Article 4

This Directive is addressed to the Member States.

Done at Brussels,

For the European Parliament
The President

For the Council
The President

Health, 6701 Rockledge Drive, Room 3190, MSC 7848, Bethesda, MD 20892, (301) 435–1507.

Name of Committee: Center for Scientific Review Special Emphasis Panel.

Date: October 11, 2001.

Time: 9:00 am to 5:00 pm.

Agenda: To review and evaluate grant applications.

Place: Holiday Inn, 8120 Wisconsin Avenue, Bethesda, MD 20814.

Contact Person: Richard Marcus, PhD, Scientific Review Administrator, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 5168, MSC 7844, Bethesda, MD 20892, (301) 435–1245, richard.marcus.@nih.gov.

Name of Committee: Center for Scientific Review Special Emphasis Panel.

Date: October 11, 2001.

Time: 1:00 pm to 2:30 pm.

Agenda: To review and evaluate grant applications.

Place: NIH, Rockledge 2, Bethesda, MD 20892, (Telephone Conference Call).

Contact Person: Victor A. Fung, PhD, Scientific Review Administrator, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 4120, MSC 7804, Bethesda, MD 20814–9692, (301) 435–3504, fungv@csr.nih.gov.

(Catalogue of Federal Domestic Assistance Program Nos. 93.306, Comparative Medicine, 93.306; 93.333, Clinical Research, 93.333, 93.337, 93.393–93.396, 93.837–93.844, 93.846–93.878, 93.892, 93.893, National Institutes of Health, HHS)

Dated: September 20, 2001.

LaVerne Y. Stringfield,

Director, Office of Federal Advisory Committee Policy.

[FR Doc. 01–24366 Filed 9–27–01; 8:45 am]

BILLING CODE 4140–01–M

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

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

EPISKIN™, EpiDerm™, and Rat Skin Transcutaneous Electrical Resistance Methods: In Vitro Test Methods Proposed for Assessing the Dermal Corrosivity Potential of Chemicals; Notice of Availability of a Background Review Document and Proposed ICCVAM Test Method Recommendations and Request for Public Comment.

Summary

The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) announces availability of a background review document (BRD) entitled "EPISKIN™, EpiDerm™, and Rat Skin Transcutaneous Electrical Resistance (TER) Methods: In Vitro Test Methods for Assessing the Dermal Corrosivity Potential of Chemicals," and proposed test method recommendations from the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) on the use of these methods. The NICEATM invites public comment on the BRD and ICCVAM recommendations.

Availability of Background Review Document and Proposed ICCVAM Recommendations

An electronic version of this BRD and proposed ICCVAM test method recommendations may be obtained from the NICEATM/ICCVAM web site at *http://iccvam.niehs.nih.gov.* For a paper copy (a limited number are available), please contact the NICEATM at (919) 541–3398 or via email at *niceatm@niehs.nih.gov.*

Request for Public Comment

NICEATM invites written public comments on the BRD on in vitro corrosivity methods and the proposed ICCVAM recommendations for these methods. The deadline for submission of comments is November 13, 2001. Comments submitted via email are preferred; the acceptable file formats are MS Word (Office 98 or older), plain text, or PDF. Comments should be sent to Dr. William Stokes, Director, NICEATM, NIEHS, MD EC–17, P.O. Box 12233, Research Triangle Park, NC, 27709; telephone 919–541–3398; fax 919–541–0947; email *niceatm@niehs.nih.gov.* Persons submitting written comments should include their contact information (name, affiliation, address, telephone/fax numbers, and email) 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* and provided to the ICCVAM. ICCVAM will consider all comments prior to finalizing its test recommendations on EpiDerm™, EPISKIN™, and Rat Skin TER. In accordance with Public Law 106–545, ICCVAM test recommendations will be forwarded to appropriate Federal agencies and will be made available to the public on the NICEATM/ICCVAM website.

Background

ICCVAM and the ICCVAM Corrosivity Working Group (CWG) recently evaluated three in vitro test methods for assessing the dermal corrosivity potential of chemicals and chemical mixtures—EpiDerm™, EPISKIN™, and Rat Skin TER. EpiDerm™ and EPISKIN™ utilize a three dimensional human skin model comprised of a reconstructed epidermis and a functional stratum corneum. The test chemical is applied to this reconstructed epidenmis for a specified time and subsequent cell viability is measured. Rat Skin TER assesses the skin corrosivity of a chemical by applying the test material to the epidermal surface of a rat skin disc for two and 24 hours; subsequently, the transcutaneous electrical resistance (TER) of the skin disc is measured. NICEATM prepared a background review document summarizing the available data and prior reviews for the three test methods, which was then considered by the CWG and ICCVAM. The CWG concluded, based on the information provided and outcomes of the previous reviews, that further evaluation by an independent scientific peer review panel did not appear necessary, and recommended that these methods undergo ICCVAM evaluation using an expedited review process (ICCVAM, 2001). ICCVAM agreed with the CWG recommendation for expedited review. This process involves the development of a draft ICCVAM position (proposed ICCVAM test recommendations) and publishing the position in the **Federal Register** for public comment. Public comments are considered by ICCVAM, and if no major problems are found, ICCVAM then finalizes its test recommendations and forwards to federal agencies for their determination of regulatory acceptability. If major problems are noted, then ICCVAM will determine an appropriate process for further evaluation, such as an independent peer review panel evaluation.

ECVAM Evaluation

The European Center for the Validation of Alternative Methods (ECVAM) conducted validation studies on these three in vitro methods (Barratt et al., 1998; Fentem et al., 1998; Liebsch et al., 2000). The ECVAM Management Team concluded that EpiDerm™, Rat Skin TER, and EPISKIN™ were scientifically valid for use as replacements for the animal test currently used to distinguish between corrosive and non-corrosive chemicals and for all chemical classes (Fentem et al., 1998; Liebsch et al., 2000).

Other Reviews

The validation status of these three methods was then evaluated by the ECVAM Scientific Advisory Committee (ESAC). The ESAC also concluded that the Rat Skin TER, EpiDerm™, and the EPISKIN™ tests were scientifically

valid for use as replacements for the animal test and were ready to be considered for regulatory acceptance (Balls and Corcelle, 1998; Balls and Hellsten, 2000). The European Scientific Committee for Cosmetic Products and Non-food Products (SCCNFP) evaluated the EPISKIN™ and Rat Skin TER and concluded that they were applicable for the safety evaluation of cosmetic ingredients or mixtures of ingredients (Anon., 1999). The European Commission subsequently adopted EpiDerm™, EPISKIN™, and Rat Skin TER (Anon., 2000).

Proposed ICCVAM Recommendations

ICCVAM proposes that these assays can be used to assess the dermal corrosion potential of chemicals in a weight-of-evidence approach in an integrated testing scheme [e.g., OECD Globally Harmonised Classification System (OECD, 1998); OECD Revised Proposals for Updated Test Guidelines 404 and 405: Dermal and Eye Corrosion/Irritation Studies (OECD, 2001a)]. These integrated testing schemes for dermal irritation/corrosion allow for the use of validated and accepted in vitro methods. In this approach, positive in vitro corrosivity responses do not generally require further testing and can be used for classification and labeling. Negative in vitro corrosivity responses shall be followed by in vivo dermal corrosion/irritation testing. (Note: The first animal used in the irritation/corrosivity assessment would be expected to identify any chemical corrosives that were false negatives in the in vitro test). Furthermore, as is appropriate for any in vitro assay, there is the opportunity for confirmatory testing if false positive results are indicated on a weight of evidence evaluation of supplemental information, such as pH, structure activity relationships (SAR), and other chemical and testing information.

