods for which the ICCVAM test recommendations may be added or substituted.

We have reviewed the "Report of the International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity" and the accompanying "Guidance Document on Using *In Vitro* Data to Estimate *In Vivo* starting Doses for Acute Oral Toxicity," and support the ICCVAM recommendations for additional research and development efforts that would further advance the usefulness of the *in vitro* methods. In particular, we look forward to reviewing the results of the ongoing collaborative validation study between the U.S. and the European Committee on the Validation of Alternative Methods (ECVAM) that would further corroborate the reliability and accuracy of these methods. As suggested in your letter, ATSDR will make information about this approach available as one of the tools that can be used to select an appropriate starting dose for acute toxicity tests.

The revised UDP was proposed as an alternative to the existing LD_{50} and brought to the ICCVAM to assess its validation status in 1999. We agree with the conclusions of the scientific peer review panel and the recommendations of the ICCVAM that the revised UDP does perform appropriately and will result in a reduction and refinement in animal usage compared to the conventional LD_{50} test (about 50 animals versus 7 animals). ATSDR is not a regulatory agency, but does have a mandate to assure the initiation of a toxicological program of research to fill research needs for the most hazardous substances found at waste sites, and to date we have identified 263 priority data needs for 60 substances. It is important to note, however, that ATSDR does not require LD_{50} data for this program, but that such data are

reported in ATSDR toxicological profiles and have possible relevance in emergency situations. Thus, we support the use of the scientifically valid revised UDP on grounds of reduction and refinement of the use of animals in research, but note that there are no ATSDR toxicological tests or test method recommendations that will be added or substituted as a result. Instead, the availability of the revised UDP as a valid test method to assess acute oral toxicity will be shared with ATSDR program staff and with various public and private sector partners who work closely with ATSDR in accomplishing its research and service missions.

In closing, we strongly support the goal of the ICCVAM to coordinate the validation of proposed new test methods throughout the federal and scientific communities. We find ourselves in a solid position to defend new test methods, such as the revised UDP, when we are assured that the methodology meets the validation criteria established by the ICCVAM and has passed the rigors of scientific peer review. We look forward with keen interest to your report on the acceptance status of the revised UDP method by the regulatory agencies. Please share this information as it becomes available.

Sincerely,

Henry Falk, M.D., M.P.H.
Assistant Administrator
Rear Admiral, U.S.P.H.S. (Retired)

cc:
Bill Cibulas, Division of Toxicology, ATSDR

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U.S. CONSUMER PRODUCT SAFETY COMMISSION
WASHINGTON, DC 20207

Todd Stevenson
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Dr. Kenneth Olden
Director
National Institutes of Health
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, N.C. 27709

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Dear Dr. Olden:

We are pleased to inform you, as required by the ICCVAM Authorization Act, that the US Consumer Product Safety Commission (Commission) voted unanimously on August 28, 2003 to approve the recommendations of ICCVAM that for the purpose of classification and labeling, the Revised Up-and-Down Procedure (UDP) be used instead of the conventional LD50 test to determine the acute oral toxicity hazard of chemicals. Further, the Commission also approved the recommendation to encourage the use of certain *in vitro* tests for determining the starting dose for acute systemic toxicity testing. The UDP can be used instead of the conventional LD50 for the purpose of classification for labeling under the Federal Hazardous Substances Act. (FHSA) (15 U.S.C. 1261-1278) Both the FHSA at 2(h)(2) and the supplemental definitions state that available data on human experience that indicate results different from those obtained in animals in the defined dosages or concentrations will always take precedence. This is true for both the conventional LD50 and the UDP. The briefing package sent to the Commission can be found on the Commission website (www.cpsc.gov) in the Library (FOIA) section at http://www.cpsc.gov/library/foia/foia03/brief/testing.pdf.

Sincerely,

Todd Stevenson

Todd Stevenson

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

DEC 2 2003

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Kenneth Olden, Ph.D.
Director
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, NC 27709

Dear Dr. Olden:

Thank you for transmitting in your letter of March 21 recommendations from the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) on test methods for acute oral systemic toxicity. Specifically, ICCVAM seeks to know if the Environmental Protection Agency (EPA) will accept use of the up-and-down procedure for determining acute oral toxicity hazard and the use of *in vitro* cytotoxicity testing as one of the tools for estimating a starting dose for conduct of *in vivo* assessments of acute oral toxicity. Acknowledgment of receipt of your letter by EPA was sent to you on May 2, 2003. The following is EPA's response regarding the use of these alternative methods in the Agency's testing programs for industrial chemicals and pesticides.

HISTORY

In 1987 the Organization for Economic Cooperation and Development (OECD) published the traditional LD50 test guideline for acute oral toxicity. A preliminary form of the up-and-down procedure (UDP) was accepted by OECD in 1997 for use in addition to the traditional test. Subsequently, OECD determined that further work was necessary on the UDP and other approved acute oral tests in order for them to be used as replacements for the traditional acute test. Accordingly, a team of regulatory and industry scientists in the United States revised the UDP guideline. EPA was instrumental in having ICCVAM review the revised UDP and this review was published in November 2001. The revised UDP and other alternatives were formally adopted by OECD in 2001. The EPA Federal Insecticide, Fungicide and Rodenticide Act Scientific Advisory Panel (FIFRA SAP) met in December 2001 to discuss the applicability of the UDP to EPA testing programs. The Panel agreed that the method generates LD50 point estimates that are usable for hazard classification purposes. The confidence limits on the point estimates can also be useful, although in some cases they may be very broad.

