



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

August 28, 2008

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Samuel H. Wilson, Ph.D.
Acting Director
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, NC 27709

Dear Dr. Wilson:

In your letter of February 28, 2008, you sent Administrator Stephen L. Johnson a report entitled, *The Interagency Coordinating Committee on the Validation of Alternative Methods Test Method Evaluation Report: In Vitro Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Tests* (NIH Publication No. 07-4519). This report contained ICCVAM recommendations for two *in vitro* alternative test methods proposed for use in estimating starting doses for acute oral (LD50-type) systemic toxicity tests conducted for hazard classification and labeling purposes. Also enclosed with your letter was a comprehensive Background Review Document containing validation study results and analyses supporting the ICCVAM recommendations for the two *in vitro* methods (NIH Publication No. 07-4518). The U.S. Environmental Protection Agency (EPA) appreciates the opportunity to review these materials and acknowledges the diligent efforts made by ICCVAM in reviewing and reporting on these methods.


Previously in December 2003, EPA responded to ICCVAM's earlier recommendations on the use of *in vitro* cytotoxicity methods (Enclosure 1). In our response, we embraced the concept of these methods in our regulatory programs, where appropriate, and also discussed the strengths and weaknesses of these methods based on the evaluation conducted by ICCVAM at that time.

Your February 28, 2008 letter to federal agencies detailing the most recent ICCVAM activities regarding the use of *in vitro* cytotoxicity methods states that the two alternatives evaluated should be considered before using animals for acute oral toxicity testing where appropriate. EPA agrees with the ICCVAM recommendations that data from the *in vitro* test methods may be used in a weight of the evidence approach, where appropriate, for determining starting doses for *in vivo* studies and we agree this may result in the reduction of animals used in this type of toxicity testing.

EPA also agrees with ICCVAM's conclusion that these *in vitro* test methods are not sufficiently accurate to replace animals for regulatory labeling and hazard classification purposes.

EPA's harmonized test guidelines for Acute Oral Toxicity (dated December 2002) currently discuss the use of *in vitro* data to estimate *in vivo* starting doses for acute oral toxicity testing and we reference the ICCVAM Guidance Document (NIH Publication No. 01-4500). The Agency looks forward to working with ICCVAM staff to update this document as necessary.

Sincerely,



Elizabeth Resek
Acting Director
Office of Science Coordination and Policy

Enclosure Attached

cc: Jim Jones
Charles Aurer
Hal Zenick
Karen Hamernik
Tina Levine
Oscar Hernandez
Julian Preston
Suzanne McMaster
Deborah McCall
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ENCLOSURE 1

DEC 2 2003

OFFICE OF
PREVENTION, PESTICIDES AND
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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 $\pm \log 5$).

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,

/s/

Joseph J. Merenda, Jr.

Director

Office of Science Coordination and Policy

cc: Jim Jones
Charles Auer
William Stokes