APPENDIX D

FEDERAL REGISTER NOTICES AND PUBLIC COMMENTS

D1 Federal Register Notices..... D-3

D2 ICCVAM Consideration of Public Comments Received in Response to Federal Register Notices D-27

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APPENDIX D1
FEDERAL REGISTER NOTICES

Federal Register Notice (**65 FR 37400**, June 14, 2000): Notice of an International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity, co-sponsored by NIEHS, NTP and the U.S. Environmental Protection Agency (EPA): Request for Data and Suggested Expert Scientists..... D-5

Federal Register Notice (**65 FR 57203**, September 21, 2000): Notice of an International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity D-9

Federal Register Notice (**66 FR 49686**, September 28, 2001): Report of the International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity; Guidance Document on Using *In Vitro* Data to Estimate *In Vivo* Starting Doses for Acute Toxicity: Notice of Availability and Request for Public Comment..... D-13

Federal Register Notice (**69 FR 11448**, March 10, 2004): Notice of the Availability of Agency Responses to ICCVAM Test Recommendations for the Revised Up-and-Down Procedure for Determining Acute Oral Toxicity and *In Vitro* Methods for Assessing Acute Systemic Toxicity..... D-15

Federal Register Notice (**69 FR 61504**, October 19, 2004): 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..... D-17

Federal Register Notice (**70 FR 14473**, March 22, 2005): Request for Nominations for an Independent Peer Review Panel To Evaluate In Vitro Testing Methods for Estimating Acute Oral Systemic Toxicity and Request for *In Vivo* and *In Vitro* Data D-19

Federal Register Notice (**71 FR 14229**, March 21, 2006): Announcement of a Peer Review Meeting on the Use of *In Vitro* Testing Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Tests..... D-21

Federal Register Notice (**71 FR 39122**, July 11, 2006): Availability of the Peer Review Panel Report on the Use of *In Vitro* Basal Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Testing D-25

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH), National Toxicology Program (NTP); Notice of an International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity, co-sponsored by NIEHS, NTP and the U.S. Environmental Protection Agency (EPA): Request for Data and Suggested Expert Scientists

SUMMARY: Pursuant to Public Law 103–43, notice is hereby given of a public meeting sponsored by NIEHS, the NTP, and the EPA, and coordinated by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). The agenda topic is a scientific workshop to assess the current status of in vitro test methods for evaluating the acute systemic toxicity potential of chemicals, and to develop recommendations for future development and validation studies. The workshop will take place on October 17–20, 2000 at the Hyatt Regency Crystal City Hotel, 2799 Jefferson Davis Highway, Arlington, VA, 22202. The meeting will be open to the public.

In preparing for this Workshop, ICCVAM is requesting: (1) Information and data that should be considered at the Workshop, including relevant data on currently available in vitro methods for assessing acute systemic toxicity; and (2) nominations of expert scientists to participate in the Workshop. An agenda, registration information, and other details will be provided in a subsequent **Federal Register** notice.

Background

ICCVAM, with participation by 14 Federal regulatory and research agencies and programs, was established in 1997 to coordinate issues relating to the development, validation, acceptance, and national/international harmonization of toxicological test methods. ICCVAM seeks to promote the scientific validation and regulatory acceptance of new and improved test methods applicable to Federal agencies, including methods that may reduce or replace animal use, or that refine protocols to lessen animal pain and distress. The Committee's functions include the coordination of interagency reviews of toxicological test methods and communication with stakeholders throughout the process of test method development and validation. The following Federal regulatory and research agencies participate:

Consumer Product Safety Commission
Department of Defense
Department of Energy
Department of Health and Human Services
  Agency for Toxic Substances and Disease Registry
  Food and Drug Administration
  National Institute for Occupational Safety and Health/CDC
  National Institutes of Health
  National Cancer Institute
  National Institute of Environmental Health Sciences
  National Library of Medicine
Department of the Interior
Department of Labor
  Occupational Safety and Health Administration
Department of Transportation
  Research and Special Programs Administration
Environmental Protection Agency

NICEATM was established in 1998 and provides operational support for the ICCVAM. NICEATM and ICCVAM collaborate to carry out activities associated with the development, validation, and regulatory acceptance of proposed new and improved test methods. These activities may include:

- Test Method Workshops, which are convened as needed to evaluate the adequacy of current methods for assessing specific toxicities, to identify areas in need of improved or new testing methods, to identify research efforts that may be needed to develop new test methods, and to identify appropriate development and validation activities for proposed new methods.
- Expert Panel Meetings, which are typically convened to evaluate the validation status of a method following the completion of initial development and pre-validation studies. Expert Panels are asked to recommend additional validation studies that might be helpful in further characterizing the usefulness of a method, and to identify any additional research and development efforts that might enhance the effectiveness of a method.
- Independent Peer Review Panel Meetings, which are typically convened following the completion of comprehensive validations studies on a test method. Peer Review Panels are asked to develop scientific consensus on the usefulness and limitations of test methods to generate information for specific human health and/or ecological risk assessment purposes. Following the independent peer review of a test method, ICCVAM forwards recommendations on its usefulness to agencies for their consideration. Federal agencies then determine the regulatory acceptability of a method according to their mandates.

Additional information about ICCVAM and NICEATM can be found at the website: *http://iccvam.niehs.nih.gov.*

Workshop Background and Scope

A. Background

Federal regulatory agencies require toxicity testing to determine the safety or hazard of various chemicals and products prior to human exposure. Agencies use this information to properly classify and label products as to their hazard potential. Acute oral toxicity determinations are currently made using animals. However, recent studies (e.g., Spielmann et al., 1999) suggest that in vitro cytotoxicity methods may be useful in predicting a starting dose for in vivo studies, and thus may potentially reduce the number of animals necessary for such determinations.

Other studies (e.g., Ekwall et al., 2000) have indicated an association between in vitro cytotoxicity and human lethal blood concentrations. However, these in vitro methods have not yet been evaluated in validation studies to determine their usefulness and limitations for generating acute toxicity testing information necessary to meet regulatory testing requirements. Additionally, other in vitro methods would likely be necessary to establish accurate dose-response relationships before such methods could substantially reduce or replace animal use for acute toxicity determinations.

This workshop will examine the status of available in vitro methods and develop recommendations for validation efforts necessary to characterize the usefulness and limitations of existing methods. Recommendations for future research and development efforts that might further enhance the usefulness of in vitro assessments of acute systemic lethal toxicity will also be developed.

B. Objectives of the Workshop

Four major topics will be addressed:

1. General cytotoxicity methods predictive of acute lethal toxicity;
2. Toxicokinetic and organ specific toxicity methods;
3. Reference chemicals for validation of the above methods; and
4. The use of quantitative structure activity relationships (QSAR) and chemical/physical properties for predicting acute lethal toxicity.

The objectives of the meeting are to:

1 a. Identify and review the status of in vitro general cytotoxicity screening methods that may reduce animal use for assessing acute systemic toxicity;

b. Identify information from in vitro methods necessary to predict acute systemic toxicity and review the status of relevant methods (*e.g.,* in vitro methods to assess gut absorption, metabolism, blood-brain barrier penetration, volume distribution to critical target organs, and specific target organ toxicity);

2. Identify candidate methods for further evaluation in prevalidation and validation studies;

3. Identify reference chemicals useful for development and validation of in vitro methods for assessing acute systemic toxicity;

4. Identify validation study designs needed to adequately characterize the proposed methods in 2.; and

5. Identify priority research efforts necessary to support the development of in vitro methods to adequately assess acute systemic toxicity. Such efforts might include incorporation and evaluation of new technologies such as gene microarrays, and development of methods necessary to generate dose response information.

C. Methods for Consideration

Given the breadth of the workshop topics, many methods are likely to be considered relevant to the discussion. Methods will include but are not limited to those proposed in the Multicentre Evaluation of In Vitro Cytotoxicity (MEIC) battery (*http://www.ctlu.se*). A background document summarizing the data and performance characteristics for available methods is being prepared by NICEATM in collaboration with the ICCVAM interagency organizing committee. Information received as a result of this **Federal Register** notice will be

considered for inclusion in the background document. In formulating its recommendations, the Workshop participants will evaluate information in the background document and relevant information from other sources.

D. Test Method Data and Information Sought

Data are sought from completed, ongoing, or planned studies that provide comparative performance data for in vitro methods compared to currently accepted in vivo methods for determining acute lethal toxicity and hazard classification. Data from test methods that provide toxicokinetic and specific target organ toxicity information are also sought. Submissions should describe the extent to which established criteria for validation and regulatory acceptance have been addressed. These criteria are provided in ''Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods,'' NIH publication 97–3981 (*http://ntp-server.niehs.nih.gov/htdocs/ICCVAM/iccvam.html*). Where possible, submitted data and information should adhere to the guidance provided in the document, ''Evaluation of the Validation Status of Toxicological Methods: General Guidelines for Submissions to ICCVAM,'' NIH Publication 99–4496, (*http://iccvam.niehs.nih.gov/doc1.htm*). Both publications are also available on request from NICEATM at the address provided below. Relevant information submitted in response to this request will be incorporated into the background material provided to Workshop participants. A preliminary list of relevant studies is provided at the end of this announcement, and public comment and suggestions for additions are invited.

NICEATM and the ICCVAM interagency workshop organizing committee will compile information on the studies to be considered at the Workshop. All data should be submitted by July 15, 2000 in order to ensure full consideration.

E. Request for Nomination of Expert Scientists for the Test Method Workshop

NICEATM is soliciting nominations for expert scientists to participate in the Workshop. (See Guidelines for Submission of Comments below). Types of expertise likely to be relevant include acute toxicity testing in animals, evaluation and treatment of acute toxicity in humans, development and use of in vitro methodologies, statistical data analysis, knowledge of chemical data sets useful for validation of acute toxicity studies, and hazard classification of chemicals and products. Expertise need not be limited to these areas, nor will these areas necessarily be included on the Panel. An appropriate breadth of expertise will be sought. If other areas of scientific expertise are recommended, the rationale should be provided.