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 (Public Law 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*.

References

Anon. EU Commission Directive 2000/33/EC of 25 April 2000 (Official Journal of the European Communities), Skin Corrosion, Rat Skin TER and Human Skin Model Assay. OJ L 136, June 8, 2000. Available: *http://embryo.ib.amwaw.edu.pl/invittox/prot/1_13620000608en00010089.pdf* [cited July 19, 2001].

Anon. Scientific Committee for Cosmetic Products, and Non-food Products intended for Consumers. Excerpts of the Outcome of Discussions Record of the 6th Plenary Meeting (SCCNFP) Brussels, Belgium. January 20, 1999. Available: *http://europa.eu.int/comm/food/fs/sc/sccp/out50_en.html* [cited July 19, 2001].

Balls M, Corcelle G. "Statement on the scientific validity of the Rat Skin Transcutaneous Electrical Resistance (TER) Test (an in vitro test for skin corrosivity) and Statement of the scientific validity of the EPISKIN™ test (an in vitro test for skin corrosivity)," dated April 3, 1998. Statement from the European Commission Joint Research Centre, Environment Institute, Ispra (VA), Italy presenting the results of the 10th ECVAM Scientific Advisory Committee (ESAC) meeting on March 31 (1998). Available: *http://www.iivs.org/news/ratskin-episkin.html* [cited July 19, 2001].

Balls M, Hellsten E. "Statement on the application of the EpiDerm™ human skin model for corrosivity testing," dated March 20, 2000. ECVAM Scientific Advisory Committee meeting, Ispra, Italy, March 14–15 (2000).

Barratt, MD, Brantom PG, Fentem JH, Gerner I, Walker AP, Worth AP. The ECVAM international validation study on in vitro tests for skin corrosivity. 1. Selection and distribution of the test chemicals. Toxicology In Vitro 12:471–482 (1998).

Fentem, JH, Archer GEB, Balls M, Botham PA, Curren RD, Earl LK, Esdaile DJ, Holzhutter H–G, Liebsch M. The ECVAM international validation study on in vitro tests for skin corrosivity. 2. Results and evaluation by the management team. Toxicology In Vitro 12:483–524 (1998).

Interagency Center for the Evaluation of Alternative Methods (ICCVAM). Procedures for test methods that have been endorsed by ECVAM (April 20, 2001). *http://iccvam.niehs.nih.gov*.

Liebsch M, Traue D, Barrabas C, Spielmann H, Uphill P, Wilkins S, McPherson JP, Wiemann C, Kaufmann T, Remmele M, Holzhutter HG. The ECVAM prevalidation study on the use of EpiDerm for skin corrosivity testing. ATLA-Alternatives to Laboratory Animals 28:371–401 (2000).

Organization for Economic Co-operation and Development (OECD). Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances, as endorsed by the 28th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, OECD, Paris, France. (November 1998) *http://www.oecd.org/ehs/Class/HCL6.htm*

OECD. OECD Revised Proposals for Updated Test Guidelines 404 and 405: Dermal and Eye Corrosion/Irritation Studies. [OECD ENV/JM/TG (2001)2]. OECD Environment Directorate, Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology. Test Guidelines Programme. Circulated in preparation for the 13th Meeting of the Working Group of the National Coordinators of the Test Guidelines Programme, OECD, Paris, France. (2001a)

Dated: September 21, 2001.

Samuel H. Wilson,

Deputy Director, National Institute of Environmental Health Sciences.

[FR Doc. 01–24371 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)

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

valid for use as replacements for the animal test and were ready to be considered for regulatory acceptance (Balls and Corcelle, 1998; Balls and Hellsten, 2000). The European Scientific Committee for Cosmetic Products and Non-food Products (SCCNFP) evaluated the EPISKIN™ and Rat Skin TER and concluded that they were applicable for the safety evaluation of cosmetic ingredients or mixtures of ingredients (Anon., 1999). The European Commission subsequently adopted EpiDerm™, EPISKIN™, and Rat Skin TER (Anon., 2000).

Proposed ICCVAM Recommendations

ICCVAM proposes that these assays can be used to assess the dermal corrosion potential of chemicals in a weight-of-evidence approach in an integrated testing scheme [e.g., OECD Globally Harmonised Classification System (OECD, 1998); OECD Revised Proposals for Updated Test Guidelines 404 and 405: Dermal and Eye Corrosion/Irritation Studies (OECD, 2001a)]. These integrated testing schemes for dermal irritation/corrosion allow for the use of validated and accepted in vitro methods. In this approach, positive in vitro corrosivity responses do not generally require further testing and can be used for classification and labeling. Negative in vitro corrosivity responses shall be followed by in vivo dermal corrosion/irritation testing. (Note: The first animal used in the irritation/corrosivity assessment would be expected to identify any chemical corrosives that were false negatives in the in vitro test). Furthermore, as is appropriate for any in vitro assay, there is the opportunity for confirmatory testing if false positive results are indicated on a weight of evidence evaluation of supplemental information, such as pH, structure activity relationships (SAR), and other chemical and testing information.

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 (Public Law 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.*

References

Anon. EU Commission Directive 2000/33/EC of 25 April 2000 (Official Journal of the European Communities), Skin Corrosion, Rat Skin TER and Human Skin Model Assay. OJ L 136, June 8, 2000. Available: *http://embryo.ib.amwaw.edu.pl/invittox/prot/1_1362000608en00010089.pdf* [cited July 19, 2001].

Anon. Scientific Committee for Cosmetic Products, and Non-food Products intended for Consumers. Excerpts of the Outcome of Discussions Record of the 6th Plenary Meeting (SCCNFP) Brussels, Belgium. January 20, 1999. Available: *http://europa.eu.int/comm/food/fs/sc/sccp/out50_en.html* [cited July 19, 2001].

Balls M, Corcelle G. "Statement on the scientific validity of the Rat Skin Transcutaneous Electrical Resistance (TER) Test (an in vitro test for skin corrosivity) and Statement of the scientific validity of the EPISKIN™ test (an in vitro test for skin corrosivity)," dated April 3, 1998. Statement from the European Commission Joint Research Centre, Environment Institute, Ispra (VA), Italy presenting the results of the 10th ECVAM Scientific Advisory Committee (ESAC) meeting on March 31 (1998). Available: *http://www.iivs.org/news/ratskin-episkin.html* [cited July 19, 2001].

Balls M, Hellsten E. "Statement on the application of the EpiDerm™ human skin model for corrosivity testing," dated March 20, 2000. ECVAM Scientific Advisory Committee meeting, Ispra, Italy, March 14–15 (2000).

Barratt, MD, Brantom PG, Fentem JH, Gerner I, Walker AP, Worth AP. The ECVAM international validation study on in vitro tests for skin corrosivity. 1. Selection and distribution of the test chemicals. Toxicology In Vitro 12:471–482 (1998).