Certain studies have suggested that *in vitro* cytotoxicity methods may be helpful for predicting *in vivo* acute toxicity. EPA co-sponsored an international workshop conducted by ICCVAM in October 2000 on the status of such *in vitro* methods to predict acute systemic toxicity. There was consensus among workshop participants that *in vitro* methods were not sufficiently developed to be able to replace acute oral animal test methods. The ICCVAM workshop report recommends that cytotoxicity measurements in either of two cell systems, BALB/C 3T3 mouse fibroblasts or normal human keratinocytes, can be used as part of the evidence for estimating a starting dose prior to conducting *in vivo* acute oral studies. Further work on the validation of such methods is proceeding through ICCVAM.

EPA has incorporated the revised UDP in its guidelines (December 2002) for use in testing pesticides and industrial chemicals, including chemicals in the EPA High Production Volume Challenge Program. This test guideline encourages the use of the cytotoxicity *in vitro* methods as a supplemental component to the *in vivo* studies to estimate starting dose. In February 2002, EPA co-sponsored an ICCVAM/ILSI (International Life Sciences Institute) training workshop to facilitate implementation of *in vitro* cytotoxicity testing as well as the UDP and other alternative tests for acute oral toxicity.

UP-AND-DOWN PROCEDURE

EPA recognizes that there are characteristics of the UDP that lend support for its use in regulatory testing although there are some shortcomings to its application as well.

Strengths

1. The UDP is the only alternative test approved by OECD that generates a point estimate of the LD50; the other two methods only generate an LD50 within a dose range.
2. The method generates usable LD50 estimates for hazard classification purposes.
3. It is unique among the methods approved by OECD in generating LD50 confidence limits.
4. Compared with the previously employed traditional LD50 test, the UDP leads to reduction in animal use and may modestly help to refine the test (e.g. reduce animal distress) by commencing dosing at levels below the anticipated LD50. Moreover, use of the OECD guideline for humane endpoints in conducting the UDP should reduce the overall suffering of the animals.
5. Default use of animals of one sex (generally female) will suffice for most purposes.

Weaknesses

1. Optimum performance of the UDP depends on availability of good prior estimates of slope and LD50 for the chemical as well as knowledge of whether metabolic is

necessary for toxic effects.

2. Not all UDP tests will provide point estimates of the LD50; when no partial kills are observed, the LD50 will be estimated within a range.
3. Due to the small number of animals tested, confidence limits on LD50 estimates may be very wide. Because the profile likelihood method used to estimate confidence limits is approximate, coverage of the confidence interval does not always correspond to its nominal value and falls below 95% for populations with shallow slopes.
4. Neither the UDP nor other acute oral toxicity alternatives accepted by OECD generate estimates of the dose response slope. This is a shortcoming in cases when acute toxicity risk assessments are necessary for human health or ecological considerations.
5. Since single animals are tested sequentially in the UDP, care must be taken to ensure that test animals remain within a usable age and weight range. These elements add to the length, complexity and cost of the test. Also, the method is not usable in those rare cases where chemicals lead to delayed death.

Recognizing the strengths and weaknesses of the UDP, EPA recommends use of the UDP to evaluate acute oral toxicity of industrial chemicals, pesticides and chemical mixtures. Steps have been taken to inform the public of this determination (Federal Register 67FR77064-77065, December 16, 2002; www.epa.gov/ckhemrtk/toxprtcl.htm).

In vitro CYTOTOXICITY METHODS FOR ACUTE ORAL TOXICITY

ICCVAM has recommended the use of *in vitro* cytotoxicity as part of the evidence for estimating a starting dose for conduct of acute oral studies. There are arguments for and against using these *in vitro* measures.

Strengths

1. There appears to be a linear log-log relationship between *in vitro* cytotoxicity (IC50) and *in vivo* lethality (LD50); the correlation is best for chemicals with moderate to low acute toxicity.
2. *In vivo* acute oral toxicity test alternatives are sensitive to the starting dose. The *in vitro* cytotoxicity level can be used as part of the weight-of-evidence for estimating a starting dose for *in vivo* acute oral studies and, thus, on average, decrease the number of animals committed to test.

Weaknesses

1. No *in vitro* system has been shown to be a valid measure of *in vivo* acute oral toxicity.
2. Cultured cell cytotoxicity is not expected to be accurate for predicting *in vivo* acute toxicity for chemicals that need to be metabolized to an active form or for agents that act through binding to cell-specific receptors.
3. The existing evaluations of *in vitro* systems use the RTECS *in vivo* LD50, which has an inherent bias of being the lowest reported toxicity value.
4. Dispersion data on the regression of *in vivo* LD50 on *in vitro* cytotoxicity (IC50) indicates that about 25% of test materials are outside of a prediction interval (constructed as ± log 5).

EPA encourages test sponsors to explore the potential benefit from using *in vitro* cytotoxicity as a part of the weight-of-evidence, including consideration of structure-activity relationships, recognition of physicochemical properties and other considerations, for estimating a starting dose for animal acute oral toxicity studies (www.epa.gov/chemrtk/toxprtcl.htm). EPA encourages receipt of such screening as part of any report submitted to the Agency.

If you have any questions, please contact Dr. Karen Hamernik at the Agency. She can be reached at 202-564-8430.

Sincerely,

Joseph J. Merenda, Jr.
Director
Office of Science Coordination and Policy

cc: Jim Jones
Charles Auer
William Stokes

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

September 9, 2003

Kenneth Olden, Ph.D.
Director
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, North Carolina 27709

cc
Olden
Stokes
Wolfe
ICCVAM
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Dear Dr. Olden:

Thank you for the opportunity to consider the recommendations of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) for two alternative toxicological test methods.

The first test recommendation, *in vitro* methods for assessing acute systemic toxicity, addresses the potential use of *in vitro* tests in determining the starting dose of agents in acute systemic toxicity tests. The recommendation is based upon (a) the *Report of the International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity*, NIH Publication No. 01-4499, and (b) the *Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity*, NIH Publication No. 01-4500.

