United States
CONSUMER PRODUCT SAFETY COMMISSION
Washington, D.C. 20207

BALLOT VOTE SHEET

DATE: August 21, 2003

TO : The Commission
Todd A. Stevenson, Secretary

FROM : W.H. DuRoss, III, General Counsel

SUBJECT: Recommended Response to ICCVAM on Acute Toxicity Testing

BALLOT VOTE DUE: AUG 29, 2003

The Commission staff, in the attached memorandum, recommends a proposed response to the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) for determining acute oral toxicity for the purpose of classification and labeling and for test tube methods of determining the starting dose for acute systemic toxicity testing. These methods could be used for determining toxicity under the Federal Hazardous Substances Act.

Please indicate your vote on the following options:

I. APPROVE THE STAFF RECOMMENDATIONS

_____ _____
(Signature) (Date)

II. DO NOT APPROVE THE STAFF RECOMMENDATIONS

_____ _____
(Signature) (Date)

OGC 6 (GH) Cleared 8/22/03
__ No Mfrs/PrvtLblrs or Products Identified
__ Excepted by _____
__ Firms Notified,
__ Comments Processed.

NOTE: This document has not been reviewed or accepted by the Commission.
Initial ___ Date 8/22/03

III. TAKE OTHER ACTION (please specify)

_____ _____

(Signature) (Date)

Attachment

UNITED STATES
CONSUMER PRODUCT SAFETY COMMISSION
WASHINGTON, DC 20207

Memorandum

Date: AUG 21 2003

TO : The Commission

THROUGH: Todd A. Stevenson, Secretary
W.H. DuRoss, III, General Counsel
Patricia M. Semple, Executive Director

FROM : Jacqueline Elder
Assistant Executive Director
Office of Hazard Identification and Reduction
Marilyn L. Wind, Ph.D.
Deputy Associate Executive Director for Health Sciences

SUBJECT : Staff Response to the ICCVAM Recommendations on the Revised Up-and-Down Procedure and *in vitro* Methods for Determining Starting Dose for Acute Toxicity Testing

This review discusses the recommendations of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) for the use of: 1) the revised Up-and-Down Procedure (UDP) for determining acute oral toxicity for the purpose of classification and labeling and 2) *in vitro*[1] methods for determining the starting dose for acute systemic toxicity testing. It further discusses whether these validated methods are acceptable in the regulatory context for the purpose of classification for labeling under the Federal Hazardous Substances Act (FHSA). (15 U.S.C. 1261-1278)

Introduction

Internationally, over the past decade, there has been a movement to refine, reduce, or replace the use of animals in toxicological testing. The National Institutes of Health Revitalization Act of 1993 (Public Law No. 103-43, Section 1301) directed the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH) to establish a program dealing with alternative test methods, those methods that refine, reduce, or replace the use of animals. The Act further directed NIEHS to establish criteria for the validation[2] and regulatory acceptance[3] of alternative testing methods and to recommend a process by which validated

[1] *In vitro* means in a test tube.
[2] Validation is the determination that a method is reliable (measure of the degree to which a test can be performed reproducibly within and among laboratories over time), repeatable (closeness of agreement of results within a laboratory when the same procedure is performed on the same substance within a given time period), and relevant (the extent to which a test method will correctly predict or measure the biological effect of interest in humans).
[3] Regulatory acceptance is how a particular test method fits into the regulatory structure at a given agency, whether it will provide the type of data mandated under that particular agency's rules.

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methods could be accepted for regulatory use. In 1994 NIEHS established ICCVAM as an *ad hoc* interagency committee to accomplish these goals. The ICCVAM Authorization Act of 2000 (Public Law 106-545) established ICCVAM as a permanent committee of the NIEHS/NIH.

ICCVAM coordinates issues of interest to more than one agency relating to the development, validation, acceptance, and national/international harmonization of toxicological test methods that are alternatives to animal tests. The ICCVAM Authorization Act of 2000 mandates that when ICCVAM has validated a test method, it must send a report detailing its review and recommendations to the appropriate Federal agencies. The Federal agencies then have 180 days to respond to ICCVAM, describing test methods for which the recommended test could be added or substituted. Two ICCVAM test recommendations have been forwarded to the Commission for action: 1) the revised UDP for determining acute oral toxicity for classification and labeling purposes and 2) *in vitro* methods for determining the starting dose for acute systemic toxicity testing. The Commission needs to respond back to ICCVAM by September 24, 2003.