Nominations should be accompanied by complete contact information including name, address, institutional affiliation, telephone number, and e-mail address. The rationale for nomination should be provided. If possible, a biosketch or a curriculum vitae should be included. To avoid the potential for candidates being contacted by a large number of nominators, candidates need not be contacted prior to nomination.

Workshop experts will be selected by an ICCVAM interagency workshop organizing committee after considering all nominations received from the public as well as nominations developed internally. All nominees will be contacted for interest and availability, and curricula vitae will be solicited from the nominees. Candidates will be required to disclose potential conflicts of interest.

Schedule for the Workshop

The Workshop will take place on October 17–20, 2000 at the Hyatt Regency Crystal City Hotel, 2799 Jefferson Davis Highway, Arlington, VA 22202. The Workshop meeting will be open to the public, limited only by space available.

Submitted methods and supporting data will be reviewed during the July to August 2000 timeframe and a background review document will be prepared by NICEATM in collaboration with the ICCVAM interagency organizing committee. The background information will be made available to Workshop experts for discussion at the meeting and will be available to the Public in advance of the Workshop.

Public Input Invited

As described above, ICCVAM invites comments on the scope and process for the review; comments on the ICCVAM preliminary list of studies for consideration; the submission of other test methods for consideration; and the nomination of experts to participate in the Workshop. Nominations must be submitted within 30 days of the publication date of this notice, and other information should be submitted by July 15, 2000.

Guidelines for Submission of Public Comment

Correspondence should be directed to Dr. William S. Stokes, NTP Interagency Center for the Evaluation of Alternative Toxicological Methods, Environmental Toxicology Program, NIEHS/NTP, MD EC–17, PO Box 12233, Research Triangle Park, NC 27709; 919–541–3398 (phone); 919–541–0947 (fax); *iccvam@niehs.nih.gov* (e-mail). Public comments should be accompanied by complete contact information including name, (affiliation, if applicable), address, telephone number, and e-mail address.

Preliminary List of Studies to be Considered for the Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity

ICCVAM has compiled a preliminary list of relevant studies. The public is invited to comment on this list, and suggestions for additions may be submitted. (See Section of this **Federal Register** announcement on Guidelines for Submission of Public Comments).

Studies that may be completed but not published are not included here. This list provides examples of studies and information that may be appropriate for consideration by the Workshop experts.

Balls, M., Blaauboer, B.J., Fentem, J.H., Bruner, L., Combes, R.D., Ekwall, B., Fielder, R.J., Guillouzo, A., Lewis, R.W., Lovell, D.P., Reinhardt, C.A., Repetto, G., Sladowski, D., Spielmann, H., and Zucco, F. (1995) Practical aspects of the validation of toxicity test procedures—The report and recommendations of ECVAM Workshop 5. ATLA 23, 129–147.

Bernson, V., Bondesson, I., Ekwall, B., Stenberg, K., and Walum, E. (1987) A multicenter evaluation study of *in vitro* cytotoxicity. ATLA, 14, 144–145.

Bondesson, I., Ekwall, B., Stenberg, K., Romert, L., and Walum, E. (1988) Instruction for participants in the multicenter evaluation study of *in vitro* cytotoxicity (MEIC). ATLA, 15, 191–193.

Bondesson, I., Ekwall, B., Hellberg, S., Romert, L., Stenberg, K., and Walum, E. (1989) MEIC—A new international multicenter project to evaluate the relevance to human toxicity of *in vitro* cytotoxicity tests. Cell Biol. Toxicol., 5, 331–347.

Clemedson, C., and Ekwall, B. (1999) Overview of the final MEIC results: I. The *in vitro-in vivo* evaluation. Toxicology In vitro, 13, 657–663.

Clemedson, C., McFarlane-Abdulla, E., Andersson, M., Barile, F.A., Calleja, M.C., Chesnea, C., Clothier, R., Cottin, M., Curren, R., Daniel-Szolgay, E., Dierickx, P., Ferro, M., Fiskesj'', G., Garza-Ocanas, L., Goamez-Lechoan, M.J., Gualden, M., Isomaa, B., Janus, J., Judge, P., Kahru, A., Kemp, R.B., Kerszman, G., Kristen, U., Kunimoto, M., Karenlampi, S., Lavrijsen, K., Lewan L., Lilius, H., Ohno, T., Persoone, G., Roguet, R.,

Romert, L., Sawyer, T., Seibert, H., Shrivastava, R., Stammati, A., Tanaka, N., Torres Alanis, O., Voss, J–U., Wakuri, S., Walum, E., Wang, X., Zucco, F., and Ekwall, B. (1996) MEIC evaluation of acute systemic toxicity. Part I. Methodology of 68 *in vitro* toxicity assays used to test the first 30 reference chemicals. ATLA, 24, Suppl. 1, 249–272.

Clemedson, C., McFarlane-Abdulla, E., Andersson, M., Barile, F.A., Calleja, M.C., Chesne, C., Clothier, R., Cottin, M., Curren, R., Dierickx, P., Ferro, M., Fiskesja, G., Garza-Ocanas, L., Gomez-Lechon, M.J., Gulden, M., Isomaa, B., Janus, J., Judge, P., Kahru, A., Kemp, R.B., Kerszman, G., Kristen, U., Kunimoto, M., Karenlampi, S., Lavrijsen, K., Lewan L., Lilius, H., Malmsten, A., Ohno, T., Persoone, G., Pettersson, R., Roguet, R., Romert, L., Sandberg, M., Sawyer, T., Seibert, H., Shrivastava, R., Sjostrom, M., Stammati, A., Tanaka, N., Torres Alanis, O., Voss, J–U., Wakuri, S., Walum, E., Wang, X., Zucco, F. and, Ekwall, B. (1996) MEIC evaluation of acute systemic toxicity. Part II. *In vitro* results from 68 toxicity assays used to test the first 30 reference chemicals and a comparative cytotoxicity analysis. ATLA, 24, Suppl. 1, 273–311.

Clemedson, C., Barile, F.A., Ekwall, B., Gomez-Lechon, M.J., Hall, T., Imai, K., Kahru, A., Logemann, P., Monaco, F., Ohno, T., Segner, H., Sjostrom, M., Valentino, M., Walum, E., Wang, X., and Ekwall, B. (1998). MEIC evaluation of acute systemic toxicity: Part III. *In vitro* results from 16 additional methods used to test the first 30 reference chemicals and a comparative cytotoxicity analysis. ATLA 26, Suppl. 1, 91–129.

Clemedson, C., Aoki, Y., Andersson, M., Barile, F.A., Bassi, A.M., Calleja, M.C., Castano, A., Clothier, R.H., Dierickx, P., Ekwall, B., Ferro, M., Fiskeso, G., Garza-Ocanas, L. Gomez-Lechoan, M.J., Gulden, M., Hall, T., Imai, K., Isomaa, B., Kahru, A., Kerszman, G., Kjellstrand, P., Kristen, U., Kunimoto, M., Karenlampi, S., Lewan, L., Lilius, H., Loukianov, A., Monaco, F., Ohno, T., Persoone, G., Romert, L., Sawyer, T.W., Shrivastava, R., Segner, H., Seibert, H., Sjostrom, M., Stammati, A., Tanaka, N., Thuvander, A., Torres-Alanis, O., Valentino, M., Wakuri, S., Walum, E., Wieslander, A., Wang, X., Zucco, F., and Ekwall, B. (1998). MEIC evaluation of acute systemic toxicity. Part IV. *In vitro* results from 67 toxicity assays used to test reference chemicals 31–50 and a comparative cytotoxicity analysis. ATLA 26, Suppl. 1, 131–183.

Clemedson, C., Barile, F.A., Chesne, C., Cottin, M., Curren, R., Ekwall, B., Ferro, M., Gomez-Lechon, M.J., Imai, K., Janus, J., Kemp, R.B., Kerszman, G., Kjellstrand, P., Lavrijsen, K., Logemann, P., McFarlane-Abdulla, E., Roguet, R., Segner, H., Seibert, H., Thuvander, A., Walum, E., and Ekwall, Bj. (2000) MEIC evaluation of acute systemic toxicity: Part VII. Prediction of human toxicity by results from testing of the first 30 reference chemicals with 27 further *in vitro* assays. ATLA 28, Suppl. 1, 161–200.

Ekwall, B. (1995) The basal cytotoxicity concept, pp 721–725. In Proceedings of the World Congress on Alternatives and Animal Use in the Life Sciences: Education, Research, Testing. Alternative Methods in Toxicology and the Life Sciences, Vol. 11. Mary Ann Liebert, New York, 1995.

Ekwall, B. (1999) Overview of the Final MEIC Results: II. The *In vitro/in vivo* evaluation, including the selection of a practical battery of cell tests for prediction of acute lethal blood concentrations in humans. Toxicol. *In vitro*, 13, 665–673.

Ekwall, B., Gomez-Lechon, M.J., Hellberg, S., Bondsson, I., Castell, J.V., Jover, R., Hogberg, J., Ponsoda, X., Stenberg, K., and Walum, E. (1990) Preliminary results from the Scandinavian multicentre evaluation of *in vitro* cytotoxicity (MEIC). Toxicol. *In vitro,* 4, 688–691.

Ekwall, B., Clemedson, C., Crafoord, B., Ekwall, Ba., Hallander, S., Walum E., and Bondesson, I. (1998) MEIC evaluation of acute systemic toxicity. Part V. Rodent and human toxicity data for the 50 reference chemicals. ATLA 26, Suppl. 2, 569–615.