The second test recommendation, a revised Up-and-Down Procedure (UDP) for determining acute oral toxicity, addresses the use of the UDP as a replacement for the conventional LD50 test to determine the acute oral toxicity hazard of chemicals. That recommendation is based on the report, *The Revised Up-and Down Procedure: A Test Method for Determining the Acute Oral Toxicity of Chemicals; Results of an Independent Peer Review Evaluation Organized by ICCVAM and NICEATM*, NIH Publication No. 02-4501.

I am pleased to provide you with this response from the Food and Drug Administration (FDA) to the first test recommendations developed and transmitted to ICCVAM agencies pursuant to Section 3(e)(4) of the ICCVAM Authorization Act of 2000 (P. L. 106-545).

FDA views the test methods for which ICCVAM recommendations were developed to have been appropriately validated according to ICCVAM procedures and considers the methods technically acceptable. The guidance document, NIH Publication No. 01-4500, describes how to use *in vitro* methods to estimate starting doses for acute oral toxicity animal tests. FDA agrees that such *in vitro* methods could help predict acute oral toxicity in animals and humans and could reduce the number of animals used for this testing. FDA supports activities (e.g., research, development, validation) that could advance the use of such *in vitro* predictive methods and will communicate that to its research and regulatory units. FDA also concurs with the conclusions of the Peer Review Panel (NIH Publication No. 02-4501) on the utility of the UPD as a substitute for the traditional LD50 test and acknowledges the potential reduction in animal usage.

UPD as a substitute for the traditional LD50 test and acknowledges the potential reduction in animal usage.

In meeting its regulatory mandate to promote and protect public health, FDA generally seeks toxicity information in order to assess a complete toxicological profile and determine a no observed effect level rather than lethality. Thus, FDA does not issue guidance documents for industry that specifically solicit data from LD50 or lethality tests. This is exemplified by the enclosed *Federal Register* notice of October 11, 1988, 53 FR 39650. Although FDA does not request acute lethality data or LD50 data, it recognizes that these ICCVAM-recommended alternative methods may have regulatory utility for those agencies that have a need for such data. The use of such methods should be encouraged to minimize the numbers of animals used and the extent of lethality.

FDA is fully committed to ICCVAM and the ICCVAM process, as shown by the energetic participation of representatives from each of FDA's research and regulatory centers and offices. FDA's dedicated ICCVAM members include Dr. Leonard Schechtman of the National Center for Toxicological Research (NCTR), Chair of ICCVAM; Dr. William Allaben (NCTR), Dr. Atin Datta (Office of Regulatory Affairs), Dr. Suzanne Fitzpatrick (Office of the Commissioner), Dr. David Hattan (Center for Food Safety and Applied Nutrition), Dr. Abigail Jacobs (Center for Drug Evaluation and Research), Dr. Devaraya Jagannath (Center for Veterinary Medicine), Dr. Raju Kammula of the Center for Devices and Radiological Health (CDRH), Dr. Richard McFarland (Center for Biologics Evaluation and Research), Dr. Martha Moore (NCTR), and Dr. Melvin Stratmeyer (CDRH).

The role played by ICCVAM is a unique one. FDA looks forward to its continuing involvement in important and far-reaching ICCVAM activities.

Sincerely,

Mark B. McClellan, M.D., Ph.D.
Commissioner of Food and Drugs

Enclosure

53 FR 39650

2 of 2 DOCUMENTS

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

AGENCY: Food and Drug Administration.

[Docket No. 86P-0224]

53 FR 39650

October 11, 1988

LD 50 Test Policy
ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is issuing this general statement of policy concerning the use of the "classical" LD 50 test by the agency. That test is not an FDA-required procedure for determining safety, and its use is not part of agency testing policy. This general statement of policy is being issued in response to a citizen petition (86P-0224/CP) submitted on May 15, 1986, by the American Society for the Prevention of Cruelty to Animals and other animal welfare organizations requesting FDA to issue a regulation or regulations concerning the subjects addressed by this policy and by other agency pronouncements on the "classical" LD 50 test.

ADDRESS: Comments on this general statement of policy should be submitted to the Dockets Management Branch (HFA-305), Food and Drug Administration, Rm. 4-62, 5600 Fishers Lane, Rockville, MD 20857.
FOR FURTHER INFORMATION CONTACT: Richard P. Bradbury, Center for Veterinary Medicine (HFV-4), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, 301-443-4557.

TEXT: SUPPLEMENTARY INFORMATION: As a part of FDA's responsibility for administration of the Federal Food, Drug, and Cosmetic Act (the act), the agency is required to evaluate safety data submitted in support of applications for research or marketing permits for products regulated by FDA, including new drugs, biological products, new animal drugs, food additives, color additives, and certain medical devices intended for human use. Because it is unreasonable that people be exposed to substances whose safety has not been established, initial safety studies, by necessity, are conducted on animals.

Safety testing has evolved over several decades. Some useful tests have been modified and retained; other safety tests have become recognized as being inappropriate or unnecessary. An example of the latter category is the "classical" LD 50 test. The "classical" LD 50 test requires large numbers of animals (usually rodents), ranging from 60 to more than 120 animals per test substance. Large numbers of animals are needed to attain a statistically precise median number with 95 percent confidence limits. Normally, the "classical" test uses six dose levels with five animals per sex per dose level. Following the receipt of a dose, all animals are observed over a period of 14 days for signs of toxicity and other effects.

The "classical" LD 50 test became generally accepted during the 1930's for standardization of toxic plant and biological extracts and other chemicals. Subsequently, FDA incorporated it into its acute toxicology testing requirement for new compounds. When the "classical" LD 50 test became generally recognized as unnecessarily precise, the agency ceased to require such data. In 1985, the agency revoked its only regulatory requirement for that test (See the Federal Register of May 10, 1985 *(50 FR 19675))*, eliminating the requirement of the "classical" LD 50 test for batch comparison of three antitumor antibiotics and providing for nonbiological alternative means of assessing batches of these antibiotics.