Federal Hazardous Substances Act Requirements

Since labeling hazardous household substances (chemical products) is mandated by the FHSA and some test methodology is discussed in this Act and its regulations, it is necessary to understand exactly what is required by the FHSA to determine whether the revised UDP would be acceptable as an alternative method. Under the FHSA, 15 U.S.C. § 1261-1275, the Commission requires precautionary labeling for hazardous household substances. A "*hazardous substance*" is defined as "*any substance or mixture of substances which is toxic...if such substance or mixture of substances may cause substantial personal injury or substantial injury during or as a proximate result of any customary or reasonably foreseeable handling or use...*"[4] The FHSA requires specific labeling for "*highly toxic*" substances or mixtures of substances and other labeling for "*toxic*" substances. For an orally toxic substance, the term "*highly toxic*" is defined in the Act as "*any substance which falls within any of the following categories: (a) Produces death within fourteen days in half or more than half of a group of ten or more laboratory white rats each weighing between two hundred and three hundred grams, at a single dose of fifty milligrams or less per kilogram of body weight, when orally administered...*"[5] In 16 CFR §1500.3 (c) (1) the definition is supplemented to give alternatives to the number of animals tested. It states, "*The number of animals tested shall be sufficient to give a statistically significant result and shall be in conformity with good pharmacological practices.*"

The definition of "*toxic*" given in the FHSA is supplemented by 16 CFR §1500.3 (c) (2) and states, "*Toxic means any substance that produces death within 14 days in half or more than half of a group of: (A) White rats (each weighing between 200 and 300 grams) when a single dose of from 50 milligrams to 5 grams per kilogram of body weight is administered orally...*" It further states, "*The number of animals tested shall be sufficient to give a statistically significant result and shall be in conformity with good pharmacological practices.*" "*Toxic*" also applies to any substance that is "*toxic*" (but not "*highly toxic*") on the basis of human experience. Both the Act

[4] The FHSA definition of "*hazardous substance*" includes substances that are corrosives, irritants, strong sensitizers, flammable, combustible, or generate pressure through decomposition, heat or other means but the only part of the definition that is pertinent to this discussion is the part dealing with substances that are "*toxic.*"

[5] There are also tests described for dermal and inhalation exposure but these are not relevant to this discussion.

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.

The revised UDP will be evaluated to determine whether it is an acceptable alternative to the conventional LD50 test (described below) described in the FHSA.

Acute Toxicity Testing

Acute toxicity testing in animals is generally the initial step in evaluating the health effects of a substance. Regulatory agencies use this information to properly classify and label materials with respect to their toxicity. Acute toxicity is generally regarded as toxicity or adverse health effects occurring within a short time (up to a 14 days) of administration of a single dose of a substance or multiple doses given within 24 hours. While animals can be exposed to substances by inhalation or dermally, this discussion will focus on orally administered substances since that is the focus of the revised UDP.

Conventional tests for acute oral toxicity focus on determining the median lethal dose (LD50), the dose which is expected to kill half the tested animals. The median lethal dose is a statistically derived value. In the past, these conventional tests sometimes utilized as many as 100 animals. Recently, several methods have been developed which use fewer animals. These methods have also broadened observations to include endpoints other than lethality. One of these methods is the revised UDP.

Internationally, there has been a focus on reducing, replacing or refining the use of animals in testing. As part of this process, the Organisation for Economic Cooperation and Development (OECD), on December 20, 2002, formally adopted the revised UDP as OECD Guideline 425 and deleted OECD Test Guideline 401, the conventional LD50 test. This means that internationally, it is expected that the conventional LD50 test will no longer be used for classification purposes and that other tests including the revised UDP will be used instead.

I. The Revised Up-and-Down Procedure (UDP)

Background

One of the alternative methods for *in vivo*[6] oral acute toxicity testing is known as the UDP. This test method was originally adopted by the OECD (OECD, 1998a) as an alternative to OECD Test Guideline 401 (TG 401), a conventional LD50 assay. At about the same time, the OECD adopted two additional *in vivo* acute toxicity protocols (OECD TG 420 and 423; OECD 1999a and 1999b). After adopting these three new alternative methods, the OECD proposed deleting OECD Test Guideline 401. Since it was necessary to revise the three methods to conform with a new globally harmonized hazard classification scheme, the U.S. Environmental Protection Agency (EPA) agreed to organize a technical task force to revise the UDP.

[6] *In vivo* means in a whole animal.

In August 1999, the EPA asked ICCVAM to conduct an independent scientific peer review of the revised UDP. ICCVAM convened an international panel to review the revised UDP in July 2000. ICCVAM and the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) then organized a second international scientific peer review meeting (August 21, 2001) to consider additional revisions to the revised UDP. Based upon the evaluation of the two peer review panels, ICCVAM developed recommendations about the use of the UDP as a substitute for the conventional LD50 test for the purposes of chemical classification. These recommendations were forwarded to the Federal agencies, including the CPSC, for their consideration and appropriate action.