Ekwall, B., Barile., F.A., Castano, A., Clemedson, C., Clothier, R.H., Dierickx, P., Ekwall, B., Ferro, M., Fiskesjo;, G., Garza-Ocanas, L., Gomez-Lechon, M-J., Gulden, M., Hall, T., Isomaa, B., Kahru, A., Kerszman, G., Kristen, U., Kunimoto, M., Karenlampi, S., Lewan, L, Loukianov, A., Ohno, T., Persoone, G., Romert, L., Sawyer, T.W., Segner, H., Shrivastava, R., Stammati, A., Tanaka, N., Valentino, M., Walum, E., and Zucco, F. (1998) MEIC evaluation of acute systemic toxicity. Part VI. Prediction of human toxicity by rodent LD50 values and results from 61 *in vitro* tests. ATLA 26, Suppl. 2, 617–658.

Ekwall, B., Clemedson, C., Ekwall, B., Ring, P., and Romert, L. (1999) EDIT: A new international multicentre programme to develop and evaluate batteries of *in vitro* tests for acute and chronic systemic toxicity. ATLA 27, 339–349.

Ekwall, B., Ekwall, B., and Sjostrom, M. (2000) MEIC evaluation of acute systemic toxicity: Part VIII. Multivariate partial least squares evaluation, including the selection of a battery cell line tests with a good prediction of human acute lethal peak blood concentrations for 50 chemicals. ATLA 28, Suppl. 1, 201–234.

Hellberg, S., Bondesson, I., Ekwall, B., Gomez-Lechon, M.J., Jover, R., Hogberg, J., Ponsoda, X., Romert, L., Stenberg, K., and Walum, E. (1990) Multivariate validation of cell toxicity data: The first ten MEIC chemicals. ATLA, 17, 237–238.

Hellberg, S., Eriksson, L., Jonsson, J., Lindgren, F., Sjostrom, M., Wold, S., Ekwall, B., Gomez-Lechon, J.M., Clothier, R., Accomando, N.J., Gimes, G., Barile, F.A., Nordin, M., Tyson, C.A., Dierickx, P., Shrivastava, R.S., Tingsleff-Skaanild, M., Garza-Ocanas, L., and Fiskesjo;, G. (1990) Analogy models for prediction of human toxicity. ATLA, 18, 103–116.

Shrivastava, R., Delomenie, C., Chevalier, A., John, G., Ekwall, B., Walum, E., and Massingham, R. (1992) Comparison of *in vivo* acute lethal potency and *in vitro* cytotoxicity of 48 chemicals. Cell Biol. Toxicol., 8(2), 157–170.

Spielmann, H., Genschow, E., Liebsch, M., and Halle, W. (1999) Determination of the starting dose for acute oral toxicity (LD50) testing in the up and down procedure (UDP) from cytotoxicity data. ATLA, 27(6), 957–966.

Walum, E, Nilsson, M, Clemedson, C. and Ekwall, B. (1995) The MEIC program and its implications for the prediction of acute human systemic toxicity, pp 275–282 In Proceedings of the World Congress on Alternatives and Animal Use in the Life Sciences: Education, Research, Testing. Alternative Methods in Toxicology and the Life Sciences, Vol. 11. Mary Ann Liebert, New York, 1995.

Dated: June 6, 2000.

Samuel H. Wilson,

Deputy Director, National Institute of Environmental Health Sciences.

[FR Doc. 00–14968 Filed 6–13–00; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH), National Toxicology Program (NTP); Notice of an International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity, co-sponsored by NIEHS, NTP and the U.S. Environmental Protection Agency (EPA): Workshop Agenda and Registration Information

SUMMARY: Pursuant to Public Law 103–43, notice is hereby given of a public meeting sponsored by NIEHS, the NTP, and the EPA, and coordinated by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). The agenda topic is a scientific workshop to assess the current status of *in vitro* test methods for evaluating the acute systemic toxicity potential of chemicals and to develop recommendations for future research, development, and validation studies. The workshop will take place on October 17–20, 2000, at the Hyatt Regency Crystal City Hotel, 2799 Jefferson Davis Highway, Arlington, VA, 22202. The meeting will be open to the public.

In a previous **Federal Register** notice (Vol. 65, No. 115, pp. 37400–37403), ICCVAM requested information and data that should be considered at the Workshop and nominations of expert scientists to participate in the Workshop. A preliminary list of relevant studies to be considered for the Workshop was also provided. As a result of this request, an ICCVAM interagency Workshop Organizing Committee has selected an international group of scientific experts to participate in this Workshop. NICEATM, in collaboration with ICCVAM, has developed a background summary of data and performance characteristics for available *in vitro* methods. This summary will be made available to invited expert scientists and the public before the Workshop. Requests for the summary can be made to the address given below. This notice provides an agenda, registration information, and updated details about the Workshop.

Workshop Background and Scope

A. Background

Acute toxicity testing is conducted to determine the hazards of various chemicals and products. This information is used to properly classify and label materials as to their lethality in accordance with an internationally harmonized system (OECD, 1998). Non-lethal endpoints may also be evaluated to identify potential target organ toxicity, toxicokinetic parameters, and dose-response relationships. While animals are currently used to evaluate acute toxicity, recent studies suggest that *in vitro* methods may also be helpful in predicting acute toxicity.

Studies by Spielmann *et al.* (1999) suggest that *in vitro* cytotoxicity methods may be useful in predicting a starting dose for *in vivo* studies, and thus may potentially reduce the number of animals necessary for such determinations. Other studies (*e.g.,* Ekwall *et al.,* 2000) have indicated an association between chemical concentrations leading to *in vitro* cytotoxicity and human lethal blood concentrations. A program to assess toxicokinetics and target organ toxicity utilizing *in vitro* methods has been proposed that may provide enhanced predictions of toxicity and potentially reduce or replace animal use for some tests (Ekwall *et al.,* 1999). However, many of the necessary *in vitro* methods for this program have not yet been developed. Other methods have not been evaluated in validation studies to determine their usefulness and limitations for generating information to meet regulatory requirements for acute toxicity testing. Development and validation of *in vitro* methods which can establish accurate dose-response relationships will be necessary before such methods can be considered for the reduction or replacement of animal use for acute toxicity determinations.

This workshop will examine the status of available *in vitro* methods for assessing acute toxicity. This includes screening methods for acute toxicity, such as methods that may be used to predict the starting dose for *in vivo* animal studies, and methods for generating information on toxicokinetics, target organ toxicity, and mechanisms of toxicity. The workshop will develop recommendations for validation efforts necessary to characterize the usefulness and limitations of these methods. Recommendations will also be developed for future mechanism-based research and development efforts that might further improve *in vitro* assessments of acute systemic lethal and non-lethal toxicity.

B. Objectives of the Workshop

Four major topics will be addressed:

- *In Vitro* Screening Methods for Assessing Acute Toxicity;

- *In Vitro* Methods for Toxicokinetic Determinations;
- *In Vitro* Methods for Predicting Organ Specific Toxicity; and
- Chemical Data Sets for Validation of *In Vitro* Acute Toxicity Test Methods.

The objectives of the meeting are to:

1. Review the status of *in vitro* methods for assessing acute systemic toxicity:

a. Review the validation status of available *in vitro* screening methods for their usefulness in estimating *in vivo* acute toxicity,

b. Review *in vitro* methods for predicting toxicokinetic parameters important to acute toxicity (*i.e.,* absorption, distribution, metabolism, elimination), and

c. Review *in vitro* methods for predicting specific target organ toxicity;

2. Recommend candidate methods for further evaluation in prevalidation and validation studies;

3. Recommend validation study designs that can be used to characterize adequately the usefulness and limitations of proposed *in vitro* methods;

4. Identify reference chemicals that can be used for development and validation of *in vitro* methods for assessing *in vivo* acute toxicity; and

5. Identify priority research efforts necessary to support the development of mechanism-based *in vitro* methods to assess acute systemic toxicity. Such efforts might include incorporation and evaluation of new technologies, such as gene microarrays, and development of methods necessary to generate dose response information.

Workshop Information

A. Workshop Agenda

Tuesday, October 17, 2000

8:30 a.m.—Opening Plenary Session
- Workshop Introduction
- Welcome from the National Toxicology Program (NTP)
- Overview of ICCVAM and NICEATM
- Acute Toxicity: Historical and Current Regulatory Perspectives
- Acute Toxicity Data: A Clinical Perspective

10:30 a.m.—*In Vitro* Approaches to Estimate the Acute Toxicity Potential of Chemicals
- Estimating Starting Doses for *In Vivo* Studies using *In Vitro* Data
- An Integrated Approach for Predicting Systemic Toxicity
- Opportunities for Future Progress

Public Comment
Breakout Groups' Charges
12:30 p.m.—Lunch Break
1:45 p.m.—Breakout Groups: Identifying What Is Needed from *In Vitro* Methods
- Screening Methods;
- Toxicokinetic Determinations;
- Predicting Organ Specific Toxicity and Mechanisms; and
- Chemical Data Sets for Validation

5:30 p.m.—Adjourn for the Day

Wednesday, October 18, 2000

8:00 a.m.—Plenary Session—Status Reports by Breakout Group Co-Chairs
9:00 a.m.—Breakout Groups: Current Status of *In Vitro* Methods for Acute Toxicity
- Screening Methods;
- Toxicokinetic Determinations;
- Predicting Organ Specific Toxicity and Mechanisms; and
- Chemical Data Sets for Validation

12:00 p.m.—Lunch Break
1:30 p.m.—Breakout Groups: Current Status of *In Vitro* Methods for Acute Toxicity (Cont'd)
5:30 p.m.—Adjourn for the Day

Thursday, October 19, 2000

8:00 a.m.—Plenary Session—Status Reports by Breakout Group Co-Chairs
9:00 a.m.—Breakout Groups: Future Directions for *In Vitro* Methods for Acute Toxicity
- Screening Methods;
- Toxicokinetic Determinations;
- Predicting Organ Specific Toxicity and Mechanisms; and
- Chemical Data Sets for Validation

12:00 p.m.—Lunch Break
1:30 p.m.—Breakout Groups: Future Directions for *In Vitro* Methods for Acute Toxicity (Cont'd)
5:30 p.m.—Adjourn for the Day

Friday, October 20, 2000

8:00 a.m.—Closing Plenary Session—Reports by Breakout Group Co-Chairs
- Screening Methods;
- Toxicokinetic Determinations;
- Predicting Organ Specific Toxicity and Mechanisms; and
- Chemical Data Sets for Validation

Public Comment
Closing Comments
12:15 p.m.—Adjourn

B. Workshop Registration

The Workshop meeting will be open to the public, limited only by the space available. Due to space limitations, advance registration is requested by October 13, 2000. Registration forms can be obtained by contacting NICEATM at the address given below or by accessing the on-line registration form at: *http://iccvam.niehs.nih.gov/invi_reg.htm.* Other relevant Workshop information (*i.e.,* accommodations, transportation, etc.) is also provided at this website.