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Interagency Center for the Evaluation of Alternative Methods (ICCVAM). Procedures for test methods that have been endorsed by ECVAM (April 20, 2001). *http://iccvam.niehs.nih.gov.*

Liebsch M, Traue D, Barrabas C, Spielmann H, Uphill P, Wilkins S, McPherson JP, Wiemann C, Kaufmann T, Remmele M, Holzhutter HG. The ECVAM prevalidation study on the use of EpiDerm for skin corrosivity testing. ATLA-Alternatives to Laboratory Animals 28:371–401 (2000).

Organization for Economic Co-operation and Development (OECD). Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances, as endorsed by the 28th Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, OECD, Paris, France. (November 1998) *http://www.oecd.org/ehs/Class/HCL6.htm*

OECD. OECD Revised Proposals for Updated Test Guidelines 404 and 405: Dermal and Eye Corrosion/Irritation Studies. [OECD ENV/JM/TG (2001)2]. OECD Environment Directorate, Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology. Test Guidelines Programme. Circulated in preparation for the 13th Meeting of the Working Group of the National Coordinators of the Test Guidelines Programme, OECD, Paris, France. (2001a)

Dated: September 21, 2001.

Samuel H. Wilson,

Deputy Director, National Institute of Environmental Health Sciences.

[FR Doc. 01–24371 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)

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

添付資料－8

Place: The George Washington University Inn, 824 New Hampshire Ave., NW, Washington, DC 20037.

Contact Person: Joseph Kimm, PHD, Scientific Review Administrator, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 5178 MSC 7844, Bethesda, MD 20892, (301) 435–1249.

Name of Committee: Integrative, Functional and Cognitive Neuroscience Integrated Review Group, Integrative, Functional and Cognitive Neuroscience 5.

Date: February 19–20, 2002.

Time: 8:30 AM to 10:30 AM.

Agenda: To review and evaluate grant applications.

Place: Governor's House Hotel, 17th & Rhode Island Avenue, NW, Washington, DC 20036.

Contact Person: John Bishop, PHD, Scientific Review Administrator, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 5180, MSC 7844, Bethesda, MD 20892, (301) 435–1250.

Name of Committee: Integrative, Functional and Cognitive Neuroscience Integrated Review Group, Integrative, Functional and Cognitive Neuroscience 4.

Date: February 20–21, 2002.

Time: 8:00 AM to 4:00 PM.

Agenda: To review and evaluate grant applications.

Place: Wyndham Washington Hotel, 1400 M Street NW, Washington, DC 20005–2750.

Contact Person: Dan Kenshalo, PHD, Scientific Review Administrator, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 5176, MSC 7844, Bethesda, MD 20892, (301) 435–1255.

Name of Committee: Center for Scientific Review Special Emphasis Panel.

Date: February 20, 2002.

Time: 8:00 AM to 10:00 AM.

Agenda: To review and evaluate grant applications.

Place: Georgetown Holiday Inn, Kaleidoscope Room, 2101 Wisconsin Ave. NW, Washington, DC 20007.

Contact Person: Dharam S. Dhindsa, DVM, PHD, Scientific Review Administrator, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 5126, MSC 7854, Bethesda, MD 20892, (301) 435–1174, *dhindsad@csr.nih.gov.*

Name of Committee: Molecular, Cellular and Developmental Neuroscience Integrated Review Group, Molecular, Cellular and Developmental Neurosciences 5.

Date: February 20–21, 2002.

Time: 8 a.m. to 5 p.m.

Agenda: To review and evaluate grant applications.

Place: Jurys Washington Hotel, Westbury Conference Room, 1500 New Hampshire Avenue, NW., Washington, DC 20036.

Contact Person: Syed Husain, PHD, Scientific Review Administrator, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 5216, MSC 7850, Bethesda, MD 20892, (301) 435–1224.

Name of Committee: Infectious Diseases and Microbiology Integrated Review Group, Bacteriology and Mycology Subcommittee 2.

Date: February 20–21, 2002.

Time: 8:30 a.m. to 3 p.m.

Agenda: To review and evaluate grant applications.

Place: The Latham Hotel, 3000 M Street, NW., Washington, DC 20007.

Contact Person: Lawrence N. Yager, PHD, Scientific Review Administrator, Center For Scientific Review, National Institutes for Health, 6701 Rockledge Drive MSC 7808, Room 4190, Bethesda, MD 20892, 301–435–0903, *yagerl@csr.nih.gov.*

Name of Committee: Integrative, Functional and Cognitive Neuroscience Integrated Review Group, Integrative, Functional and Cognitive Neuroscience 1.

Date: February 20–21, 2002.

Time: 8:30 a.m. to 5 p.m.

Agenda: To review and evaluate grant applications.

Place: Wyndham Washington Hotel, 1400 M Street NW., Washington, DC 20005–2750.

Contact Person: Gamil C. Debbas, PHD, Scientific Review Administrator, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 5170, MSC 7844, Bethesda, MD 20892, (301) 435–1018.

Name of Committee: Biophysical and Chemical Sciences Integrated Review Group, Medicinal Chemistry Study Section.

Date: February 20–21, 2002.

Time: 8:30 a.m. to 4 p.m.

Agenda: To review and evaluate grant applications.

Place: Holiday Inn Bethesda, 8120 Wisconsin Avenue, Bethesda, MD 20814.

Contact Person: Ronald J. Dubois, PHD, Scientific Review Administrator, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 4156, MSC 7806, Bethesda, MD 20892, (301) 435–1722.

Name of Committee: Center for Scientific Review Special Emphasis Panel.

Date: February 20, 2002.

Time: 10 a.m. to 12 p.m.

Agenda: To review and evaluate grant applications.

Place: Georgetown Holiday Inn, kaleidoscope Room, 2101 Wisconsin Ave. NW., Washington, DC 20007.

Contact Person: Dharam S. Dhindsa. DVM, PHD, Scientific Review Administrator, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 5126, MSC 7854, Bethesda, MD 20892, (301) 435–1174, *dhindsad@csr.nih.gov.*

Name of Committee: Center for Scientific Review Special Emphasis Panel

Date: February 20, 2002.

Time: 11:00 AM to 5:00 PM.

Agenda: To review and evaluate grant applications.

Place: Governor's House Hotel, 17th & Rhode Island Avenue, NW., Washington, DC 20036.

Contact Person: John Bishop, PhD., Scientific Review Administrator, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 5180, MSC 7844, Bethesda, MD 20892, (301) 435–1250.

Name of Committee: Center for Scientific Review Special Emphasis Panel.

Date: February 20, 2002.

Time: 2:00 PM to 3:30 PM.

Agenda: To review and evaluate grant applications.

Place: Holiday Inn Georgetown, 2101 Wisconsin Avenue, NW., Washington, DC 20007.

Contact Person: Zakir Bengali, PhD, Scientific Review Administrator, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 5150, MSC 7842, Bethesda, MD 20892, (301) 435–1742.

Name of Committee: Center for Scientific Review Special Emphasis Panel.

Date: February 20, 2002.

Time: 7:00 PM to 10:00 PM.

Agenda: To review and evaluate grant applications.

