ICCVAM Evaluation of *In Vitro* Methods for Assessing the Dermal Corrosivity Potential of Chemicals: EPISKIN™, EpiDerm™ (EPI-200 Model), and Rat Skin Transcutaneous Electrical Resistance (TER)

Public Law 106-545 directs the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) to evaluate new, revised and alternative test methods, and to develop and forward test recommendations to appropriate Federal agencies. ICCVAM recently evaluated and developed test recommendations for three *in vitro* methods for assessing the dermal corrosivity potential of chemicals. The methods are:

- EPISKIN™
- EpiDerm™ (EPI-200)
- Rat Skin Transcutaneous Electrical Resistance (TER) Assay

Draft proposed test recommendations were developed by the ICCVAM Corrosivity Working Group (CWG), which is composed of Federal Agency scientists who have experience and/or expertise with corrosivity testing. These proposed recommendations were endorsed by ICCVAM and made available with background review materials for a 45-day public comment period as announced in a September 28, 2001 *Federal Register* notice (NIEHS 2001, Appendix D). Written public comments were received from 15 individuals and six organizations; these comments are provided in Appendix E. The comments were considered by the CWG, which then drafted final test recommendations that were forwarded to and approved by ICCVAM in May 2002.

1.1 Introduction

ICCVAM has developed test recommendations for the use of three *in vitro* test methods to assess the dermal corrosivity potential of chemicals and chemical mixtures: EpiDerm™ (EPI-200), EPISKIN™, and the Rat Skin TER assay. Validation studies for these methods were conducted by the European Centre for the Validation of Alternative Methods (ECVAM) (Baratt et al., 1998; Fentem et al., 1998; Liebsch et al., 2000). The validation status of these three methods has been evaluated by the ECVAM Scientific Advisory Committee (ESAC) (Balls and Corcelle, 1998; Balls and Hellsten, 2000), and EPISKIN™ and Rat Skin TER have also been evaluated by the European Commission's Scientific Committee for Cosmetic Products and Non-food Products (SCCNFP) (SCCNFP, 1998). The three methods have been adopted for regulatory use within the European Union (EU) by the European Commission (EU, 2000). The EPISKIN™ human skin model is commercially available from EPISKIN SNC, Lyon, France, a wholly owned subsidiary of L'OREAL. EpiDerm™ (EPI-200) is commercially available from MatTek Corporation, Ashland, MA, USA. In the TER assay, transcutaneous electrical resistance is measured using an AIM electronic databridge 401 or 6401, which is commercially available from H. Tinsley and Co., New Addington, Croydon, Surrey, UK.

ICCVAM Expedited Review Process

ICCVAM used an expedited test method review process to consider these three methods because they had already been evaluated by ECVAM (ICCVAM, 2001). The ICCVAM CWG considered background review documents prepared by the NTP

Interagency Center for the Evaluation of Alternative Methods (NICEATM) for each of the three corrosivity methods. Based on the information provided and previous reviews, a formal independent scientific peer review panel evaluation was not considered necessary. In accordance with the expedited review process procedures, the CWG developed proposed test recommendations which were reviewed and endorsed by ICCVAM. A *Federal Register* notice (September 28, 2001, Vol. 66, No. 189, pp.49685-6) announced the availability and requested public comment on the proposed recommendations and the test method background review documents (Appendix D). These public comments are discussed below in Section 1.2.3 of this document and are provided in Appendix E. Following receipt and consideration of public comments, ICCVAM prepared final recommendations on these methods. In accordance with Public Law 106-545, these ICCVAM recommendations will be forwarded to U.S. agencies for their consideration and acceptance where appropriate.

1.2 Background

1.2.1 ECVAM Evaluation

Validation studies on these three *in vitro* assays were conducted by ECVAM (Barratt et al., 1998; Fentem et al., 1998; Liebsch et al., 2000). Based on the results, which met pre-study acceptance criteria of no more than 20% false negatives and no more than 20% false positives, the ECVAM Study Management Team concluded that EpiDerm™ (EPI-200), 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 for all chemical classes (Fentem et al., 1998; Liebsch et al., 2000). Of the three test methods, only EPISKIN™ was able to distinguish between chemicals in the EU skin corrosion hazard classes (R35 and R34) and for two of the three United Nations (UN) packing group classifications (I and II/III) (Fentem et al., 1998).[1] A detailed review of these validation studies is described in this final report (ICCVAM, 2002).