C. Public Comment

The Public is invited to attend the Workshop and the number of observers will be limited only by the space available. Two formal public comment sessions on Tuesday, October 17th and Friday, October 20th will provide an opportunity for interested persons or groups to present their views and comments to the Workshop participants (please limit to one speaker per group). Additionally, time will be allotted during each of the Breakout Group sessions for general discussion and comments from observers and other participants. The Public is invited to present oral comments or to submit comments in writing for distribution to the Breakout Groups to NICEATM at the address given below by October 13, 2000. Oral presentations will be limited to seven minutes per speaker to allow for a maximum number of presentations. Individuals presenting oral comments are asked to provide a hard copy of their statement at registration. For planning purposes, persons wishing to give oral comments are asked to check the box provided on the Registration Form, although requests for oral presentations will also be accepted on-site (subject to availability of time). Persons registering for oral comments or submitting written remarks are asked to include their contact information (name, address, affiliation, telephone, fax, and e-mail).

Guidelines for Requesting Registration Form and Submission of Public Comment

Requests for registration information and submission of public comments should be directed to the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods, Environmental Toxicology Program, NIEHS/NTP, MD EC–17, PO Box 12233, Research Triangle Park, NC 27709; 919–541–3398 (phone); 919–541–0947 (fax); *iccvam@niehs.nih.gov* (e-mail). Public comments should be accompanied by complete contact information including name, (affiliation, if applicable), address, telephone number, and e-mail address.

References

- OECD (Organisation for Economic Cooperation and Development). (1998). Harmonized integrated hazard classification system for human health and environmental effects of chemical substances. OECD, Paris. (website: *http://www.oecd.org//ehs/Class/HCL6.HTM*)
- Spielmann, H., Genschow, E., Leibsch, M., and Halle, W. (1999) Determination of the starting dose for

acute oral toxicity (LD50) testing in the up and down procedure (UDP) from cytotoxicity data. ATLA, 27(6), 957–966.

- Ekwall, B., Ekwall, B., and Sjorstrom, M. (2000) MEIC evaluation of acute systemic toxicity: Part VIII. Multivariate partial least squares evaluation, including the selection of a battery of cell line tests with a good prediction of human acute lethal peak blood concentrations for 50 chemicals. ATLA, 28, Suppl. 1, 201–234.
- Ekwall, B., Clemedson, C., Ekwall, B., Ring, P., and Romert, L. (1999) EDIT: A new international multicentre programme to develop and evaluate batteries of *in vitro* tests for acute and chronic systemic toxicity. ATLA 27, 339–349.

Dated: September 12, 2000.

Samuel H. Wilson,

Deputy Director, National Institute of Environmental Health Sciences.

[FR Doc. 00–24244 Filed 9–20–00; 8:45 am]

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

National Institute of Environmental Health Sciences (NIEHS); National Toxicology Program (NTP)

Report of the International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity; Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity: Notice of Availability and Request for Public Comment.

Summary

Notice is hereby given of the availability of the reports entitled, ''Report of the International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity'' NIH Publication 01–4499 and ''Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity'' NIH Publication 01–4500. The Report provides conclusions and recommendations from expert scientists based on their review of current in vitro methods for assessing acute toxicity at an October 17–20, 2000 workshop. The workshop was organized by the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). The Guidance Document

provides Standard Operating Procedures (SOPs) for performing two in vitro basal cytotoxicity assays and describes how to use this in vitro data to predict starting doses for in vivo acute oral toxicity studies.

Availability of the Documents

To receive a copy of either report, please contact NICEATM at P.O. Box 12233, MD EC–17, Research Triangle Park, NC 27709 (mail), 919–541–3398 (phone), 919–541–0947 (fax), or *niceatm@niehs.nih.gov* (email). The reports are also available on the ICCVAM/NICEATM website at *http://iccvam.niehs.nih.gov.*

Request for Public Comments

NICEATM invites written public comments on the Workshop Report and the Guidance Document. Comments should be sent to NICEATM by November 13, 2001. Comments submitted via e-mail are preferred; the acceptable file formats are MS Word (Office 98 or older), plain text, or PDF. Comments should be sent to Dr. William S. Stokes, Director, NICEATM, NIEHS, MD EC–17, PO Box 12233, Research Triangle Park, NC, 27709; telephone 919–541–2384; fax 919–541–0947; e-mail *niceatm@niehs.nih.gov.* Persons submitting written comments should include their contact information (name, affiliation, address, telephone and fax numbers, and e-mail) and sponsoring organization, if any. Public comments received in response to this **Federal Register** notice will be posted on the NICEATM/ICCVAM web site (*http://iccvam.niehs.nih.gov*).

Background

The International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity was held October 17–20, 2000, at the Hyatt Regency Crystal City Hotel, 2799 Jefferson Davis Highway, Arlington, VA 22202. The workshop was organized by the NICEATM and ICCVAM, and sponsored by the NIEHS, the NTP, and U.S. EPA. The objectives of the workshop were (1) to assess the current validation status of in vitro test methods that might be useful for assessing the acute systemic toxicity potential of chemicals and (2) to develop recommendations for future research, development, and validation studies that might further enhance the use of in vitro methods for this purpose.

A **Federal Register** notice (Vol. 65, No. 115, pp. 37400–37403, June 14, 2000) requested information and data that should be considered at the workshop, and nominations of expert scientists to participate in the workshop. A second **Federal Register** notice (Vol. 65, No. 184, pp. 57203–57205, September 21, 2000) announced availability of the workshop agenda, registration information, and a background summary of available in vitro methods.

At the workshop, the invited expert scientists were divided into four breakout groups as follows:

Breakout Group 1: In Vitro Screening Methods for Assessing Acute Toxicity
Breakout Group 2: In Vitro Methods for Toxicokinetic Determinations
Breakout Group 3: In Vitro Methods for Predicting Organ-Specific Toxicity
Breakout Group 4: Chemical Data Sets for Validation of In Vitro Acute Toxicity Test Methods

Each breakout group subsequently prepared a written report that represented the consensus of the invited scientists assigned to that group and these reports are included in the Workshop Report. It also includes as appendices: A detailed workshop agenda; summary minutes of plenary sessions and public comments; the background document for workshop participants; a NICEATM summary of the Multicenter Evaluation of In Vitro Cytotoxicity (MEIC); a summary of Federal regulations on acute toxicity; related **Federal Register** notices; and ICCVAM test method recommendations. The ICCVAM test recommendations were developed following the workshop to forward to Federal agencies in accordance with Pub. L. 106–545.

The Breakout Group on In Vitro Screening Methods recommended preparation of a document that would provide guidance on how to use in vitro data to estimate starting doses for in vivo acute toxicity studies. Three scientists subsequently collaborated with the NICEATM to develop a ''Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity''. The Guidance Document provides SOPs for conducting two in vitro cytotoxicity tests (the BALB/c 3T3 Neutral Red Uptake (NRU) and the Normal Human Keratinocyte (NHK) NRU assays) and instruction for using these assays to estimate starting doses for in vivo testing. The Guidance Document also includes the ZEBET (German National Centre for the Documentation and Evaluation of Alternatives to Animal Experimentation) Registry of Cytotoxicity (RC) Regression Analysis that provides a mathematical relationship between acute oral systemic rodent toxicity and in vitro basal cytotoxicity using data for 347 chemicals (Halle, 1998; Spielmann et al., 1999). The Guidance Document expands on an approach suggested by Spielmann and colleagues that—as an initial step—the relationship found with the RC data be used to predict starting doses for subsequent in vivo acute lethality assays.

Additional Information About ICCVAM and NICEATM

ICCVAM, with 15 participating Federal agencies, was established in 1997 to coordinate interagency issues on toxicological test method development, validation, regulatory acceptance, and national and international harmonization. The ICCVAM Authorization Act of 2000 (Pub. L. 106–545) formally authorized and designated ICCVAM as a permanent committee administered by the NIEHS with specific duties that include the technical evaluation of new and alternative testing methods. ICCVAM is charged with developing test recommendations based on those technical evaluations, and forwarding these to Federal agencies for their consideration. The NICEATM was established in 1998 to coordinate and facilitate ICCVAM activities, to provide peer review for validation activities and to promote communication with stakeholders. The NICEATM is located at the NIEHS, Research Triangle Park, NC. Additional information concerning ICCVAM and NICEATM can be found on the ICCVAM/NICEATM web site at *http://iccvam.niehs.nih.gov.* In accordance with Public Law 106–545, the Workshop Report and the Guidance Document will be forwarded with ICCVAM test recommendations to Federal agencies for their consideration.

References

Halle, W. 1998. Toxizitätsprüfungen in Zellkulturen für eine Vorhersage der akuten Toxizität (LD_{50}) zur Einsparung von Tierversuchen. Life Sciences/ Lebenswissenschaften, Volume 1, 94 pp., Jülich: Forschungszentrum Jülich.