For several years, FDA has initiated or participated in activities to clarify that the "classical" LD 50 test is not an FDA-required procedure for determining the safety of products regulated by the agency, and that its use is not part of agency testing policy. In 1983, the agency sponsored an Acute Studies Workshop (Ref. 1), which was open to the public, to discuss agency testing requirements including the uses of and the rationale for LD 50 tests in acute toxicity studies. The discussions at the workshop revealed that although FDA regulations require acute toxicity data for new compounds, they do not require that such data include the results of the "classical" LD 50 test.

In January 1984, the agency established a Steering Committee on Animal Welfare Issues to determine, among other things, whether FDA was indirectly perpetuating the use of the "classical" LD 50 test. The Committee's Final Report to the Commissioner, Food and Drug Administration (Ref. 2) discusses this issue in great detail. The report concludes that, in general, the agency does not directly or indirectly perpetuate the use of LD 50 determinations by statistically precise methods. The report also concludes that the "classical" LD 50 test was not required by FDA in quality control procedures (with the exception noted above), and that its use is not encouraged in agency testing policy for assessing the acute toxicity of new chemicals.

On May 15, 1986, in a citizen petition (86P-0224/CP) submitted by the American Society for the Prevention of Cruelty to Animals and 20 cosponsors, petitioners requested that FDA issue regulations to:

1. Require all FDA centers to promptly complete revisions of guideline test protocols for acute toxicity, making clear that the "classical" LD 50 test is not an FDA-required procedure for determining safety, and that data gathered from the "classical"LD 50 test will not be used or considered by FDA for determining safety of compounds, drugs, or products, after 1 year from the date of promulgation of the regulation or regulations;

2. Inform all persons submitting acute toxicity data to FDA that the "classical" LD 50 test is no longer considered scientifically necessary, wastes animal life, and is not required; and that the "classical" LD 50 test will not be used by FDA for determining safety after 1 year from the date of promulgation of the regulation or regulations;

3. Describe and define acceptable alternative testing methods to replace the "classical" LD 50 ; and

4. Prohibit FDA from using or conducting the "classical" LD 50 test within its own centers including, but not limited to, the National Center for Toxicological Research.

In a letter dated November 12, 1986 (Ref. 4), the agency denied the petition on the grounds that regulations are neither appropriate nor necessary to grant the relief requested. The agency denied petitioners' first and second requests insofar as they sought to bar FDA from accepting or reviewing data from the "classical" LD 50 test. Under the act, the agency may not refuse to accept or review data, including acute toxicity data from the "classical" LD 50 test, if they are relevant to a decision FDA must make on the safety of a regulated article. For example, the agency could not refuse to accept or review acute toxicity data showing a significant histopathological change in an internal organ resulting from the administration of one nonlethal dose of a noncorrosive compound. Thus, FDA cannot revise guideline test protocols or regulations to state that it will never use or consider any "classical" LD 50 data in making safety determinations. The agency stated, however, that it would publish in the Federal Register a notice explaining that the "classical" LD 50 test is not a required procedure for use in safety determinations within the agency. FDA further stated that it had been and would be imlementing most of the requests by policy statements, guideline modifications, and other publications, and in discussions with representatives of regulated industry, rather than by regulations.

The scientific community agrees that the "classical" LD 50 test is not necessary for determining acute toxicity. In agreement, FDA has adopted the policy that the "classical" LD 50 test is not a required toxicity study. The agency supports efforts to eliminate continued conduct of the "classifical" LD 50 test and to reduce the numbers of animals used in acute toxicity testing without sacrificing information necessary in the interest of human safety.

This policy will be further emphasized by the agency through its inclusion in the FDA Staff Manual Guide, in agency safety testing guidelines, in agency publications, and through discussions by agency officials and personnel with representatives of the regulated industry, as appropriate.

References

The following information has been placed on display in the Dockets Management Branch (HFA-305), Food and Drug Administration, Rm. 4-62, 5600 Fishers Lane, Rockville, MD 20857, and may be seen by interested persons between 9 a.m. and 4 p.m. Monday through Friday.

1. "Report on Acute Studies Workshop Sponsored by the Food and Drug Administration," November 9, 1983.

2. "Final Report to the Commissioner, Food and Drug Administration, Agency Steering Committee on Animal Welfare Issues," August 15, 1984.

3. Citizen Petition 86P-0224.

4. Letter from John M. Taylor, FDA, to Barbara K. Pequet, American Society for the Prevention of Cruelty to Animals, November 12, 1986.

Interested persons may submit to the Dockets Management Branch (address above) written comments regarding this general statement of policy. Two copies of any comments are to be submitted, except that individuals may submit one copy. Comments are to be identified with the docket number found in brackets in the heading of this document. Received comments may be seen in the Dockets Management Branch between 9 a.m. and 4 p.m., Monday through Friday.

Dated: October 5, 1988.
John M. Taylor,

Associate Commissioner for Regulatory Affairs.
[FR Doc. 88-23504 Filed 10-6-88; 4:11 pm]

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DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
National Institutes of Health
National Cancer Institute
Bethesda, Maryland 20892

August 25, 2003

Kenneth Olden, Ph.D.
Director
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, NC 27709

Dear Dr. Olden:

I am responding on behalf of the National Cancer Institute (NCI) to the test recommendations of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). The ICCVAM Authorization Act (P.L. 106-545) Section 5 APPLICATION (a) states that this Act does not apply to "...research related to the causes, diagnosis, treatment, control or prevention of physical or mental diseases or impairments of human or animals." Since the mission of the NCI is biomedical research related to the causes, diagnosis, treatment, control or prevention of cancer, AIDS and other diseases in humans, the ICCVAM recommendations do not apply ordinarily to the research activities.