Place: La Jolla Coves Suites, 1155 Coast Blvd., La Jolla, CA 92037.

Contact Person: Eileen W. Bradley, DSC, Scientific Review Administrator, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 5120, MSC 7854, Bethesda, MD 20892, (301) 435–1179, bradleye@csr.nih.gov.

Name of Committee: Center for Scientific Review Special Emphasis Panel.

Date: February 20, 2002.

Time: 7:00 PM to 10:00 PM.

Agenda: To review and evaluate grant applications.

Place: La Jolla Coves Suites, 1155 Coast Blvd., La Jolla, CA 92037.

Contact Person: Lee Rosen, PhD., Scientific Review Administrator, Center for Scientific Review, National Institutes of Health, 6701 Rockledge Drive, Room 5116, MSC 7854, Bethesda, MD 20892, (301) 435–1171.

(Catalogue of Federal Domestic Assistance Program Nos. 93.306, Comparative Medicine, 93.306, 93.333, Clinical Research, 93.333, 93.337, 93.393–93.396, 93.837–93.844, 93.846–93.878, 93.892, 93.893, National Institutes of Health, HHS)

Dated: January 29, 2002.

LaVerne Y. Stringfield,

Director, Office of Federal Advisory Committee Policy.

[FR Doc. 02–2899 Filed 1–30–02; 8:45 am]

BILLING CODE 4140–01–M

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

National Institute of Environmental Health Sciences (NIEHS); National Toxicology Program (NTP); Report on the Revised Up-and-Down Procedure: A Test Method for Determining the Acute Oral Toxicity of Chemicals; Notice of Availability and Request for Public Comments

SUMMARY: Notice is hereby given of the availability of the report entitled, "The Revised Up-and-Down Procedure: A

Test Method for Determining the Acute Oral Toxicity of Chemicals," NIH Publication 02–4501. The report contains the final test recommendations on the " Revised Up-and-Down Procedure" (Revised UDP) by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), the results of an independent scientific peer review evaluation of the Revised UDP, and the final test guideline for the Revised UDP. The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) is seeking public comment on this report on behalf of the ICCVAM prior to transmittal to US Federal agencies in accordance with Pub. L. 106–545. The report and public comments will be transmitted to appropriate Federal agencies following this public comment period.

Availability of the Report

The report is available electronically (PDF and HTML) on the ICCVAM/NICEATM Web site, *http://iccvam.niehs.nih.gov*. A limited number of printed reports are available. To receive a printed copy, please contact NICEATM at PO Box 12233, MD EC–17, Research Triangle Park, NC 27709 (mail), 919–541–2384 (phone), 919–541–0947 (fax), or *niceatm@niehs.nih.gov* (e-mail).

Request for Public Comments

NICEATM invites written public comments on the report. Comments should be sent to NICEATM no later than March 25, 2002. 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, at PO Box 12233, MD EC–17, Research Triangle Park, NC 27709 (mail), 919–541–0947 (fax), or *niceatmcomments@niehs.nih.gov* (e-mail). 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 by the above deadline will be posted on the ICCVAM/NICEATM Web site, *http://iccvam.niehs.nih.gov*, and forwarded to the appropriate Federal agencies with the report.

Background

The Organisation for Economic Cooperation and Development (OECD) Test Guidelines Program (TG 425; OECD 1998) adopted the UDP in 1998. The U.S. Environmental Protection Agency (EPA) subsequently determined it was necessary to revise the UDP to: (1) Conform to a newly harmonized global hazard classification scheme for acute toxicity (OECD, 2001) and (2) ensure that regulatory and testing needs would be met with the Revised UDP prior to OECD's proposed deletion of the conventional acute oral toxicity test (OECD, 1987). In August 1999, the EPA asked ICCVAM to evaluate the validation status of the Revised UDP as a substitute for the existing conventional LD50 test (U.S. EPA 870.1100, 1998; OECD Test Guideline (TG) 401, 1987).

The Revised UDP test method submitted to ICCVAM for evaluation included three components:

- A Primary Test for estimating the median lethal dose using sequential testing.
- A Limit Test for evaluating substances anticipated to have minimal or no toxicity.
- A Supplemental Test to determine the slope and confidence interval (CI) for the dose-response curve.

An initial **Federal Register** notice (Vol. 65, No. 34, pp. 8385–8386, February 18, 2000) requested data and the nomination of expert scientists to participate in the independent scientific peer review evaluation of the Revised UDP. A second **Federal Register** notice (Vol. 65, No. 106, pp. 35109–35110, June 1, 2000) announced the peer review panel meeting, availability of a background review document on the Revised UDP, and requested public comments.

The first meeting of the Panel to evaluate the Revised UDP was held on July 25, 2000. The public meeting was organized by the ICCVAM and NICEATM and was sponsored by the NIEHS, NTP, and EPA. The Panel evaluated the extent to which the Revised UDP addresses established validation and acceptance criteria (ICCVAM, 1997) and developes conclusions regarding the usefulness and limitations of the Revised UDP.

The Panel agreed that the Primary and Limit tests would perform as good or better than the respective existing conventional LD50 and limit tests. They also agreed that the revised test methods would reduce animal use compared to the current test methods. The Panel provided other recommendations for revision of the Revised UDP test guideline and did not recommend the UDP Supplemental Test.

Based on the Panel's July 25, 2000 conclusions and recommendations, the EPA UDP Technical Task Force modified the UDP Primary and Limit Tests and removed the UDP Supplemental Test. A computational procedure was added to calculate the confidence interval (CI) for the estimated LD50. The EPA also developed a software program that would calculate subsequent test doses, determine when to stop the test, estimate the LD50, and calculate a CI for the LD50. The publicly available software was developed to mitigate complexity for the user and to facilitate correct performance of the Revised UDP.

A **Federal Register** notice (Vol. 66, No. 121, pp. 33550–33552, June 22, 2001) requested public comment and announced availability of the revised draft test guideline for the Revised UDP, the procedure for calculating the confidence interval for the estimated LD50, and the software program. A subsequent **Federal Register** notice (Vol. 66, No. 133, pp. 36294–36295, July 11, 2001) announced a second public meeting of the UDP Panel.

The second meeting of the UDP Panel was held by teleconference on August 21, 2001. The Panel reviewed and endorsed modifications to the Revised UDP, the CI procedure, and the software program. The Panel recommended additional clarifications to the Revised UDP. Written reports of the Panel meetings are included in the final report.

Following the August 21st meeting, the EPA UDP Technical Task Force revised the UDP Guideline in response to the Panel's recommendations. A discussion of software program limitations and information about using *in vitro* cytotoxicity data to estimate starting doses for *in vivo* studies were added. An ICCVAM Acute Toxicity Working Group and the ICCVAM reviewed and endorsed the final Revised UDP Test Guideline, and developed and adopted ICCVAM test method recommendations for the Revised UDP. In accordance with P.L. 106–545, the ICCVAM test recommendations will be forwarded to appropriate Federal agencies for acceptance consideration.

The final report comprises two volumes. The first volume (143 pages) includes the final ICCVAM test method recommendations on the Revised UDP procedure, the final Revised UDP Test Guideline, and the two peer review panel meeting reports. Volume 2 (291 pages) contains an updated background review document and other information considered by the Panel for the July 2000 meeting. Following receipt of public comments, the report will be forwarded to Federal agencies in accordance with Pub. L. 106–545.