Spielmann, H., E. Genschow, M. Liebsch, and W. Halle. 1999. Determination of the starting dose for acute oral toxicity (LD_{50}) testing in the up and down procedure (UDP) from cytotoxicity data. ATLA 27: 957–966.

Dated: September 18, 2001.

Samuel H. Wilson,

Deputy Director, National Institute of Environmental Health Sciences.

[FR Doc. 01–24370 Filed 9–27–01; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

National Institute of Environmental Health Sciences (NIEHS); National Toxicology Program (NTP); Notice of the Availability of Agency Responses to ICCVAM Test Recommendations for the Revised Up-and-Down Procedure for Determining Acute Oral Toxicity and In Vitro Methods for Assessing Acute Systemic Toxicity

Summary

The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) announces the availability of Federal agency responses to Interagency Coordinating Committee on the Validation of Alternative Methods

(ICCVAM) test recommendations for: (1) The revised Up-and-Down Procedure (UDP) for determining acute oral toxicity and (2) *in vitro* methods for assessing acute systemic toxicity. Pursuant to sections 3 of the ICCVAM Authorization Act of 2000 [Pub. L. 106–545 (42 U.S.C. 285l–4)], ICCVAM is required to make final ICCVAM test recommendations and the responses from agencies regarding such recommendations available to the public.

Availability of Agency Responses

The agency responses to the ICCVAM test recommendations and other current information relevant to these test recommendations are available electronically (PDF and HTML formats) on the NICEATM/ICCVAM Web site at *http://iccvam.niehs.nih.gov.* Hard copy versions of these responses can be requested by contacting NICEATM at P.O. Box 12233, MD EC–17, Research Triangle Park, NC 27709 (mail), 919–541–2384 (telephone), 919–541–0947 (fax), or *niceatm@niehs.nih.gov.*

In summary, the Federal agencies agreed that the UDP had been adequately validated as a replacement for the conventional LD50 test and indicated to the extent applicable, that they will encourage the use of *in vitro* tests for determining starting doses for acute systemic toxicity testing.

ICCVAM Recommmendations

NICEATM announced availability of the ICCVAM recommendations for the UDP on February 7, 2002 (**Federal Register** Vol. 67, No. 26, pages 5842–5844). ICCVAM recommends based upon 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 the ICCVAM and NICEATM,* NIH Publication No. 02–4501, that the UDP be used instead of the conventional LD50 test to determine the acute oral toxicity hazard of chemicals for hazard classification and labeling purposes.

NICEATM announced availability of the ICCVAM recommendations for the *in vitro* methods for assessing acute systemic toxicity on September 28, 2001 (**Federal Register** Vol. 66, No. 189, pages 49686–49687). ICCVAM recommends based upon the reports, *Report of the International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity,* NIH Publication No. 01–4499, and the *Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity,* NIH Publication No. 01–4500, that the *in vitro* methods be considered as a tool for estimating starting doses for animal tests of acute systemic toxicity.

Background Information on ICCVAM and NICEATM

The NIEHS established the ICCVAM in 1997 to coordinate the interagency technical review of new, revised, and alternative test methods of interagency interest, and to coordinate cross-agency issues relating to the validation, acceptance, and national/international harmonization of toxicological testing methods. ICCVAM was established as a permanent interagency committee of the NIEHS under the NICEATM on December 19, 2000, by the ICCVAM Authorization Act of 2000 (Pub. L. 106–545, available at *http://iccvam.niehs.nih.gov/about/PL106545.pdf*). The Committee is composed of representatives from fifteen Federal regulatory and research agencies that use or generate toxicological information. ICCVAM promotes the scientific validation and regulatory acceptance of toxicological test methods that will improve agencies' ability to accurately assess the safety or hazards of chemicals and various types of products, while refining (less pain and distress), reducing, and replacing animal use wherever possible. NICEATM administers the ICCVAM and provides scientific and operational 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.*

Dated: March 2, 2004.

Samuel H. Wilson,

Deputy Director, National Institute of Environmental Health Sciences.

[FR Doc. 04–5321 Filed 3–9–04; 8:45 am]

BILLING CODE 4140–01–P

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/.*

Dated: October 6, 2004.

Samuel H. Wilson,

Deputy Director, National Institute of Environmental Health Sciences.

[FR Doc. 04–23335 Filed 10–18–04; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

National Toxicology Program; National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM); Request for Nominations for an Independent Peer Review Panel To Evaluate In Vitro Testing Methods for Estimating Acute Oral Systemic Toxicity and Request for In Vivo and In Vitro Data

AGENCY: National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH), HHS.

ACTION: Request for nominations for an independent peer review panel and request for *in vivo* and *in vitro* data.

SUMMARY: The NTP Interagency Center for Evaluation of Alternative Toxicological Methods (NICEATM) in collaboration with the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) is planning to convene an independent peer review panel (hereafter, Panel) to evaluate the validation status of two *in vitro* cytotoxicity assays for estimating *in vivo* acute oral toxicity. The Panel will evaluate the usefulness, limitations, accuracy, and reliability of these test methods for their intended purpose. NICEATM requests nominations of expert scientists for consideration as potential Panel members. ICCVAM will consider the conclusions and recommendations from the Panel in developing test method recommendations and performance standards for these test methods. Data from standard *in vivo* acute oral toxicity testing and *in vitro* cytotoxicity testing also is requested.

DATES: Nominations and data should be received by noon on May 6, 2005.

ADDRESSES: Nominations and data should be sent by mail, fax, or e-mail to Dr. William S. Stokes, Director of NICEATM, at NICEATM, NIEHS, P.O. Box 12233, MD EC–17, Research Triangle Park, NC 27709, (phone) 919–541–2384, (fax) 919–541–0947, (e-mail) *niceatm@niehs.nih.gov.* Courier address: NICEATM, 79 T.W. Alexander Drive,

Building 4401, Room 3128, Research Triangle Park, NC 27709.

FOR FURTHER INFORMATION CONTACT: NICEATM, NIEHS, P.O. Box 12233, MD EC–17, Research Triangle Park, NC 27709, (phone) 919–541–2384, (fax) 919–541–0947, (e-mail) *niceatm@niehs.nih.gov.*

SUPPLEMENTARY INFORMATION:

Background

NICEATM and the European Committee on the Validation of Alternative Methods (ECVAM) conducted a collaborative validation study to independently evaluate the usefulness of two *in vitro* basal cytotoxicity assays proposed for estimating *in vivo* rat acute oral toxicity. Neutral red uptake assays using both a mouse cell line (*i.e.,* BALB/c 3T3 fibroblasts) and a primary human cell type (*i.e.,* normal human epithelial keratinocytes) were evaluated in a multi-laboratory validation study. Cytotoxicity results are proposed for use in predicting starting doses for *in vivo* acute oral lethality assays, which may reduce the number of animals required for such determinations.

NICEATM is preparing Background Review Documents on the two *in vitro* test methods that will contain comprehensive summaries of available data, an analysis of the accuracy and reliability of standardized test method protocols, and related information characterizing the current validation status of these assays. Once completed, the Background Review Documents will be provided to the Panel and made available to the public. Meeting information, including date and location, and public availability of the Background Review Documents will be announced in a future **Federal Register** notice and posted on the ICCVAM/NICEATM Web site (*http://iccvam.niehs.nih.gov*).

Request for the Nomination of Scientists for the Peer Review Panel

NICEATM invites nominations of scientists with relevant knowledge and experience to serve on the Panel. Areas of relevant expertise include, but are not limited to: physiology and pharmacology, acute systemic toxicity testing in animals, evaluation and treatment of acute toxicity in humans, development and use of *in vitro* methodologies, biostatistical data analysis, knowledge of chemical data sets useful for validation of acute toxicity studies, and hazard classification of chemicals and products. Each nomination should include the person's name, affiliation, contact information (*i.e.* mailing address, e-mail address, telephone and fax numbers), and a brief summary of relevant experience and qualifications. Nominations should be sent to NICEATM by mail, fax, or e-mail within 45 days of the publication of this notice. Correspondence should be directed to Dr. William Stokes, Director, NICEATM, at the address given above.

Request for Data

NICEATM invites the submission of data from standard *in vivo* acute oral toxicity testing and *in vitro* cytotoxicity testing. Two previous requests for existing *in vivo* and *in vitro* acute toxicity data have been made (**Federal Register,** Vol. 69, No. 201, pp. 61504–5, October 19, 2004 and Vol. 65, No. 115, pp. 37400–3, June 14, 2000). *In vivo* and *in vitro* acute toxicity testing data for chemicals or products should be sent to NICEATM by mail, fax, or e-mail to the address given above. Data submitted by the deadline listed in this notice will be considered during an evaluation of the validation status of the two cytotoxicity methods, anticipated in late 2005; however, data will be accepted at any time. 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 class.
- 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.

Background Information on ICCVAM and NICEATM

ICCVAM is an interagency committee composed of representatives from 15 Federal regulatory and research agencies that use or generate toxicological information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability and promotes the scientific validation and regulatory acceptance of toxicological test methods that more accurately assess the safety and hazards of chemicals and products and that refine, reduce, and replace animal use. The ICCVAM Authorization Act of 2000 (Pub. L. 106–545, available at *http://iccvam.niehs.nih.gov/about/PL106545.htm*) establishes ICCVAM as a permanent interagency committee of the NIEHS under the NICEATM. NICEATM administers the ICCVAM and provides scientific and operational support for 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.*

Dated: March 11, 2005.

Samuel H. Wilson,

Deputy Director, National Institute of Environmental Health Sciences.

[FR Doc. 05–5564 Filed 3–21–05; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Toxicology Program (NTP), NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM); Announcement of an Independent Scientific Peer Review Meeting on the Use of In Vitro Testing Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Tests and Request for Comments

AGENCY: National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH).

ACTION: Meeting Announcement and Request for Comment.