The NCI rarely performs any lethality studies in animals and does not usually conduct acute oral toxicity studies as defined by ICCVAM. However, in the spirit of ICCVAM, NCI researchers will use the revised Up-and-Down Procedure (UDP) whenever possible if it does not compromise the scientific validity of the research and the in vitro method for estimating starting dose when appropriate. UDP is currently in use by NCI researchers.

Consideration of the applicable ICCVAM recommendations in all animal-related activities conducted or supported by NCI is consistent with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and Section 404c of the NIH Revitalization Act of 1993 (P.L. 103-43).

NCI remains committed to animal welfare efforts consistent with sound research design.

Sincerely,

Andrew C. von Eschenbach, M.D.
Director
National Cancer Institute

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

National Institutes of Health
National Institute of
Environmental Health Sciences
P. O. Box 12233
Research Triangle Park, NC 27709

Date: September 9, 2003

To: The Record

From: Director, NIEHS

Subject: NIEHS Response to Test Recommendations on Acute Toxicity from the Interagency Coordinating Committee on the Validation of Alternative Methods

On March 21, 2003, at the request of the Secretary of the Department of Health and Human Services, I forwarded toxicological test recommendations from the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) to 14 Federal Agencies for their consideration. The recommendations were developed and transmitted pursuant to Section 3(e)(4) of the ICCVAM Authorization Act of 2000 (P. L. 106-545). Pursuant to Sections 4(a) and 4(d) of the ICCVAM Authorization Act, agencies are required to review ICCVAM test recommendations and notify ICCVAM in writing of their findings, including identification of relevant test methods for which the ICCVAM test recommendations may be added or substituted. This memorandum provides the NIEHS response to the ICCVAM test recommendations.

NIEHS has reviewed the ICCVAM test recommendations that were provided for 1) the revised Up-and-Down Procedure (UDP) and 2) *in vitro* methods for estimating the starting dose for acute oral toxicity studies. NIEHS agrees that the UDP significantly reduces the number of animals required to estimate the median oral lethal dose of chemicals, and that the *in vitro* methods can be helpful in further reducing the number of animals required for such studies. NIEHS has determined that it does not currently use or specify any test methods for which the test recommendations may be added or substituted. Furthermore, NIEHS is not a regulatory agency, and therefore does not promulgate regulatory testing requirements for which the recommendations may be applicable. NIEHS does conduct toxicity testing as part of its National Toxicology Program activities; however, acute oral toxicity testing to estimate median lethal doses is not normally performed. If, for some unforeseen reason, such data are required in the future, the NIEHS intends to follow the recommendations of the ICCVAM on this matter and use the UDP and the *in vitro* methods where appropriate.

NIEHS scientists and the NIEHS Institutional Animal Care and Use Committee (IACUC) have been informed about the availability of these two alternative test methods and advised that they should be considered when planning and reviewing animal studies involving acute systemic toxicity in order to minimize animal use and to reduce pain and distress. The IACUC has also been asked to ensure that these alternative methods are considered in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and applicable USDA Animal Welfare Act regulations.

NIEHS has also reviewed the ICCVAM recommendations for research, development, and validation efforts that could advance the use of *in vitro* methods for assessing acute systemic toxicity. NIEHS through its National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) has initiated a validation study to standardize and evaluate the usefulness of two *in vitro* basal cytotoxicity assays for predicting starting doses for *in vivo* acute oral toxicity studies and for predicting lethal concentrations in humans. Expert scientists participating in the ICCVAM International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity recommended the study. The project is co-funded by the U.S. Environmental Protection Agency and is being conducted in collaboration with the European Commission's European Center for the Validation of Alternative Methods (ECVAM).

NIEHS remains committed to the development, validation, and regulatory acceptance of scientifically sound alternative testing methods that will provide improved protection of human and animal health and the environment, and that will provide for improved animal welfare.

Ken Olden

Kenneth Olden, Ph.D., Sc.D.

cc:
Dr. Leonard Schechtman
Dr. William Stokes
Dr. Elias Zerhouni

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20892

www.nih.gov

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Kenneth Olden, Ph.D.
Director
National Institute of
Environmental Health Sciences
P.O. Box 12233, B1-02
Research Triangle Park, North Carolina 27709

Dear Dr. Olden:

Thank you for your March 21 letter forwarding the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) test recommendations, sent on behalf of the Secretary of the Department of Health and Human Services. Pursuant to Sections 4(a) and 4(d) of the ICCVAM Authorization Act (P.L. 106-545), I am responding on behalf of the National Institutes of Health (NIH), with the exception of the National Library of Medicine (NLM), National Cancer Institute (NCI), and National Institute of Environmental Health Sciences (NIEHS). I understand that NLM, NCI, and NIEHS will respond directly to your letter regarding the applicability of ICCVAM Test recommendations in their respective programs.

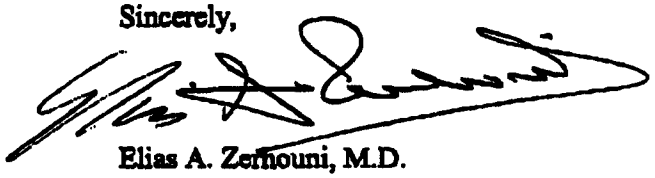
Section 5(a) of the ICCVAM Authorization Act states that this Act does not apply to "...research related to the causes, diagnosis, treatment, control or prevention of physical or mental diseases or impairments of humans or animals." Since the mission of the NIH is biomedical research related to the causes, diagnosis, treatment control or prevention of physical or mental diseases or impairments of humans, the ICCVAM recommendations do not apply ordinarily to the research activities conducted by the Institutes and Centers of the NIH.