Additional Information About ICCVAM and NICEATM

The NICEATM and ICCVAM were established to facilitate development, validation, and regulatory acceptance of improved toxicological methods that predict human health risks while reducing, refining, and/or replacing animal tests and to promote communication with stakeholders. The NICEATM coordinates activities for the ICCVAM and is located at the NIEHS, Research Triangle Park, NC. ICCVAM, with 15 participating Federal agencies, originally established in 1997, was formally authorized and designated as a permanent interagency coordinating committee by the ICCVAM Authorization Act of 2000 (Pub. L. 106–545). ICCVAM's duties include the technical evaluation of new and alternative testing methods, the development of test recommendations based on those technical evaluations, and the forwarding of its test recommendations to Federal agencies for their consideration. The ICCVAM also coordinates interagency issues on toxicological test method development, validation, regulatory acceptance, and national and international harmonization. Additional information about ICCVAM and NICEATM can be found on the ICCVAM/NICEATM Web site at *http://iccvam.niehs.nih.gov.*

References

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). 1997. 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. National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Available: *http://iccvam.niehs.nih.gov/docs/guidelines/validate.pdf* (cited October 1, 2001).

National Institute of Environmental Health Sciences (NIEHS). 2000a. National Toxicology Program: Request for Data and Nomination of Expert Scientists to Participate in the Independent Peer Review Evaluation of the Revised Up-and-Down Procedure for Assessing Acute Oral Toxicity. Evaluation of the Up-and-Down Procedure. 65 FR 8385. February 18, 2000.

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OECD. 1998. OECD Guideline for the Testing of Chemicals, Revised Test Guideline 425, Acute Oral Toxicity, Up-and-Down Procedure. OECD, Paris. Available: *http://www.oecd.org//ehs/test/health.htm* (cited October 1, 2001).

OECD. 2001. Harmonized Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures. ENV/JM/MONO(2001)6. OECD, Paris. Available: *http://www.oecd.org/ehs/class/HCL6.htm* (cited December 13, 2001).

OECD. 1987. OECD Guideline for the Testing of Chemicals, Test Guideline 401, Acute Oral Toxicity. OECD, Paris. Available: *http://www.oecd.org//ehs/test/health.htm* (cited October 1, 2001).

U.S. EPA. 1998. Health Effects Test Guidelines, OPPTS 870.1100, Acute Oral Toxicity. Washington, DC, U.S. Environmental Protection Agency. Available: *http://www.epa.gov/docs/OPPTS Harmonized/870 Health Effects Test Guidelines/Series/*(cited October 1, 2001).

Dated: January 11, 2002.

Samuel H. Wilson,

Deputy Director, National Institute of Environmental Health Sciences.

[FR Doc. 02–2905 Filed 2–6–02; 8:45 am]

BILLING CODE 4140–01–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

National Toxicology Program (NTP); The NTP Annual Plan for Fiscal Year 2001; Notice of Availability and Request for Public Comments

SUMMARY: The *NTP Annual Plan for Fiscal Year 2001* outlines the NTP research program for studying the toxicity of physical and chemical agents and for developing methods for toxicological evaluations. The Report also provides information about efforts to develop and validate alternative and improved methods and identifies NTP resource allocations.

Background

The NTP was established within the Public Health Service of the Department of Health and Human Services (DHHS) in November 1978. The NTP is an interagency program whose mission is to evaluate agents of public health concern by developing and applying the tools of modern toxicology and molecular biology. In carrying out its mission, the NTP has several goals to:

- Broaden the spectrum of toxicological information obtained on selected chemicals;
- Develop and validate more sensitive and specific test methods;
- Develop improved strategies for generating scientific data that strengthen the scientific foundation for risk assessments; and
- Communicate NTP plans and results to government agencies, the medical and scientific communities, and the public.

A balanced program was created that uses chronic exposure studies, short-term exposure studies, the collection and application of mechanistic information, model development, alternative methods, and human studies. Scientific activities are divided into several major program areas: Carcinogenesis, risk assessment research, alternative test systems, and toxicology. Toxicology covers activities in immunotoxicology, neurobehavioral toxicology, reproductive and developmental toxicology, respiratory toxicology and phototoxicology. Program and project leaders along with contact information are provided in the plan.

The NTP is an interagency program headquartered at the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health. The NIEHS along with the National Center for Toxicological Research of the Food and Drug Administration and the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention are NTP core agencies. The Director of the NIEHS is also the NTP Director.

The NTP receives advice from two primary external advisory groups. The NTP Executive Committee provides primary program oversight and links DHHS health research institutes and centers with Federal health regulatory agencies. This effort helps to ensure that the NTP's basic and applied toxicology research and development activities are responsive to regulatory and public health needs.

The NTP Board of Scientific Counselors provides scientific oversight

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以降のページは雑誌/図書等に掲載された論文となりますので、
「添付資料」をご参照ください。

「添付資料」

In vitro toxicity screens may aid 30% reduction in test animals – ICCVAM

The Rose Sheet. Vol.22 No.42, p9, 2001

DRAFT TG 430
27 March 2002

OECD/OCDE

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

DRAFT PROPOSAL FOR A NEW GUIDELINE 430

In Vitro Skin Corrosion: Transcutaneous Electrical Resistance Test (TER)

INTRODUCTION

1. Skin corrosion refers to the production of irreversible tissue damage in the skin following the application of a test material [as defined by the Globally Harmonised System for the Classification and Labelling of Chemical Substances and Mixtures (GHS)] (1). This Test Guideline does not require the use of live animals for the assessment of skin corrosivity.

2. The assessment of skin corrosivity has typically involved the use of laboratory animals (2). Concern for the pain and suffering of animals involved with this procedure has been addressed in the 1992 revision of Guideline 404 that allows for the determination of skin corrosion by using alternative, *in vitro,* methods, avoiding pain and suffering.

3. The principal obstacle to completely replacing *in vivo* testing for skin corrosion in Guideline 404 has been the lack of formal, independent, validation of *in vitro* tests. A first step towards defining alternative tests that could be used for skin corrosivity testing for regulatory purposes was the conduct of prevalidation studies (3). Following this, a formal validation study of *in vitro* methods for assessing skin corrosion (4)(5) was conducted (6)(7)(8). The outcome of these studies and other published literature led to the recommendation of two equivalent tests as replacements for the *in vivo* skin corrosivity test (9): the human skin model test (see Test Guideline 431) and the transcutaneous electrical resistance test (this Guideline).

DEFINITIONS

4. Definitions used are provided in the Annex.

INITIAL CONSIDERATIONS

5. A validation study and other published studies have shown that the rat skin transcutaneous electrical resistance (TER) assay (10)(11) is able to reliably discriminate between known skin corrosives and non-corrosives (5)(9).

6. The test described in this guideline allows the discrimination between corrosive and non-corrosive chemical substances and mixtures. It does not provide information on skin irritation, nor does it allow the sub-categorisation of corrosive substances as permitted in the Globally Harmonised Classification System (GHS) (1).