SUMMARY: NICEATM in collaboration with the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) announces a public, independent, scientific peer review meeting to evaluate the validation status of the *in vitro* 3T3 and normal human keratinocyte (NHK) neutral red uptake (NRU) basal cytotoxicity test methods for estimating starting doses for *in vivo* acute oral toxicity tests. These two *in vitro* cytotoxicity test methods are proposed as adjuncts to the *in vivo* acute oral toxicity tests to refine (i.e., to lessen or avoid pain and distress) and/or reduce animal use. At this meeting, a scientific peer review panel (''Panel'') will peer review the background review document (BRD) on the 3T3 and NHK cytotoxicity test methods, evaluate the extent that the BRD addresses established validation and acceptance criteria, and provide comment on the draft ICCVAM recommendations on the proposed use of these test methods, draft test method protocols, and draft performance standards. NICEATM requests public comments on the BRD, draft ICCVAM test method recommendations, draft test method protocols, and draft performance standards.

DATES: The meeting will be held on May 23, 2006, from 8:30 a.m. to 5 p.m. The meeting is open to the public with attendance limited only by the space available. In order to facilitate planning for this meeting, persons wishing to attend the meeting are asked to register via the ICCVAM/NICEATM Web site (*http://iccvam.niehs.nih.gov*) by May 12, 2006.

ADDRESSES: The meeting will be held at the National Institutes of Health (NIH), Natcher Conference Center, 45 Center Drive, Bethesda, MD 20892.

FOR FURTHER INFORMATION CONTACT: Correspondence should be sent by mail, fax, or email to Dr. William S. Stokes, NICEATM Director, NIEHS, P.O. Box 12233, MD EC–17, Research Triangle Park, NC 27709, (phone) 919–541–2384, (fax) 919–541–0947, (e-mail) *niceatm@niehs.nih.gov*, Courier address: NICEATM, 79 T.W. Alexander Drive, Building 4401, Room 3128, Research Triangle Park, NC 27709.

SUPPLEMENTARY INFORMATION:

Background

In September 2001, ICCVAM recommended that *in vitro* basal cytotoxicity test methods be considered as tools for estimating starting doses for *in vivo* acute systemic toxicity studies (**Federal Register** Vol. 66, No. 189, pp. 49686–7, 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 procedures for two *in vitro* basal cytotoxicity test methods and instructions for using these test methods to estimate starting doses for *in vivo* testing.

U.S. Federal agencies' responses to the ICCVAM recommendations from the International Workshop were announced in 2004 (**Federal Register** Vol. 69, No. 47, pp. 11448–9, March 10, 2004). The U.S. Federal agencies agreed to encourage, to the extent applicable, the use of *in vitro* tests for determining starting doses for acute oral systemic toxicity testing. Furthermore, the U.S. Environmental Protection Agency (EPA) specifically encouraged those participating in the High Production Volume Challenge Program to consider using the recommended *in vitro* test methods as a supplemental component when conducting any new *in vivo* acute oral toxicity studies for the program (*http://www.epa.gov/chemrtk/toxprtow.htm*).

In 2002, NICEATM and the European Committee on the Validation of Alternative Methods began a collaborative validation study to independently evaluate the usefulness of two *in vitro* basal cytotoxicity test methods proposed for estimating starting doses for *in vivo* rodent acute oral toxicity tests. *In vitro* NRU cytotoxicity test methods using either BALB/c 3T3 fibroblasts, a mouse cell line, or NHK cells, primary human epidermal cells, were evaluated in a multi-laboratory international validation study. During the pre-validation phases of the study, the test method protocols were standardized further and revised to improve their intra- and inter-laboratory reproducibilities. NICEATM recommended using the revised test method protocols (**Federal Register**, Vol. 69, No. 201, pp. 61504–5, October 19, 2004) rather than the standard procedures outlined in the guidance document (ICCVAM, 2001b). During the validation study, 72 reference chemicals were tested using the 3T3 and NHK NRU test methods. The *in vitro* NRU cytotoxicity test results were used to estimate acute oral LD_{50} values, which in turn were used to identify the starting doses for simulated acute oral toxicity testing using the Up-and-Down Procedure (UDP; EPA 2002; OECD 2001a) and the Acute Toxic Class method (ATC; OECD 2001b). The *in vivo* test simulations were used to compare the number of animals used and the number of deaths expected to occur when starting with the default starting doses versus using a starting dose based on *in vitro* cytotoxicity data.

To assist in an evaluation of the usefulness of these two *in vitro* NRU basal cytotoxicity test methods for estimating starting doses for *in vivo*. acute oral toxicity tests, NICEATM requested the submission of existing *in vivo* and *in vitro* acute toxicity data (**Federal Register,** Vol. 69, No. 201, pp. 61504–5, October 19, 2004 and Vol. 65, No. 115, pp. 37400–3, June 14, 2000). In 2005, NICEATM announced a request for nominations of scientists to serve on the Panel and again requested existing *in vivo* and *in vitro* data (**Federal Register** Vol. 70, No. 54, pp. 14473–4, March 22, 2005).

Expert Panel Meeting

The purpose of this meeting is the scientific peer review evaluation of the validation status of the 3T3 and NHK NRU basal cytotoxicity test methods to determine starting doses for the UDP and ATC acute oral toxicity test methods in order to refine and reduce the use of animals. The Panel will first peer review the BRD on the 3T3 and NHK cytotoxicity test methods and then evaluate the extent that the BRDs address established validation and acceptance criteria (*Validation and Regulatory Acceptance of Toxicological Test Methods: A Report of the ad hoc Interagency Coordinating Committee on the Validation of Alternative Methods,* NIH Publication No. 97–3981, *http://iccvam.niehs.nih.gov*). The Panel will also be asked to provide comment on the draft ICCVAM test method recommendations, draft standardized test method protocols, and draft performance standards. Information about the Panel meeting, including a roster of the members of the Panel and the agenda, will be made available two weeks prior to the meeting on the ICCVAM/NICETATM Web site (*http://iccvam.niehs.nih.gov*) or can be obtained after that date by contacting NICEATM (see **FOR FURTHER INFORMATION CONTACT** above).

Attendance and Registration

The public Panel meeting will take place May 23, 2006, at the NIH Campus, Natcher Conference Center, Bethesda, MD (a map of the NIH Campus and other visitor information are available at *http://www.nih.gov/about/visitor/index.htm*). The meeting will begin at 8:30 a.m. and conclude at approximately 5 p.m. Persons needing special assistance, such as sign language interpretation or other reasonable accommodation in order to attend, should contact 919–541–2475 voice, 919–541–4644 TTY (text telephone), through the Federal TTY Relay System at 800–877–8339, or by e-mail to *niehsoeeo@niehs.nih.gov*. Requests should be made at least seven business days in advance of the event.

Availability of the BRD and Draft ICCVAM Recommendations

NICEATM prepared a BRD on the 3T3 and NHK NRU basal cytotoxicity test methods that contains comprehensive summaries of the data generated in the validation study, an analysis of the accuracy and reliability of the two test methods, a simulation analysis of the refinement and reduction in animal use that would occur if these tests were used as adjuncts to the UDP and ATC acute oral systemic toxicity test methods, and related information characterizing the validation status of these assays. The BRD, draft ICCVAM test method recommendations, draft test method protocols, and draft test method performance standards will be provided to the Panel and made available to the public. Copies of these materials can be obtained from the ICCVAM/NICEATM Web site (*http://iccvam.niehs.nih.gov*) or by contacting NICEATM (see **FOR FURTHER INFORMATION CONTACT** above).

Request for Comments

NICEATM invites the submission of written comments on the BRD, draft ICCVAM test method recommendations, draft test method protocols, and draft test method performance standards. When submitting written comments, it is important to refer to this **Federal Register** notice and include appropriate contact information (name, affiliation, mailing address, phone, fax, email and sponsoring organization, if applicable). Written comments should be sent by mail, fax, or email to Dr. William Stokes, Director of NICEATM, at the address listed above not later than May 5, 2006. All comments received will be placed on the ICCVAM/NICEATM website and made available to the Panel, ICCVAM agency representatives, and attendees at the meeting.

This meeting is open to the public and time will be provided for the presentation of public oral comments at designated times during the peer review. Members of the public who wish to present oral statements at the meeting (one speaker per organization) should contact NICEATM (see **FOR FURTHER INFORMATION CONTACT** above) no later than May 12, 2006. Speakers will be assigned on a consecutive basis and up to seven minutes will be allotted per speaker. Persons registering to make comments are asked to provide a written copy of their statement by May 12, 2006, so that copies can be distributed to the Panel prior to the meeting or if this is not possible to bring 40 copies to the meeting. Written statements can supplement and expand the oral presentation. Each speaker is asked to

provide contact information (name, affiliation, mailing address, phone, fax, email and sponsoring organization, if applicable) when registering to make oral comments.

Summary minutes and a final report of the Panel will be available following the meeting at the ICCVAM/NICEATM Web site (*http://iccvam.niehs.nih.gov*). ICCVAM will consider the conclusions and recommendations from the Panel and any public comments received in finalizing test method recommendations and performance standards for these test methods.

Background Information on ICCVAM and NICEATM

ICCVAM is an interagency committee composed of representatives from 15 U.S. Federal regulatory and research agencies that use or generate toxicological information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability and promotes the scientific validation and regulatory acceptance of toxicological test methods that more accurately assess the safety and hazards of chemicals and products while refining (less pain and distress), reducing, and replacing animal use. The ICCVAM Authorization Act of 2000 (Pub. L. 106–545, available at *http://iccvam.niehs.nih.gov/about/PL106545.htm*) establishes ICCVAM as a permanent interagency committee of the NIEHS under the NICEATM. NICEATM administers the ICCVAM and provides scientific and operational support for ICCVAM-related activities. NICEATM and ICCVAM work collaboratively to evaluate new and improved test methods applicable to the needs of U.S. Federal agencies. Additional information about ICCVAM and NICEATM can be found at the ICCVAM/NICEATM Web site: *http://iccvam.niehs.nih.gov.*

References

EPA. 2002a. Health Effects Test Guidelines OPPTS 870.1100 Acute Oral Toxicity. EPA 712–C–02–190. Washington, DC: U.S. Environmental Protection Agency.