The NIH does support researchers or contractors conducting toxicological evaluations needed for safety assessment and preclinical development of experimental therapeutics or biological compounds (e.g., imaging agents). These researchers conduct preliminary dose-range finding toxicity studies by a variety of methods. These methods are selected on a case-by-case basis based on chemical structure, availability of previous data (e.g,. animal efficacy data), and other information. Wherever scientifically appropriate, ICCVAM-recommended methods are recognized as valuable in predicting dose levels before moving into full toxicology assays required by the Food and Drug Administration.

Consideration of the applicable ICCVAM recommendations in all animal-related activities conducted or supported by NIH is consistent with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and Section 404C of the NIH Revitalization Act of 1993 (P.L. 103-43).

In closing, I want to acknowledge the contributions of NIH staff and NIH-supported researchers and contractors to ICCVAM's efforts. Numerous members of the NIH community participated in the development and validation of these first ICCVAM recommendations. NIH remains committed to animal welfare efforts consistent with sound research design.

Sincerely,



Elias A. Zerhouni, M.D.
Director

FAX FAX FAX

NATIONAL INSTITUTES OF HEALTH
Office of the Director

EXECUTIVE SECRETARIAT
Building 1, Room B1-56
Fax #: (301) 496 - 8276

Date: Sept 9, 2003

Number of pages: 3
(including this cover sheet)

From: **Karen Mason**
NIH/OD/Executive Secretariat
Phone: (301) 496 -1461

To: **Donna Shields, NIEHS**

Notes or special instructions:

Donna,

The original is in the mail. Please remember to send me a copy of NIEHS's ICCVAM response. I need it for my file. Thanks!

Karen M

Karen Mason

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Centers for Disease Control
and Prevention (CDC)
National Institute for Occupational
Safety and Health
Pittsburgh Research Laboratory
P.O. Box 18070
Pittsburgh, PA 15236-0070

September 30, 2003

Kenneth Olden, Ph.D.
Director
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, North Carolina 27709

Dear Dr. Olden:

The National Institute for Occupational Safety and Health (NIOSH) has reviewed the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) toxicological test recommendations on *in vitro* methods for acute systemic toxicity and the revised Up-and-Down Procedure (UDP) per your letter dated March 21, 2003. NIOSH is not an agency which "*carries out a program that requires or recommends acute or chronic toxicological testing*" as stipulated under Section 4(a) of Public Law 106-545, and as such is not required to report. However, NIOSH is a member agency of ICCVAM, and strongly supports the adoption of validated alternative tests. NIOSH concurs with the ICCVAM test method evaluation that both of these test methods are valid; the *in vitro* method can be used to select an appropriate starting dose for acute toxicity tests, and the UDP can be used instead of the conventional LD50.

NIOSH does not currently conduct acute oral toxicity testing, and thus the test methods are not directly applicable to NIOSH programs. However, we will notify all NIOSH personnel of the availability of the new test methods via agency-wide intranet notices and agency news letters. NIOSH's Health Effects Laboratory Division does conduct toxicity testing and may apply information provided by tests that have been acted on by ICCVAM.

In addition, NIOSH will post a notice of concurrence with these ICCVAM test recommendations on our internet Web site so that outside agencies or institutions that contribute data or results considered by NIOSH in evaluations of hazards or risk assessments will be notified of NIOSH's endorsement of these two test methods.

Thank you for providing these first two ICCVAM toxicological test recommendations for our review.

Sincerely yours,

John Howard

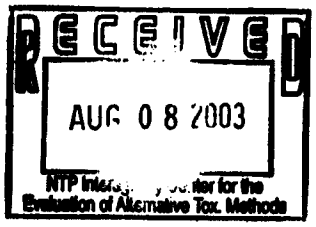
John Howard, M.D.
Director

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
National Library of Medicine
Bethesda MD 20894

August 8, 2003



Kenneth Olden, Ph.D.
Director
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, North Carolina 27709

Dear Ken:

The National Library of Medicine (NLM) has reviewed the toxicological test recommendations from the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), which you sent to us on March 21, 2003.

Pursuant to the ICCVAM Authorization Act of 2000 (Public Law 106-545), the NLM is responding to the ICCVAM recommendation that the Up-and-Down procedure be used in place of the conventional LD50 procedure to reduce and refine animal use. NLM has no regulatory or guidance responsibilities directly applicable to the recommended testing methods. NLM, however, will facilitate access to the validated methods and published test results through its databases.

Best wishes.

Yours truly,

Donald A.B. Lindberg, M.D.
Director

U.S. Department of Labor

Assistant Secretary for
Occupational Safety and Health
Washington, D.C. 20210

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Kenneth Olden, Ph.D.
Director
National Institute of
Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, N.C. 27709

Dear Dr. Olden:

Thank you for your letter of March 21 to the Occupational Safety and Health Administration (OSHA) in which you forwarded toxicological recommendations from the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) for our consideration. Please accept my apologies for the delay in response.

In your letter, you request that we review the ICCVAM test recommendations on the "Up-and-Down Procedure (UDP)" for acute oral toxicity testing and identify relevant test methods at OSHA for which the ICCVAM test recommendations may be added or substituted. We have reviewed the method and agree with you that this method may be a useful tool in toxicity testing and will likely reduce and refine animal use. As you may know, OSHA does not require or enforce toxicity testing as a part of its activities. Therefore, at this time we have no relevant test methods for which the ICCVAM test recommendations may be added or substituted. However, OSHA does use the results of toxicity testing conducted by others in a variety of ways, and we appreciate the advances in test methods in the ICCVAM recommendations.

Thank you for your valuable work in this field. We look forward to continued participation on the ICCVAM.