1.2.2 Relevant Comments from an OECD Expert Consultation Meeting

In 1999, the Organisation for Economic Co-operation and Development (OECD) proposed a draft test guideline (TG) describing the Rat Skin TER assay and a generic *in vitro* skin model assay (OECD, 1999). A generic skin model assay procedure was proposed rather than the specific EPISKIN™ and EpiDerm™ (EPI-200) test method protocols because of OECD's policy not to adopt TGs for tests that require equipment or material that can only be obtained from unique sources. OECD requested review of the draft TG by member countries in 2000. Extensive comments were received, and an Extended Expert Consultation Meeting was convened in Berlin, Germany on November 1-2, 2001 to address these comments and other technical issues.

[1] UN packing group classifications I, II, and III are assigned based on the capacity of a chemical, when tested on the intact skin of rabbits, to produce skin corrosion following exposure intervals of 3 minutes, 1 hour, or 4 hours, respectively (Fentem et al., 1998). Current EU regulations require classification of chemicals according to certain risk phrases, such as those assigned based on whether the chemical causes corrosion following a 3-minute application (R35 – "causes severe burns"; analogous to packing group I) or 4 hours (R34 – "causes burns"; analogous to packing groups II and III) (Barratt et al., 1998; Fentem et al., 1998). Internationally harmonized classification schemes for corrosivity, which include the UN packing group classifications, have recently been adopted (OECD, 2001a).

The meeting experts agreed to prepare two separate test guidelines, one for the TER, and one for the human skin test model. With regard to use of these methods, the Expert Meeting participants agreed that, in the majority of all applications, the *in vitro* skin corrosion tests would be applied as one of the initial steps of a tiered approach. Consequently, false negative predictions are likely to be detected when the test chemical is tested on the first rabbit for skin irritation (OECD, 2002c). The deliberations at the meeting did not change the general procedures for the generic human skin model assay; however, the following revisions were proposed for the TER assay:

Rat Skin TER Assay

- Substances with a resistance value greater than 5 kΩ are considered non-corrosive. Most test substances typically have produced resistance values in two ranges, <3 kΩ (positive) and >10 kΩ (negative). It was recommended that if the resistance value for a test substance is close to the 5 kΩ decision criteria, a judgment of whether to classify the substance as positive or negative should consider a weight-of-evidence strategy or assume the more conservative approach, based on regulatory needs. If classified as positive, the standard positive confirmatory dye-binding test to demonstrate physical destruction of the stratum corneum should be conducted to avoid a false positive classification.
- Several critical aspects of the test system were defined, including the surface area of skin used, the use of magnesium sulfate ($MgSO_4$) as the electrochemical solution for measuring resistance, and the age of the animals.

Two revised draft test guidelines were subsequently circulated for comment in March 2002, and further revised for consideration at the Test Guideline Program National Coordinators Meeting in June, 2002. Both guidelines were accepted pending further revisions agreed on at the meeting (personal communication, June 2002, Angela Auletta, U.S. EPA, Washington, D.C.).

1.2.3 Public Comments

Twenty-one public comments were received in response to the September 28, 2001 *Federal Register* notice. Three of the 21 responses provided general comments about the Background Review Document (BRD), stating that it was well organized, comprehensive and clearly written. The remaining comments addressed specific aspects of the proposed test recommendations as discussed below.

Integrated testing scheme vs. stand-alone

Seventeen of the 21 public responses disagreed with or stated opposition to the proposed ICCVAM recommendation that these three *in vitro* methods should be used in the context of a weight-of-evidence approach in an integrated scheme, where negative *in vitro* corrosivity responses would be followed by *in vivo* dermal irritation/ corrosion testing. Three of the 21 comments stated that the three *in vitro* tests should be used as stand-alone tests, such that negative results would be classified as non-corrosives without further confirmatory testing.