ICCVAM. 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/.* OECD. 2001a. Guideline for Testing of Chemicals, 425, Acute Oral Toxicity—Up-and-Down Procedure. Paris France: OECD. Available at: *http://www.oecd.org* [accessed June 2, 2004]. OECD. 2001b. Guideline For Testing of Chemicals, 423, Acute Oral Toxicity—Acute Toxic Class Method. Paris France: OECD.

Dated: March 9, 2006.

Samuel H. Wilson,

Deputy Director, National Institute of Environmental Health Sciences and National Toxicology Program.

[FR Doc. E6–4075 Filed 3–20–06; 8:45 am]

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

National Toxicology Program (NTP), NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM); Availability of Peer Review Panel Report on the Use of *In Vitro* Basal Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Testing and Request for Comments

AGENCY: National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH).

ACTION: Request for comments.

SUMMARY: The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), in collaboration with the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), organized an independent, scientific peer review meeting on May 23, 2006, to evaluate the validation status of the *in vitro* 3T3 and normal human keratinocyte (NHK) neutral red uptake (NRU) basal cytotoxicity test methods. These two *in vitro* cytotoxicity test methods are proposed as adjuncts (for the purpose of determining the starting dose) to *in vivo* acute oral toxicity tests. The peer review report from this meeting, entitled *Peer Review Panel Evaluation of the Use of In Vitro Basal Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Testing*, is now available. The report contains (1) a summary of the peer review evaluation and (2) the peer review panel's (Panel) conclusions on the draft ICCVAM test method recommendations regarding the proposed usefulness, limitations, and validation status of the 3T3 and NHK cytotoxicity test methods. The NICEATM invites public comment on the Panel's conclusions on the draft ICCVAM test method recommendations. Copies of the Panel report may be obtained on the ICCVAM/NICEATM Web site at *http://iccvam.niehs.nih.gov*, or by contacting NICEATM at the address given below.

DATES: Written comments should be received at NICEATM by August 25, 2006.

ADDRESSES: Public comments and any other correspondence should be sent by mail, fax, or e-mail to Dr. William S. Stokes, NICEATM, NIEHS, P. O. Box 12233, MD EC–17, Research Triangle Park, NC, 27709, (phone) 919–541–2384, (fax) 919–541–0947, (e-mail) niceatm@niehs.nih.gov.

SUPPLEMENTARY INFORMATION:

Background

The 3T3 and NHK cytotoxicity test methods are proposed as adjuncts (for the purpose of determining the starting dose) to *in vivo* acute oral toxicity test methods (*i.e.*, the Up-and-Down Procedure [EPA 2002a; OECD 2001a], the Acute Toxic Class method [OECD 2001b]) to refine (*i.e.*, to lessen or avoid pain and distress) and/or reduce animal use. Both *in vitro* cytotoxicity test methods have been assessed in a NICEATM and European Centre on the Validation of Alternative Methods (ECVAM) collaborative independent validation study. At this peer review meeting, the Panel reviewed the background review document (BRD) on the 3T3 and NHK cytotoxicity test methods and evaluated the extent that established validation and acceptance criteria had been adequately addressed for the intended purpose of the test methods. The Panel also provided comments on draft ICCVAM recommendations regarding the proposed use of these test methods, draft test method protocols, draft performance standards, and draft recommended future studies. The Panel's conclusions and recommendations on the two *in vitro* cytotoxicity test methods are described in the *Peer Review Panel Evaluation of the Use of In Vitro Basal Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Testing* (available at *http://iccvam.niehs.nih.gov/*).

Prior to the Panel meeting, NICEATM issued **Federal Register** notices to (1) recommend that in vitro basal cytotoxicity test methods be considered as tools for estimating starting doses for in vivo acute systemic toxicity tests (66FR49686), (2) announce a request for nominations for Panel members and submission of existing in vivo and in vitro data (70FR14473), (3) announce the independent peer review meeting on the use of the 3T3 and NHK cytotoxicity test methods for estimating starting doses for acute oral systemic toxicity tests, and (4) request comments on the draft BRD and draft ICCVAM recommendations (71FR14229). All **Federal Register** notices, the draft BRD, and the draft ICCVAM recommendations are available at *http://iccvam.niehs.nih.gov/*.

Request for Comments

NICEATM invites the submission of written comments on the Panel's

conclusions on the draft ICCVAM test method recommendations. When submitting written comments please refer to this **Federal Register** notice and include appropriate contact information (name, affiliation, mailing address, phone, fax, e-mail and sponsoring organization, if applicable). All comments received by the deadline listed above will be placed on the ICCVAM/NICEATM Web site and made available to ICCVAM. In addition, there will be an opportunity for oral public comments on the draft ICCVAM test method recommendations for the 3T3 and NHK cytotoxicity test methods during a teleconference meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) scheduled for August 3, 2006. Details of the SACATM teleconference are published as a separate **Federal Register** notice (available at *http://ntp.niehs.nih.gov/go/frn*). Any written comments on the Panel report received prior to July 25, 2006, will be distributed to SACATM.

ICCVAM will consider the Panel report along with SACATM and public comments received on that report as it prepares final ICCVAM recommendations for the 3T3 and NHK cytotoxicity test methods. An ICCVAM test method evaluation report, which will include the final ICCVAM recommendations, will be forwarded to the appropriate federal agencies for their consideration. This report also will be available to the public on the ICCVAM/NICEATM website and by request from NICEATM.

Background Information on ICCVAM, NICEATM, and SACATM

ICCVAM is an interagency committee composed of representatives from 15 federal regulatory and research agencies that use or generate toxicological information. ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability and promotes the scientific validation and regulatory acceptance of toxicological test methods that more accurately assess the safety and hazards of chemicals and products and that refine, reduce, or replace animal use. The ICCVAM Authorization Act of 2000 [42 U.S.C. 285l-3(d)] establishes ICCVAM as a permanent interagency committee of the NIEHS under NICEATM. NICEATM administers ICCVAM and provides scientific and operational support for 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 ICCVAM-NICEATM Web site (*http://iccvam.niehs.nih.gov*).

SACATM was established January 9, 2002, to fulfill section 3(d) of the ICCVAM Authorization Act of 2000 and is composed of scientists from the public and private sectors (**Federal Register**: March 13, 2002: Vol. 67, No. 49, page 11358). SACATM provides advice to the Director of the NIEHS, ICCVAM, and NICEATM regarding statutorily mandated duties of ICCVAM and activities of NICEATM. Additional information about SACATM, including the charter, roster, and records of past meetings can be found at *http://ntp.niehs.nih.gov/go/167*.

References

EPA. 2002. Health Effects Test Guidelines OPPT 870.1100 Acute Oral Toxicity. EPA 712–C–02–190. Washington, DC: U.S. Environmental Protection Agency. Available at: *http://www.epa.gov/opptsfrs/publications/*.

ICCVAM. 2003. ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods. NIH Publication No. 03–4508. Research Triangle Park, NC: NIEHS. Available at: http://iccvam.niehs.nih.gov.

OECD. 2001a. Guideline for Testing of Chemicals, 425, Acute Oral Toxicity—Up-and-Down Procedure. Paris, France:OECD. Available at: *http://www.oecd.org*.

OECD. 2001b. Guideline for Testing of Chemicals, 423, Acute Oral Toxicity—Acute Toxic Class Method. Paris, France:OECD. Available at: *http://www.oecd.org*.

Dated: June 30, 2006.

Samuel H. Wilson,

Deputy Director, National Institute of Environmental Health Sciences and National Toxicology Program.

[FR Doc. E6–10789 Filed 7–10–06; 8:45 am]

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APPENDIX D2

ICCVAM CONSIDERATION OF PUBLIC COMMENTS RECEIVED IN RESPONSE TO FEDERAL REGISTER NOTICES

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In response to eight *Federal Register* (*FR*) notices that were released between June 2000 and July 2006, 298 public comments were received. Comments received in response to the *FR* notices and/or were related to those *FR* notices can be obtained on CD ROM upon request to The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) by mail, fax, or email (NICEATM, NIEHS, P.O. Box 12233, MD EC-17, Research Triangle Park, NC 27709, (phone) 919-541-2384, (fax) 919-541-0947, (email) niceatm@niehs.nih.gov). The following sections, delineated by *FR* notice, provide a brief discussion of the public comments received in response to three of the published *FR* notices.

1.0 Public Comments Received in Response to *FR* Notice Released on March 22, 2005 (Volume 70, Number 54; pages 14473-14474)

NICEATM, in an *FR* notice (70 *FR* 54:14473-14474, March 22, 2005) requested nominations of scientific experts for consideration as part of an independent peer review panel to evaluate the validation status of two *in vitro* cytotoxicity assays for estimating *in vivo* oral toxicity. One comment was received in response to this request and stated that animal testing should be stopped and more accurate and humane methods should be used.

The ICCVAM appreciates the comment received. It should be noted that ICCVAM does not determine whether a test method is acceptable for use by U.S. Federal agencies or the international regulatory community. ICCVAM develops and forwards recommendations on the usefulness and limitations of the proposed test methods to each U.S. Federal agency for its review. Based on their specific statutory mandates, each U.S. Federal agency will consider ICCVAM's recommendations and then make a determination as to the acceptability of the test methods.

2.0 Public Comments Received in Response to *FR* Notice Released on March 21, 2006 (Volume 71, Number 54; pages 14229-14231)

NICEATM, in an *FR* notice (71 *FR* 54:14229-14231, March 21, 2006) requested comments on (1) the draft BRD being forwarded to the Scientific Peer Review Panel, (2) the draft ICCVAM test method recommendations, (3) draft test method protocols, and (4) draft performance standards. In response to this *FR* notice, 297 comments were received.