Sincerely,

John L. Henshaw

United States Department of Agriculture

Marketing and Regulatory Programs

Animal and Plant Health Inspection Service

Washington, DC 20250

SEP 22 2003

Dr. Kenneth Olden
Director, National Institute
of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, NC 27709

Dear Dr. Olden:

Thank you again for your letter of March 21, 2003, to the U.S. Department of Agriculture (USDA) concerning toxicological test recommendations forwarded from the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). Our Agency received this letter April 7, 2003, and provided a preliminary response on May 2, 2003.

The test recommendations on *in vitro* methods for predicting acute systemic toxicity were distributed to, and reviewed by, scientists at our Center for Veterinary Biologics (CVB). The CVB regulates veterinary biologics (vaccines, bacterins, antisera, diagnostic kits, and other products of biological origin) to ensure they are pure, safe, potent, and effective. The CVB is the only regulatory unit within USDA that requires animal testing. They determined these test methods:

1. A cytotoxicity assay procedure used to select an appropriate starting dose for testing the acute oral lethality potential of chemicals in humans and animals, and

2. the revised Up-and-Down Procedure, a substitute for an existing LD_{50} test for assessing the acute oral toxicity of chemicals, — do not apply to the safety testing done under the mandates of the Virus-Serum-Toxin Act for veterinary biologicals. As noted by the absence any USDA regulations listed in Appendix F of the National Institutes of Health (NIH) Publication No. 01-4499, *Report of the International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity* and Appendix Q of NIH Publication No. 02-4501, *The Revised Up-and-Down Procedure: A Test Method for Determining the Acute Oral Toxicity of Chemicals*, our Agency does not recommend or require this type of testing.

We appreciate the effort put forth by ICCVAM to generate these recommendations. Links to these test methods have been posted on the website of the Animal Welfare Information Center (National Agricultural Library, Agricultural Research Service) at http://www.nal.usda.gov/awic/alternatives/alternat.htm# as a resource for investigators considering alternatives to painful or distressful procedures in animals.

We encourage the development and use of methods that reduce, refine, or replace animal testing while ensuring the scientifically valid results necessary for regulatory testing requirements. We look forward to receiving more such recommendations.

Sincerely,

Bobby R. Acord
Administrator

cc:
Rick Hill, VS/CVB, 510 S. 17th Street, Suite 104, Ames, IA 50010

OFFICE OF THE DIRECTOR OF
DEFENSE RESEARCH AND ENGINEERING
3040 DEFENSE PENTAGON
WASHINGTON, D.C. 20301-3040

SEP 30 2003

Dr. Kenneth Olden
Director
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, NC 27709

Dear Dr. Olden:

I am replying to your letter dated March 21, 2003 requesting the Department of Defense's (DoD) review of toxicological test recommendations from the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). The Department endorses the ICCVAM recommendations and will promote the use of these acute oral toxicity test methods that provide alternatives to animal use in research.

By endorsing the ICCVAM recommendations, DoD agrees with ICCVAM's determinations that the following two test methods are scientifically valid for their intended use: (1) a toxicological test method, "The Revised Up-and-Down Procedure: A Test Method for Determining the Acute Oral Toxicity of Chemicals," and (2) in vitro methods to predict acute oral toxicity. When these test methods are appropriate to support the research goals conducted or supported by the Department, they will be accepted.

The Department will use a variety of mechanisms to promote and encourage the use of these test methods. Education of Department scientists and veterinarians will be accomplished through activities such as newsletters and training sessions for both Institutional Animal Care and Use Committee (IACUC) members and laboratory personnel. Extramural scientists supporting defense research will be informed of these alternative test methods through their interactions with DoD personnel and IACUCs. In addition the Department will also publish acceptability of these methods on the DoD web site for animal use (www.dtic.mil/biosys/org/au.html) and in the DoD Animal Care and Use Report. The report is an annual summary of DoD animal use in research and highlights the Department's efforts to promote and implement animal use alternatives (the reports can be found on the DoD web site for animal use). The DoD takes very seriously our responsibility to develop and encourage methods that replace, reduce, and refine the use of animals in research.

I appreciate your leadership role in the federal government's effort to identify and validate alternatives to using animals in research. The ICCVAM has been instrumental in facilitating federal-wide endorsement of alternatives to animal use.

Sincerely,

Charles J. Holland
Deputy Under Secretary of Defense
(Science and Technology)

From: Frazier, Marvin
Sent: Wednesday, September 24, 2003 5:20 PM
To: McCarley, Debbie (NIH/NIEHS)
Subject: RE: DOE Response to the ICCVAM Test Recommendations

Kenneth Olden, Ph.D.
Director
National Institute of Environmental Health Services
P. O. Box 12233
Research Triangle Park, North Carolina 27709

Dear Ken:

Thank you for your letter on March 21and the enclosed Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) test recommendation documents (In Vitro Methods for Assessing Acute Systemic Toxicity and Revised Up- and Down Procedure for Determining Acute Oral Toxicity of Chemicals). Pursuant to the ICCVAM Authorization Act of 2000 (Public Law 106-545), the Department of Energy (DOE) is responding tothe ICCVAM test recommendations in developing test alternatives to achieve the goals of 3Rs (Replacement, Reduction, and Refinement). These documents are helpful in formulating DOE response.

As you know, DOE has no regulatory or guidance responsibilities directly applicable to the ICCVAM test recommendations. However, DOE will continue to support the goal of ICCVAM and disseminate the test recommendations to all DOE Labs and contractors engaged in research activities using animals as test objects.