Revised UDP Test Protocol

The revised UDP assay has two components: a primary test utilizing six to fifteen animals and a limit test utilizing three to five animals. The limit dose is primarily used when it is sufficient to determine or confirm that the LD50 is above or below a defined limit (2000 or 5000 mg/kg).[7,8]

The primary UDP test procedure calls for sequentially administering concentrations of a test substance differing by a constant factor to one animal at a time. Whether a dose is adjusted up or down depends on the outcome of the previous dose. Because the testing scheme is complex, a computer program was developed which, when results from animals tested are entered, will indicate what the dose should be for the next animal to be tested. The program also will tell the tester when to stop testing and will calculate the LD50 and its confidence interval (CI). The UDP can provide a LD50 value useful for classification and labeling with the use of six to fifteen animals.

The limit dose is also a sequential test. If the first animal tested at the limit dose dies, then the primary test is conducted to determine the LD50. If the first animal survives, additional animals are dosed one at a time. Survival of three animals or death of three animals determines the outcome of the test. Using this test method, up to five animals are required to determine if the LD50 is above or below 5000 mg/kg, the limit dose required under the FHSA.

Validation of the UDP

The revised UDP was validated using computer simulations that were equivalent to testing thousands of animals. Computer simulations were done for a large number of LD50's, with several different slopes[9] assumed for each LD50, and using a thousand or more simulated animals for each combination of LD50 and slope. These computer

[7] The FHSA requires labeling chemical substances with an LD50 of less than 5000 mg/kg. The limit dose test is important, therefore, for determining whether or not a substance needs to be labeled.

[8] The complete test guideline can be found at http://iccvam.niehs.nih.gov/docs/EPA/870r_1100.pdf

[9] The slope of the LD50 curve is an indication of the change in deaths relative to dose. A steep slope indicates that a small change in dose can have a large effect on deaths.

simulations were done for both the revised UDP as well as other *in vivo* tests for determining the LD50.

UDP Performance

The second peer review panel convened by ICCVAM concluded the following:

- 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."

Although there are no data specifically addressing the reliability and repeatability of the revised UDP, the ICCVAM peer review panel concluded that this was not a problem. Since the dosing method and observations for both the conventional LD50 test and the revised UDP are similar and since the conventional LD50 has been standardized to minimize variability, the Panel concluded that there was no reason to assume that the inter- and intra- laboratory variability would be different.

II. Use of *In Vitro* Test Methodologies for Determining Starting Dose for Acute Toxicity Testing

ICCVAM states that there are certain *in vitro* test methods that would be appropriate to use to determine the starting dose for the UDP test. This recommendation resulted from an international workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity, which was held October 17-20, 2000.

Background

The international workshop dealt with a broad range of topics, but one of the four breakout groups specifically addressed *in vitro* screening methods. This group was charged with evaluating the available data supporting the use of basal cytotoxicity methods[10] which use cell lines in tissue culture, and recommending whether and how

[10] Cytotoxicity is defined as the adverse effects resulting from interference with structures and processes essential for cell survival, proliferation, and/or function. Basal cell functions are functions that virtually all cells possess and for most chemicals, toxicity is a result of non-specific alterations in these cellular functions.

these methods might be used to reduce and refine animal use for acute toxicity testing. They concluded that *in vitro* cytotoxicity data could be useful in estimating starting doses for *in vivo* acute toxicity testing, which will reduce the number of animals required for such determinations. This conclusion was primarily based upon work done at the Multicentre Evaluation of *In Vitro* Cytotoxicity (MEIC) and the German Center for the Documentation and Validation of Alternative Methods (ZEBET). The MEIC program coordinated the generation of *in vitro* toxicity data for 50 chemicals in 96 laboratories and correlated the findings with data from human poisoning reports. Zebet used data from the Registry of Cytotoxicity, which contains a regression analysis of *in vitro* cytotoxicity IC50[11] values and rodent LD50 values for 347 chemicals, to determine starting doses for LD50 tests.

The working group also suggested that a guidance document be prepared to provide practical guidance on how to generate and use basal cytotoxicity data to predict starting doses for *in vivo* acute toxicity assays. The guidance document was written by scientists working with NICEATM and was thoroughly reviewed by scientists involved with ICCVAM and the international workshop. The guidance document is comprehensive and provides the following information:

- background information on the correlation between *in vitro* cytotoxicity and acute lethality,
- a description of the basic elements of *in vitro* assays for cytotoxicity, and
- a description of the use of the Registry of Cytotoxicity prediction model to evaluate candidate cytotoxicity assays.

In addition, the guidance document describes two candidate cytotoxicity tests recommended for use. These are the neutral red uptake assays (NRU) using the mouse fibroblast cell line BALB/c 3T3 and a test using normal human keratinocytes (NHK). Protocols for these tests, additional guidance for implementing the protocols, and a standard template for data collection are also described.