7. For a full evaluation of local skin effects after a single dermal exposure, it is recommended to follow the sequential testing strategy as appended to Test Guideline 404 (2). This testing strategy includes

This Draft Guideline is open for public comment until 19th April 2002

Comments should be submitted as follows:

1. If you are a citizen of an OECD Member country or a non-member country participating in the work on Test Guidelines (see list) and:

- working in the public sector (local/ federal government, academia) please submit your comments to your National Co-ordinator (see list);

- working in the private sector (industry, contract laboratory) please submit to your comments either to your National Co-ordinator (see list) to the Business and Industry Advisory Council (BIAC) to OECD;

- commenting on behalf of a public interest group, please submit your comments to your National Co-ordinator (see list) or to the EEB (for environmental interest groups) or ICAPO (for animal welfare interest groups).

2. If you are not a citizen of an OECD Member country or a non-member country participating in the work on Test Guidelines (see list) please submit your comments directly to the Secretariat.

For inquiries related to the work on Test Guidelines, please contact the Head of the Test Guidelines Programme

the conduct of *in vitro* tests for skin corrosion (as described in this guideline) and skin irritation before considering testing in live animals.

PRINCIPLE OF THE TEST

8. The test material is applied for up to 24 hours to the epidermal surfaces of skin discs in a two-compartment test system in which the skin discs function as the separation between the compartments. The skin discs are taken from humanely killed rats aged 28-30 days. Corrosive materials are identified by their ability to produce a loss of normal stratum corneum integrity and barrier function, which is measured as a reduction in the TER below a threshold level (10). For rat TER, a cut-off value of 5kΩ has been selected based on extensive data for a wide range of chemicals where the vast majority of values were either clearly well above (often > 10 kΩ), or well below (often < 3 kΩ) this value (10). Generally, materials which are non-corrosive in animals but are irritating or non-irritating do not reduce the TER below this cut-off value. Furthermore, use of other skin preparations or other equipment may alter the cut-off value, necessitating further validation.

9. A dye-binding step is incorporated into the test procedure for confirmation testing of positive results in the TER. The dye-binding step determines if the increase in ionic permeability is due to physical destruction of the stratum corneum. The TER method utilizing rat skin has shown to be predictive of *in vivo* corrosivity in the rabbit assessed under OECD guideline 404 (2). It should be noted that the *in vivo* rabbit test is highly conservative with respect to skin corrosivity and skin irritation when compared with the human skin patch test (12).

PROCEDURE

Animals

10. Rats are the species of choice because the sensitivity of their skin to chemicals in this test has been previously demonstrated (10). The age (when the skin is collected) and strain of the rat is particularly important to ensure that the skin is in the dormant phase before adult hair growth begins.

11. The dorsal and flank hair from young, approximately 22 day-old, rats (Wistar-derived or a comparable strain), is carefully removed with small clippers. Then, the animals are washed by careful wiping, whilst submerging the clipped area in antibiotic solution (containing, for example, streptomycin, penicillin, chloramphenicol, and amphotericin, at concentrations effective in inhibiting bacterial growth). Animals are washed with antibiotics again on the third or fourth day after the first wash and are used within 3 days of the second wash, when the stratum corneum has recovered from the hair removal.

Preparation of the skin discs

12. Animals are humanely killed when 28-30 days old; this age is critical. The dorsal skin of each animal is then removed and stripped of excess fat by carefully peeling it away from the skin. Skin discs, with a diameter of approximately 20 mm each, are removed. The skin may be stored before disks are used where it is shown that positive and negative control data are equivalent to that obtained with fresh skin.

13. Each skin disc is placed over one of the ends of a PTFE (polytetrafluoroethylene) tube, ensuring that the epidermal surface is in contact with the tube. A rubber 'O' ring is press-fitted over the end of the tube to hold the skin in place and excess tissue is trimmed away. Tube and 'O' ring dimensions are shown

in Figure 2. The rubber 'O' ring is then carefully sealed to the end of the PTFE tube with petroleum jelly. The tube is supported by a spring clip inside a receptor chamber containing $MgSO_4$ solution (154 mM) (Figure 1). The skin disc should be fully submerged in the $MgSO_4$ solution. As many as 10-15 skin discs can be obtained from a single rat skin.

14. Before testing begins, the electrical resistance of two skin discs is measured as a quality control procedure for each animal skin. Both discs should give resistance values greater than 10 kΩ for the remainder of the discs to be used for the test. If the resistance value is less than 10 kΩ, the remaining discs from that skin should be discarded.

Application of the test and control substances

15. Concurrent positive and negative controls should be used for each study to ensure adequate performance of the experimental model. Skin discs from a single animal should be used. The suggested positive and negative control substances are 10M hydrochloric acid and distilled water, respectively.

16. Liquid test substances (150 µL) are applied uniformly to the epidermal surface inside the tube. When testing solid materials, a sufficient amount of the solid is applied evenly to the disc to ensure that the whole surface of the epidermis is covered. Deionised water (150 µL) is added on top of the solid and the tube is gently agitated. In order to achieve maximum contact with the skin, solids may need to be warmed to 30^0 C to melt or soften the test substance, or ground to produce a granular material or powder.

17. Three skin discs are used for each test and control substance. Test substances are applied for 24 hours. The test substance is removed by washing with a jet of tap water at up to 30^0 C until no further material can be removed.

TER measurements

18. The TER is measured by using a low-voltage, alternating current databridge (13). The databridge measures inductance, capacitance and resistance up to values of 2000H, 2000µF, and 2MΩ, respectively at frequencies of 100Hz or 1kHz, using series or parallel values. For the purposes of the TER corrosivity assay measurements are recorded in resistance, at a frequency of 100Hz and using series values. Prior to measuring the electrical resistance, the surface tension of the skin is reduced by adding a sufficient volume of 70% ethanol to cover the epidermis. After a few seconds, the ethanol is removed from the tube and the tissue is then hydrated by the addition of 3mL $MgSO_4$ solution (154mM). The databridge electrodes are placed on either side of the skin disc to measure the resistance in kΩ/skin disc (Figure 1). Electrode dimensions and the length of the electrode exposed below the crocodile clips are shown in Figure 2. The clip attached to the inner electrode is rested on the top of the PTFE tube during resistance measurement to ensure that a consistent length of electrode is submerged in the $MgSO_4$ solution. The outer electrode is positioned inside the receptor chamber so that it rests on the bottom of the chamber. The distance between the spring clip and the bottom of the PTFE tube is maintained as a constant (Figure 2), because this distance affects the resistance value obtained.

19. If the measured resistance value is greater than 20 kΩ, this may be due to the remains of the test substance coating the epidermal surface of the skin disc. Further removal of this coating can be attempted, for example, by sealing the PTFE tube with a gloved thumb and shaking it for approximately 10 seconds; the $MgSO_4$ solution is discarded and the resistance measurement is repeated with fresh $MgSO_4$.

20. The properties and dimensions of the test apparatus and the experimental procedure used may influence the TER values obtained. The 5 kΩ corrosive threshold was developed from data obtained with the specific apparatus and procedure described in this Guideline. Different threshold and control values may apply if the test conditions are altered or a different apparatus is used. Therefore, it is necessary to calibrate the methodology and resistance threshold values by testing a series of reference standards chosen from the chemicals used in the validation study (4)(5), or from similar chemical classes to the chemicals being investigated. A set of suitable reference chemicals is shown in Table 1.