Sincerely,

Marvin E. Frazier, Director
Life Sciences Division
US DOE

United States Department of the Interior

U.S. GEOLOGICAL SURVEY
Office of the Director
Reston, Virginia 20192

In Reply Refer To:
Mail Stop 300
#20030295

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Olden
Stokes
Wolfe
ICCVAM file
ES 7/22

Dr. Kenneth Olden
Director, National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, North Carolina 12233

Dear Dr. Olden:

We are responding to your letter of March 21, 2003, to Secretary Norton on testing recommendations developed by the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM), pursuant to Section 3(e)(4) of the ICCVAM Act of 2000 (Public Law 106-545). As you know, the Department of the Interior (DOI) has very limited regulatory authority related to chemical registration. We conduct some registration studies on therapeutics for aquaculture and have a substantial effort that assesses the hazards of various environmental contaminants (e.g., pesticides, industrial chemicals, metals) to natural resources (invertebrates, fish, wildlife and their supporting habitat). The documents describing *In Vitro Methods for Assessing Acute Systemic Toxicity* and the *Revised Up-and-Down Procedure for Determining Acute Oral Toxicity of Chemicals* were reviewed by scientific staff of the Bureau of Land Management, U.S. Fish and Wildlife Service, National Park Service, and the U.S. Geological Survey (USGS). Below please find comments on application and utility of these methods by the Department.

In Vitro Methods for Assessing Acute Systemic Toxicity -
The development of *in vitro* methods for the prediction of acute oral toxicity of chemicals is a worthy initiative that is gaining international momentum. At present, this effort is focused on cytotoxicity studies in mammalian cell culture lines, and the extrapolation of these data to domesticated laboratory rodents, and humans. Much of our work is focused on invertebrates, fish and wildlife whose responsiveness to toxic agents is often different from that of laboratory mammals, both from a sensitivity and mechanistic standpoint. Unfortunately, efforts to develop *in vitro* methods to predict acute oral toxicity in these nontraditional test species have been hampered for several reasons. Invertebrate and fish cell lines generally exhibit poor responsiveness to xenobiotics, and research with cell lines from other groups of species is too limited to draw conclusions about their potential utility. Scientists within the DOI recognize these problems and recommend additional work with nontraditional cell lines and test species. The development and validation of methods for extrapolation of laboratory mammal acute toxicity data to invertebrates, fish and wildlife is also warranted. Such data would potentially Reduce the number of animals used in *in vivo* acute toxicity studies and might also assist in *ecotoxicological risk assessments.*

Revised Up-and-Down Procedure for Determining Acute Oral Toxicity of Chemicals -
There are practical concerns limiting the number of animal subjects used in acute oral toxicity tests. However, the weak quality of inference obtained when using the Revised Up-and-Down Procedure may have serious natural resource consequences. Estimating the median lethal dose from six or seven animals (National Institutes of Health Publication Number 02-4501: Executive Summary and Appendix F) is dubious for species whose sensitivity and response characteristics are poorly known. If inadequate numbers of individuals are used to generate acute toxicity data, the precision of the trial (confidence interval about the median lethal dose estimate) is so poor that one could argue that the test subjects were wasted. The Revised Up-and-Down Procedure does not generate a slope of the dose-response relationship and thus has very limited utility in describing this relationship and ecological effects at concentrations found in the environment. However, the Revised Up-and-Down Procedure could serve as a corroborative method in studies comparing toxicity among species whose sensitivity and response characteristics are well known.

In view of the limited authority of DOI in the area of chemical registration, we do not have relevant test methods for which the new ICCVAM test regulations can be substituted. Nonetheless, we can report that the Up-and-Down Procedure has been used by some scientists in the USGS involved in registration studies on therapeutics for potential use in aquaculture. Use of the Revised Up-and-Down Procedure will be considered by scientists within DOI for range finding and pilot studies, although thorough ecological risk assessments require more rigorous test methods. Thank you for providing us the opportunity to comment on these new toxicological testing recommendations.

Sincerely,

Charles G. Groat
Director

U.S. Department
of Transportation
**Research and
Special Programs
Administration**

400 Seventh St., S.W.
Washington, D.C. 20590

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Gen
Oldenburg

cc/
ICCVAM file
Stokes
Portier
Wolfe
ES
5/13

Mr. Kenneth Olden, Ph.D.
Director
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, NC 27709

Dear Mr. Olden:

Thank you for your letter to Secretary Mineta which forwarded toxicological test recommendations to the U.S. Department of Transportation (DOT) from the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). The DOT was one of several Federal agencies that received the toxicological test recommendations for consideration. Your letter was referred to the Research and Special Programs Administration (RSPA), the agency within DOT responsible for regulations on the transportation of hazardous materials and participation in ICCVAM.

As requested, we reviewed the toxicological test recommendations. Based on ICCVAM's work, in March 2002 the Expert from the United States of America transmitted a proposal to the United Nations Economic and Social Council's Committee of Experts on the Transport of Dangerous Goods and on the Globally Harmonized System of Classification and Labeling of Chemicals to amend the definition for acute oral toxicity in the United Nations Recommendations on the Transport of Dangerous Goods (UN Recommendations). The proposal would allow the use of three alternative Organization for Economic Co-operation and Development (OECD) Test Guidelines (TGs) that have been developed and implemented to replace TG 401 (Acute Oral Toxicity). The three TGs are the Fixed Dose Procedure (FDP, TG 420), the Acute Toxic Class Method (ATCM, TG 423), and the Up-and-Down Procedure (UDP, TG 425).

The proposal was considered and adopted by the UN Committee. The amended definition for "LD50 for acute oral toxicity" will appear in the thirteenth revised edition of the UN Recommendations which is scheduled to be published later this year. Subsequently, the DOT will issue a notice of proposed rulemaking (NPRM) to propose amending the Hazardous Materials Regulations (HMR) to harmonize the HMR with the UN Recommendations. The NPRM will include a proposal to amend the definition for LD50 acute oral toxicity to reflect the ICCVAM recommendation.

I hope this information is useful. If we can be of further assistance in this matter, please contact Ms. Patricia Klinger, Director of External Communications, at (202) 366-4831.

Sincerely yours,

Samuel G. Bonasso
Acting Administrator