DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

National Institute of Environmental Health Sciences (NIEHS); National Toxicology Program (NTP); NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM): Availability of Updated Standardized *In Vitro* Cytotoxicity Test Method Protocols for Estimating Acute Oral Systemic Toxicity; Request for Existing *In Vivo* and *In Vitro* Acute Toxicity Data

Summary: NICEATM announces the availability of two updated standardized in vitro cytotoxicity test method protocols to estimate acute oral systemic toxicity in rodents. These two test methods were previously recommended by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) for selecting starting doses for in vivo acute oral systemic toxicity tests (**Federal Register** Vol. 66, No. 189, pages 49686–49687, September 28, 2001). This approach can reduce the number of animals required for acute oral toxicity testing. NICEATM also requests the submission of existing and future data on chemicals and products tested for both acute oral systemic toxicity and in vitro cytotoxicity using the standardized test method protocols mentioned in this notice. These data will be used to further evaluate the usefulness and limitations of cytotoxicity methods for estimating in vivo acute oral toxicity. The data will also be used to establish a database to support the investigation of other test methods necessary to improve the accuracy of in vitro assessments of acute systemic toxicity.

Availability of Standardized Test Method Protocols for Estimating Starting Doses for *In Vivo* Acute Oral Toxicity Tests

Updated standardized protocols for two neutral red uptake assays using either BALB/c 3T3 cells or normal human keratinocytes are now available at: *http://iccvam.niehs.nih.gov/methods/invitro.htm.* These test method protocols have been improved to maximize intra- and inter-laboratory reproducibility and are currently being used for the final phase of a joint NICEATM-European Center for the Validation of Alternative Methods (ECVAM) validation study. NICEATM recommends that these updated test method protocols be used in place of standard operating procedures previously recommended by ICCVAM for two cytotoxicity test methods to estimate starting doses for in vivo acute oral toxicity tests (ICCVAM, 2001b).

Submission of Chemical and Protocol Information/Test Data

In vivo and in vitro acute toxicity testing data for chemicals or products should be sent by mail, fax or e-mail to NICEATM [Dr. William S. Stokes, Director, NICEATM, NIEHS, PO Box 12233, MD EC–17, Research Triangle Park, NC 27709, (phone) 919–541–2384, (fax) 919–541–0947, (e-mail) *iccvam@niehs.nih.gov*]. Data will be accepted at any time. Data submitted within the next 9 months will be considered during an evaluation of the validation status of the two cytotoxicity methods anticipated in late 2005. Chemical and protocol information/test data submitted in response to this notice may be incorporated in future NICEATM and ICCVAM reports and publications as appropriate.

When submitting chemical and protocol information/test data, please reference this **Federal Register** notice and provide appropriate contact information (name, affiliation, mailing address, phone, fax, e-mail, and sponsoring organization, as applicable).

NICEATM prefers data to be submitted as copies of pages from study notebooks and/or study reports, if available. Raw data and analyses available in electronic format may also be submitted. Each submission for a chemical should preferably include the following information, as appropriate:

- Common and trade name
- Chemical Abstracts Service Registry Number (CASRN)
- Chemical and/or product class
- Commercial source
- In vitro basal cytotoxicity test protocol used
- In vitro cytotoxicity test results
- In vivo acute oral toxicity test protocol used
- Individual animal responses at each observation time (if available)
- The extent to which the study complied with national or international Good Laboratory Practice (GLP) guidelines
- Date and testing organization

Those persons submitting data on chemicals tested for in vitro basal cytotoxicity are referred to the standard test-reporting template recommended for the High Production Volume (HPV) program at *http://www.epa.gov/chemrtk/toxprtow.htm* or at *http://iccvam.niehs.nih.gov/methods/invitro.htm.* In vivo data for the same chemicals should be reported as recommended in the test reporting section of the current Environmental Protection Agency (EPA) guideline for acute oral toxicity (EPA, 2002).

Submitted data will be used to further evaluate the usefulness and limitations of in vitro cytotoxicity data for estimating acute oral toxicity, and will be included in a database to support the investigation of other test methods necessary to improve the accuracy of in vitro assessments of acute systemic toxicity.

History

In September 2001, the ICCVAM recommended that in vitro cytotoxicity test methods be considered as a tool for estimating starting doses for in vivo acute systemic toxicity testing studies (**Federal Register** Vol. 66, No. 189, pages 49686–49687, September 28, 2001.) The recommendations were based on the Report of the International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity (ICCVAM, 2001a). The Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity (ICCVAM, 2001b) was

also made available at that time. The guidance document provided standard operating procedures for two cytotoxicity test methods and instructions for using these assays to estimate starting doses for in vivo testing.

Federal agency responses to the ICCVAM test method recommendations were announced on March 10, 2004 (**Federal Register** Vol. 69, No. 47, pages 11448–11449). Federal agencies agreed to encourage, to the extent applicable, the use of in vitro tests for determining starting doses for acute systemic toxicity testing. Furthermore, EPA specifically encouraged those participating in the HPV Challenge Program to consider using the recommended in vitro tests as a supplemental component in conducting any new in vivo acute oral toxicity studies for the program (*http://www.epa.gov/chemrtk/toxprtow.htm*).

A NICEATM–ECVAM validation study was initiated in 2002 to evaluate the usefulness of the two neutral red uptake cytotoxicity assays currently available for predicting starting doses for in vivo acute oral toxicity tests. During the pre-validation phases of the study, the test method protocols were further standardized and revised to improve their intra- and inter-laboratory reproducibility. NICEATM recommends using the revised test method protocols rather than the standard operating procedures outlined in the guidance document (ICCVAM, 2001b.) The guidance document should be consulted for the procedure for calculating starting doses using in vitro cytotoxicity data.

Background Information on ICCVAM and NICEATM

ICCVAM is an interagency committee composed of representatives from fifteen Federal regulatory and research agencies that use, generate, or disseminate toxicological information. ICCVAM promotes the development, validation, regulatory acceptance, and national and international harmonization of toxicological test methods that more accurately assess the safety or hazards of chemicals and products, and test methods that refine, reduce and replace animal use. The ICCVAM Authorization Act of 2000 (available at *http://iccvam.niehs.nih.gov/about/PL106545.htm*) established ICCVAM as a permanent interagency committee of the NIEHS under the NICEATM. NICEATM administers the ICCVAM and provides scientific support for ICCVAM and ICCVAM-related activities. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods applicable to the needs of Federal agencies. Additional information about ICCVAM and NICEATM can be found at the following Web site: *http://iccvam.niehs.nih.gov/.*

References

EPA. 2002. Health Effects Test Guidelines, OPPTS 870.1100, Acute Oral Toxicity, EPA 712–C–02–190. Available at: *http://www.epa.gov/opptsfrs/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Series/870–1100.pdf.*

ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods). 2001a. Report of the international workshop on in vitro methods for assessing acute systemic toxicity. NIH Publication 01–4499. Research Triangle Park, NC: National Institute for Environmental Health Sciences. Available at: *http://iccvam.niehs.nih.gov/.*

ICCVAM. 2001b. Guidance document on using in vitro data to estimate in vivo starting doses for acute toxicity. NIH Publication 01–4500. Research Triangle Park, NC: National Institute for Environmental Health Sciences. Available at: *http://iccvam.niehs.nih.gov/.*

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