The full guidance document can be found at http://iccvam.niehs.nih.gov/ methods /invidocs /guidance/iv_guide.pdf

ICCVAM Conclusions

After reviewing the reports of both peer review panels, the report from the international workshop on *in vitro* toxicity testing, and the guidance document, ICCVAM concluded the following:

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.*

[11] The IC50 is the dose which kills 50% of the cells in an *in vitro* test.

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 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."*

ICCVAM Recommendations

ICCVAM stated that it agrees with the peer review panel that the revised UDP test guideline is acceptable as a substitute for the conventional LD50 test to determine acute oral toxicity for the purpose of hazard classification and for obtaining certain information on acute toxicity. ICCVAM, therefore, recommends *"that the final revised UDP test guideline should: (1) replace the current OECD UDP test guideline (TG 425; OECD, 1998) and (2) be used instead of the conventional LD50 test to determine the acute oral toxicity hazard of chemicals."*

ICCVAM further states that "*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.*" They refer to the Guidance Document on Using *In Vitro* Data to Estimate *In Vivo* Starting Doses for Acute Toxicity, NIH Publication 01-4500.

Discussion

Staff agrees with the peer review panels and ICCVAM that the revised UDP test is an appropriate method for generating a point estimate for the LD50 for use in hazard classification. Limit dose testing as required by the FHSA may be conducted at 5000 mg/kg using the revised UDP. In addition to providing the necessary information for the appropriate labeling of chemicals, the UDP also reduces the use of animals and for those that remain may help to decrease pain and distress. The supplementary definition at 15CFR §1500.3 for "*toxic*" and "*highly toxic*" which states, "*the number of animals tested shall be sufficient to give a statistically significant result and shall be in conformity with good pharmacological practices*" indicates that as long as a scientifically valid method is used to determine the LD50, the exact number of animals specified does not have to be used. Staff believes that the revised UDP is a scientifically valid method for determining the LD50 and, in fact, might provide better data than the conventional LD50 tests because it uses sequential rather than fixed dosing and it uses a detailed computer program to determine doses and calculate the LD50. There is Commission precedent for changing the type of testing recommended for determining an LD50. In 1984, the Commission recommended, in its animal testing policy,[12] use of a test which reduced the number of animals used for acute toxicity determination to 10 or sometimes 20.[13] The revised UDP will give a more accurate LD50 than the test recommended in 1984. None of the specifications in the protocol for the revised UDP is in conflict with the FHSA.

ICCVAM indicated that the revised UDP might not be appropriate for chemicals causing delayed deaths (especially after five days). Staff does not believe this is a concern since the UDP protocol calls for humanely sacrificing any animal that is moribund and counting such an animal as a mortality. Thus, the symptoms of severe illness are sufficient to make a finding.

Staff also agrees with the recommendation of the international workshop and ICCVAM that based upon the work done by MEIC and ZEBET, the BALB/c 3T3 and NHK NRU assays are useful as one step in helping to determine a starting dose for the revised UDP. Use of these *in vitro* tests will reduce the use of animals still further.

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[12] Federal Register 49(105):22522-3, May 30, 1984.

[13] This test requires testing ten animals at 5000mg/kg. If not more than one animal dies, no further testing is required. If more than five die, ten animals are tested at 50 mg/kg. While this test does not result in an exact LD50, it provides sufficient information to determine whether a substance needs to be labeled and if it needs to be labeled as "*toxic*" or "*highly toxic*" under the FHSA.

Options

The Commission can vote to:

1. Accept the ICCVAM recommendations and instruct staff to draft a letter to ICCVAM indicating acceptance of its recommendations.

2. Reject the ICCVAM recommendations and instruct staff to draft a letter to ICCVAM indicating rejection of its recommendations.

Recommendation

Staff recommends that the Commission accept the ICCVAM recommendations since the revised UDP might provide better data on the LD50 than the conventional LD50 test or the one recommended in the Commission's 1984 Animal Testing Policy. Further, since the conventional LD50 test is no longer accepted globally, acceptance of the revised UDP will harmonize the Commission's policy with the OECD policy. Staff also recommends that the Commission accept the ICCVAM recommendation that the BALB/c 3T3 and NHK NRU assays are useful in helping to determine a starting dose for the revised UDP.

Staff will draft a letter to ICCVAM indicating the Commission's action with regard to the ICCVAM recommendation. The ICCVAM website (http://iccvam.niehs.nih.gov/home.htm) will link to the Commission website where we would post our acceptance or non-acceptance of the UDP and the use of two basal toxicity tests to determine the starting dose for the UDP. In the section of the ICCVAM website, Pertinent Regulations, Guidelines and Laws (http://iccvam.niehs.nih.gov/agencies/regs.htm), there will be an announcement of the Commission action on the acceptance or non-acceptance of the UDP and the use of two basal cytotoxicity assays to determine the starting dose for the UDP. Once ICCVAM receives responses from all the agencies, it will publish a Federal Register notice announcing all the agencies' responses.