Of the comments received, 296 comments stated that there was a consensus at the workshop in 2000 (*In Vitro* Methods for Assessing Acute Systemic Toxicity) that cell-based methods could be used immediately to reduce the number of animals killed and could potentially be validated as replacements to current acute systemic toxicity test methods, given the proper funding and effort. However, the comments stated that announcement for the Peer Review Panel meeting scheduled for 2006 did not mention the potential of using these cell-based methods as potential replacement methods.

ICCVAM considered all the recommendations from the 2000 workshop in developing its own recommendations for activities (ICCVAM 2001a). The ICCVAM recommendations were forwarded to U.S. Federal agencies, along with the workshop report (ICCVAM 2001a)

and the *Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity* (ICCVAM 2001b). Consistent with the workshop recommendations, ICCVAM recommended that the near-term focus for validation should be on characterizing the usefulness of two standardized *in vitro* assays using rodent and human cells in predicting acute toxicity with a broader range of chemicals than had been previously tested . Therefore, the current evaluation focused on the use of these two *in vitro* methods for estimating starting doses for acute oral systemic toxicity tests.

Of the comments received, 23 stated that it was time to refine and implement non-animal, cell-based methods to replace current systemic acute toxicity test method protocols. ICCVAM appreciates the comments received. It should be noted that ICCVAM does not determine whether a test method is acceptable for use by U.S. Federal agencies or the international regulatory community. ICCVAM develops and forwards recommendations on the usefulness and limitations of the proposed test methods to each U.S. Federal agency for its review. Based on their specific statutory mandates, each U.S. Federal agency considers ICCVAM's recommendations and then determines the acceptability of the test methods.

Of the comments received, two focused on the rationale for ICCVAM to not consider or implement the recommendations of the participants of the *International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity* (ICCVAM 2001a). ICCVAM notes that the participants of the workshop made the following recommendations (among others):

- *In vitro* cytotoxicity data should be used to predict starting doses for *in vivo* lethality studies.
- Test laboratories should evaluate and compare the performance of several *in vitro* cytotoxicity tests with the existing RC data.
- A prevalidation study should be initiated as soon as possible to evaluate various cell types, exposure periods, and endpoint measurements as predictors of acute toxicity. The assay, or battery of assays, determined to be the best predictor of *in vivo* lethality could then be optimized further to identify, standardize, and validate simple predictive systems for gut absorption, blood-brain barrier passage, kinetics, and metabolism.
- In the longer-term, preferably as a parallel activity, there should be a focus on the development and validation of human *in vitro* test systems for predicting human acute toxicity.
- The evaluation and ultimate acceptance of *in vitro* assays for human acute toxicity will need a larger reference database than is presently available for validation purposes.

ICCVAM considered these as well as other recommendations from the workshop in developing its own recommendations. The ICCVAM recommendations were forwarded to U.S. Federal agencies along with the workshop report and *Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity* (ICCVAM 2001b). Consistent with the workshop recommendations, ICCVAM recommended that the near-term focus for validation should be on characterizing the usefulness of two standardized *in vitro* assays using rodent and human cells in predicting acute toxicity with a broader range of chemicals than had been previously tested. The NICEATM/ECVAM validation study was

based on this recommendation and its goals and purpose are entirely consistent with the workshop recommendations. Research activities to identify appropriate *in vitro* absorption, distribution, metabolism, and excretion systems was identified as a longer-term objective. NICEATM proceeded with the validation study to establish the utility of setting the starting dose across the range of GHS hazard classification, and to establish a high quality database as a foundation for the development of other *in vitro* tests that could be used, along with *in vitro* basal cytotoxicity test methods, to improve the prediction of *in vivo* acute toxicity.

ICCVAM received a comment that the NICEATM/ECVAM validation study objectives appeared to be a mixture of partly conflicting goals (e.g., validating the RC prediction model, assessing the boundaries of applicability, and assessing the predictive capacity of LD_{50} point measures). As stated in the BRD, ICCVAM notes that the study objectives were to:

- Further standardize and optimize the *in vitro* NRU basal cytotoxicity protocols using 3T3 and NHK cells to maximize test method reliability (intralaboratory repeatability, intra- and inter-laboratory reproducibility)
- Assess the accuracy of the two standardized *in vitro* 3T3 and NHK NRU basal cytotoxicity test methods for estimating rodent oral LD_{50} values across the five United Nations (UN) GHS categories of acute oral toxicity, as well as unclassified toxicities (GHS; UN 2005)
- Estimate the reduction and refinement in animal use achievable from using the *in vitro* 3T3 and NHK NRU basal cytotoxicity test methods to identify starting doses for *in vivo* acute oral toxicity tests, assuming that no other information were available
- Develop high quality *in vivo* acute oral lethality and *in vitro* NRU cytotoxicity databases that can be used to support the investigation of other *in vitro* test methods necessary to improve the prediction of *in vivo* acute oral lethality

ICCVAM received a comment focused on the selection of the test chemicals for the validation study. The comment noted that these chemicals were not appropriate to achieve the main goal of the validation study (i.e., verification or falsification of the RC prediction model). ICCVAM appreciates the comment but notes that the verification of falsification of the RC prediction model was not a goal of this effort (see above).

ICCVAM received a comment regarding the variability of *in vitro* data obtained during Phase I and Phase II of the validation study. The comment stated that the *in vitro* test protocols were optimized, and that the necessity of this step was questionable. The comment recommended that the outcomes from this study be compared with other interlaboratory validation studies that have used the 3T3 NRU standard protocol. ICCVAM notes that the test acceptance criteria for the VC OD and placement of the cytotoxicity points were revised after it was noted that good dose-response data were obtained even in tests that failed the original criteria. Thus, to increase the test method experimental success rate, the criteria were revised. These changes did not alter the performance of the test methods.

Regarding the variability of the *in vitro* data, this comment appears to refer to the difference between the 3T3 NRU and NHK NRU IC_{50} values since no such variation occurred across laboratories for the same cell type. ICCVAM notes that it should not be a surprise that, for

some chemicals, large variation exists for IC_{50} results obtained using different cell lines even when using very similar test protocols. Such data are important for characterizing which cell line(s) may be optimal for *in vitro* cytotoxicity testing and for identifying chemicals that may require additional evaluation.

ICCVAM received a comment regarding the variability of the *in vivo* reference data. The comment noted that there had been extensive efforts by ICCVAM to obtain multiple *in vivo* LD_{50} values per test chemical. The comment noted that while most validation studies assess the variability of the *in vivo* data to analyze the performance of the alternative methods, this type of analysis was not present in the BRD. ICCVAM appreciates the comments and notes that the BRD analyzed the variation of *in vivo* data in Section 4 (ICCVAM 2006). Table 4-2 in the BRD provides the ratio of the maximum to the minimum acceptable LD_{50} for each chemical (ICCVAM 2006).

ICCVAM received a comment stating that the evaluation of the two *in vitro* assays was highly biased by the unbalanced selection of chemicals used in the validation study. The commenter stated that all calculations (e.g., the contingency tables for prediction of the GHS classes) were influenced by the bias in the chemical selection, so that even the strength of the prediction model (correct prediction of the absence of toxicity) was lost. The commenter stated that a thorough discussion of the influence of chemical selection on the study outcome should be included.

ICCVAM agrees with the comment that the selection of chemicals and their fit to the regression being evaluated affects the accuracy of GHS category predictions. Even though the selection of chemicals and their fit to the regressions affects the accuracy of GHS category predictions, the analyses provide a valid comparison of the test methods to one another and of the regressions to one another.

One comment stated that the results of the current study should be correlated to the results and information obtained from previous studies. ICCVAM agrees and notes that Section 9 of the BRD provides a literature review of studies most relevant to the NICEATM/ECVAM validation study. The literature review addresses (a) the use of *in vitro* NRU cytotoxicity test methods for correlations with rodent lethality and other toxicities and (b) the use of *in vitro* basal cytotoxicity to predict starting doses for acute oral lethality assays.

ICCVAM received a comment related to (a) the draft ICCVAM recommendation proposing that the RC should be revised and (b) the draft minimum performance standards. ICCVAM appreciates the comment received and notes that the proposed revisions were based on a variety of factors, were independent of each other, and are justified based on the breadth of the RC database. Furthermore, ICCVAM notes that the draft performance standards take into account the technical aspects of the test methods and proposes reference substances compatible with the RC regression after excluding substances without rat LD_{50} data and those with known mechanisms of action that are not expected to be active in the 3T3 and NHK cell cultures.

3.0 Public Comments Received in Response to *FR* Notice Released on July 11, 2006 (Volume 71, Number 132; pages 39122-39123)

NICEATM, in an *FR* notice (71 *FR* 132:39122-39123, Jul 11, 2006) requested comments on the Panel's conclusions on the draft ICCVAM test method recommendations. In response to this *FR* notice, one comment was received.

The comment stated that there was concern that despite near unanimous agreement at the 2000 workshop that the cell-based methods could be used immediately to set the starting dose for oral toxicity tests and that given appropriate effort and funding these method could be validated as a replacement measure, there has been little progress on the issue. There was concern that the Peer Panel Report did not require the use of the *in vitro* methods to estimate a starting dose, due to the understandable contention that significant information may already be available on the chemical or its class. The commentor stated that companies should be encouraged to use the non-animal methods to obtain another level of comfort with using and reading data generated by them. The comment stated that, based on the available scientific evidence, the Peer Panel Report should address expedient steps to replace lethal dose animal tests at the extremes of toxicity.

ICCVAM appreciates the comments provided. ICCVAM notes that the Peer Panel Report contains the conclusions of the Peer Review Panel and the document would not be edited by ICCVAM. However, the Peer Panel Report and all the comments received in response to the published *FR* notices were considered by ICCVAM during the development of the ICCVAM Test Method Evaluation Report.

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