Dye Binding Methods

21. Exposure of certain non-corrosive materials can result in a reduction of resistance below the cut-off of 5 kΩ allowing the passage of ions through the stratum corneum, thereby reducing the electrical resistance (5). For example, neutral organics and chemicals that have surface-active properties (including detergents, emulsifiers and other surfactants) can remove skin lipids making the barrier more permeable to ions. Thus, if the TER values of test substances are less than or equal to 5 kΩ in the absence of visual damage, an assessment of dye penetration should be carried out on the control and treated tissues to determine if the TER values obtained were the result of increased skin permeability, or skin corrosion (3)(5). In case of the latter where the stratum corneum is disrupted, the dye sulforhodamine B, when applied to the skin surface rapidly penetrates and stains the underlying tissue. This particular dye is stable to a wide range of chemicals and is not affected by the extraction procedure described below.

Sulforhodamine B dye application and removal

22. Following TER assessment, the magnesium sulfate is discarded from the tube and the skin is carefully examined for obvious damage. If there is no obvious major damage, Sulforhodamine B dye (Acid Red 52; C.I. 45100; CAS number 3520-42-1), 150µL of a 10% (w/v) dilution in distilled water, is applied to the epidermal surface of each skin disc for 2 hours. These skin discs are then washed with tap water at up to room temperature for approximately 10 seconds to remove any excess/unbound dye. Each skin disc is carefully removed from the PTFE tube and placed in a vial (e.g. a 20mL glass scintillation vial) containing deionised water (8mL). The vials are agitated gently for 5 minutes to remove any additional unbound dye. This rinsing procedure is then repeated, after which the skin discs are removed and placed into vials containing 5ml of 30% (w/v) sodium dodecyl sulphate (SDS) in distilled water and are incubated overnight at 60^0 C.

23. After incubation, each skin disc is removed and discarded and the remaining solution is centrifuged for 8 minutes at 21^0 C (relative centrifugal force ~175). A 1mL sample of the supernatant is diluted 1 in 5 (v/v) [i.e. 1mL + 4mL] with 30% (w/v) SDS in distilled water. The optical density (OD) of the solution is measured at 565nm.

Calculation of dye content

24. The sulforhodamine B dye content per disc is calculated from the OD values (5)(sulforhodamine B dye molar extinction coefficient at 565nm = 8.7×10^4; molecular weight = 580). The dye content is determined for each skin disc by the use of an appropriate calibration curve and a mean dye content is then calculated for the replicates.

Interpretation of results

25. The mean TER results are accepted if the concurrent positive and negative control values fall within the acceptable ranges for the method in the testing laboratory. The acceptable resistance ranges for

the methodology and apparatus described above are given in the following table:

Control	Substance	Resistance range (kΩ)
Positive	10M Hydrochloric acid	0.5 - 1.0
Negative	Distilled water	10 - 25

26. The mean dye binding results are accepted on condition that concurrent control values fall within the acceptable ranges for the method. Suggested acceptable dye content ranges for the control substances for the methodology and apparatus described above are given below:

Control	Substance	Dye content range (µg/disc)
Positive	10M Hydrochloric acid	40 - 100
Negative	Distilled water	15 - 35

27. The test substance is considered to be non-corrosive to skin:

i) if the mean TER value obtained for the test substance is greater than 5 kΩ, or

ii) the mean TER value is less than or equal to 5 kΩ, and

- the skin disk is showing no obvious damage, and
- the mean disc dye content is well below the mean disc dye content of the 10M HCl positive control obtained concurrently (see paragraph 26 for acceptable ranges).

28. The test substance is considered to be corrosive to skin:

i) if the mean TER value is less than or equal to 5 kΩ and the skin disk is obviously damaged, or

ii) the mean TER value is less than or equal to 5 kΩ, and
- the skin disk is showing no obvious damage, but
- the mean disc dye content is greater than or equal to the mean disc dye content of the 10M HCl positive control obtained concurrently (see paragraph 26 for positive control values).

DATA AND REPORTING

Data

29. Resistance values (kΩ) and mean dye content values (µg/disc), where appropriate, for the test material, as well as for positive and negative controls should be reported in tabular form (individual trial data and means ± S.D.), including data for replicates/repeat experiments, mean and individual values.

Test report

30. The test report must include the following information:

Test and Control Substances:
- identification data and CAS number, if known;
- physical nature and purity;
- physico-chemical properties relevant to the conduct of the study;
- treatment of the test/control substances prior to testing, if applicable (e.g., warming, grinding);
- stability, if known.

Test Animals:
- strain and sex used;
- age of the animals when used as donor animals;
- source, housing condition, diet, etc.;
- details of the skin preparation.

Test Conditions:
- calibration curves for test apparatus;
- calibration curves for dye binding test performance;
- details of the test procedure used for TER measurements;
- details of the test procedure used for the dye binding assessment;
- description of any modification of the test procedure;
- description of evaluation criteria used.

Results:
- tabulation of data from the TER and dye binding assay for individual animals and individual skin samples;
- description of any effects observed.

Discussion of the results.

Conclusions.

LITERATURE

(1) OECD (2001). Harmonised Integrated Hazard Classification System for Chemical Substances and Mixtures. http://www1.oecd.org/ehs/Class/HCL6.htm

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Table 1: Reference Chemicals

1,2-Diaminopropane	CAS-No. 78-90-0	Severely Corrosive
Acrylic Acid	CAS-No. 79-10-7	Severely Corrosive
2-tert. Butylphenol	CAS-No. 88-18-6	Corrosive
Potassuim hydroxide (10%)	CAS-No. 1310-58-3	Corrosive
Sulfuric acid (10%)	CAS-No. 7664-93-9	Corrosive
Octanoic acid (caprylic acid)	CAS-No. 124-07-02	Corrosive
4-Amino-1,2,4-triazole	CAS-No. 584-13-4	Not corrosive
Eugenol	CAS-No. 97-53-0	Not corrosive
Phenethyl bromide	CAS-No. 103-63-9	Not corrosive
Tetrachloroethylene	CAS-No. 127-18-4	Not corrosive
Isostearic acid	CAS-No. 30399-84-9	Not corrosive
4-(Methylthio)-benzaldehyde	CAS-No. 3446-89-7	Not corrosive

Most of the chemicals listed are taken from the list of chemicals selected for the ECVAM international validation study (4). Their selection is based on the following criteria:

i) equal number of corrosive and non-corrosive substances;

ii) commercially available substances covering most of the relevant chemical classes;

iii) inclusion of severely corrosive as well as less corrosive substances in order to enable discrimination based on corrosive potency;

iv) choice of chemicals that can be handled in a laboratory without posing **other serious** hazards than corrosivity.

Figure 1: Apparatus for the rat skin TER assay

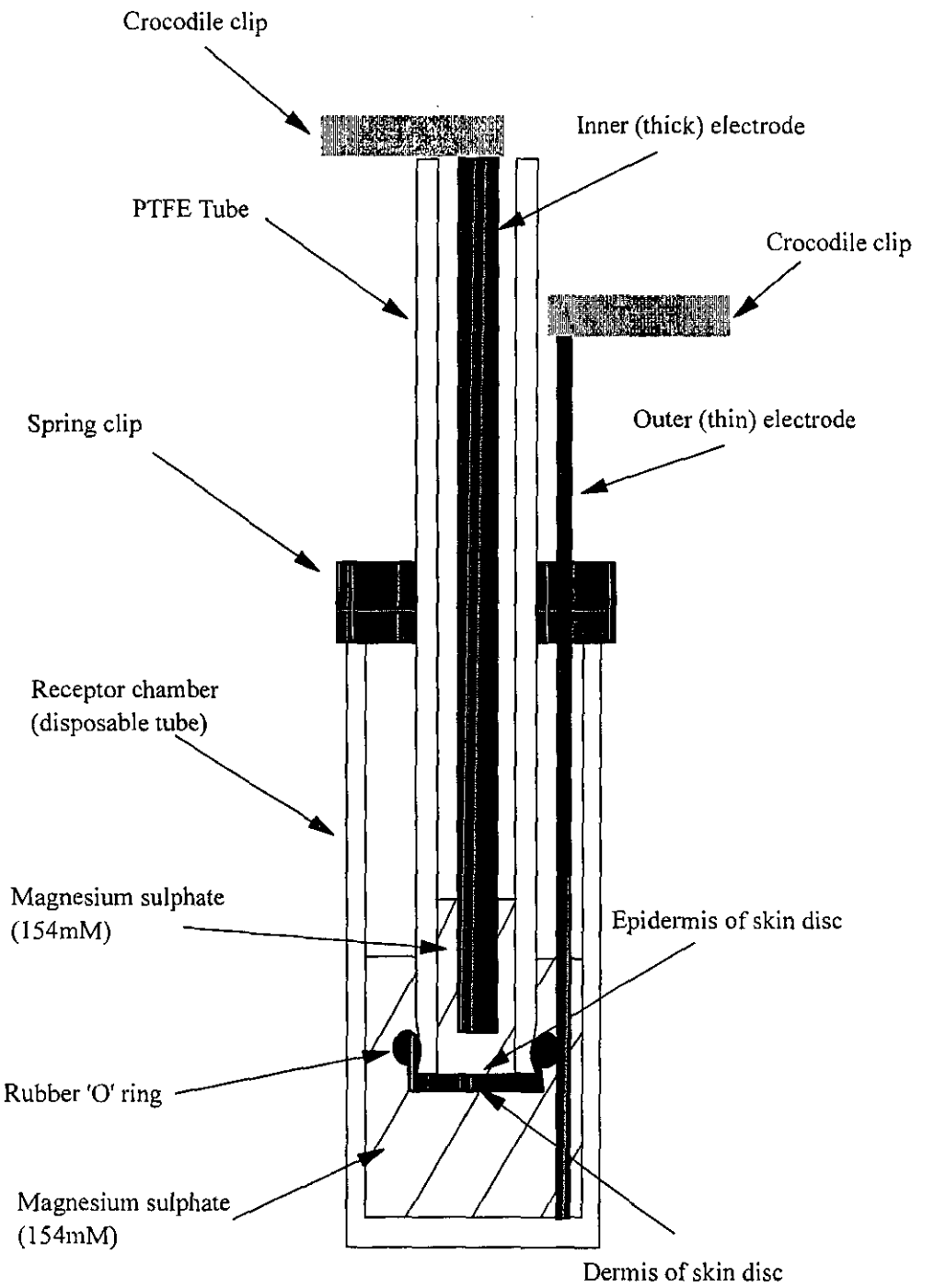
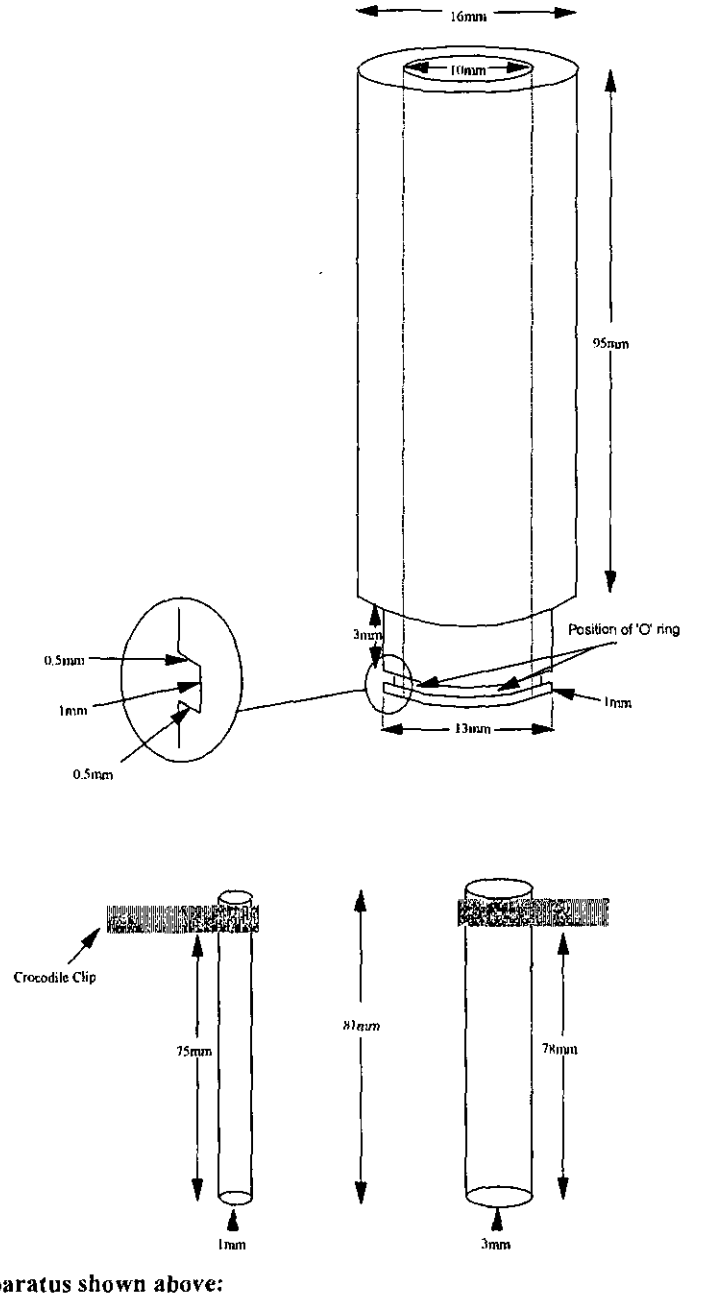


Figure 1: Apparatus for the rat skin TER assay

Figure 2: Dimensions of the polytetrafluoroethylene (PTFE) and receptor tubes and electrodes used



Critical factors of the apparatus shown above:

- the inner diameter of the PTFE tube,
- the length of the electrodes relative to the PTFE tube and receptor tube, such that the skin disc is not touched by the electrodes and that a standard length of electrode is in contact with the $MgSO_4$ solution,
- the amount of $MgSO_4$ solution in the receptor tube should give a depth of liquid, relative to the level in the PTFE tube, as shown in Figure 1,
- the skin disk should be fixed well enough to the PTFE tube, such that the electrical resistance is a true measure of the skin properties.