ICCVAM recognizes that it would be highly desirable to completely replace animals for corrosivity testing. However, the current performance characteristics resulting from validation studies of these *in vitro* assays do not adequately support their use as stand-alone assays for hazard classification. Specifically, the results of the ECVAM validation studies indicate that significant false negative results may occur with these

assays (12% for TER; 13% for EpiDerm™ (EPI-200); 17% for EPISKIN™) (Fentem et al., 1998; Liebsch et al., 2000). In such instances, a false negative result for a corrosive chemical would result in erroneous classification as a non-corrosive. Accordingly, the corrosive chemical would not be labeled with appropriate hazard warnings of corrosivity. Serious and irreversible damage can result from human exposure to corrosive substances, including dermal ulceration and scarring. Given that results of dermal corrosion are often used by regulators to identify corrosives to the eye, false negative responses in the *in vitro* dermal corrosion tests will fail to identify potential serious effects to the eye for 12-17% of true dermal corrosives. Therefore, this level of error was not considered by ICCVAM to provide adequate protection for public health and safety. ICCVAM is also cognizant of the fact that nearly all regulatory authorities that require corrosive testing also require a determination of dermal irritation potential if substances are not found to be corrosive. Current international guidance and test guidelines for dermal irritation/corrosion call for sequential testing, so that if a corrosive substance is erroneously identified in the *in vitro* test as non-corrosive, it will be detected as corrosive in an *in vivo* irritancy test (EPA, 1998; OECD 2001a, OECD 2001b; Worth, et al. 1998). *In vitro* tests for irritancy are being developed and may be coupled with *in vitro* corrosion tests. Such test strategies will need to be evaluated for their ability to correctly identify corrosive and irritant chemicals that produce false negative results in such *in vitro* tests. Thus, as outlined in Section 1.3, ICCVAM concludes that the false negative rates obtained in these three *in vitro* assays preclude their use as stand-alone assays. Instead, these assays should be considered as screens, where positive results are classified as corrosives and negative results require further testing for corrosive potential.

General test method guideline vs. specific validated test method protocols

One comment suggested using a general "skin model corrosivity test" description rather than the specific test method protocols for EpiDerm™ (EPI-200) and EPISKIN™. The basis for this suggestion was: 1) the 2 assays are similar with regard to test material exposure, endpoints, prediction models, and predictive power; and 2) this would better allow the future use of other skin models that are similar with regard to structure and function and that perform comparably to these previously validated skin models. The respondent also acknowledged that this would require the development of structural and performance criteria, including a set of reference chemicals, to evaluate such new skin models.

While ICCVAM recognizes the increased flexibility of general test method descriptions, it also recognizes the critical importance of determining the acceptability of validated specific protocols for which the reliability and performance characteristics have been carefully determined. The use of protocols that adhere to a general test method description but have not been adequately validated could lead to erroneous results. Therefore, ICCVAM is only recommending validated, specific test method protocols. However, ICCVAM appreciates that similar test methods could be found to be acceptable if adequate performance and reliability are demonstrated for a standardized test method protocol in appropriate validation studies. The provision of a list of reference chemicals and minimum performance criteria would

certainly be helpful to those interested in validating such models in the future.

Concern about limited availability

Another comment suggested revising the BRD and related documents to remove any reference to EPISKIN™, or alternatively, to include a qualifying statement regarding the current commercial unavailability of this human skin model. The basis for the comment was to avoid recommending a test method that is not otherwise commercially available. ICCVAM has added a statement regarding the current availability of each assay.

1.3 ICCVAM Test Method Recommendations

EPISKIN™, EpiDerm™ (EPI-200), and Rat Skin Transcutaneous Electrical Resistance (TER)

Based on evaluation of the ECVAM validation studies and other available data, ICCVAM concludes that there are sufficient data to substantiate the use of these three *in vitro* assays for assessing the dermal corrosion potential of chemicals in a weight-of-evidence approach in an integrated testing scheme (EPA, 1996; OECD, 2001c; OECD, 2001d; OECD, 2001e; OECD, 2001f; Worth, et al. 1998). EPISKIN™, EpiDerm™ (EPI-200), and Rat Skin TER are not appropriate methods for assessing irritation. 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 irritation/corrosion testing. (Animals 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 test system, there is the opportunity for confirmatory testing if false positive results are indicated based on a weight-of-evidence evaluation of supplemental information, such as pH, structure-activity relationships (SAR), and other chemical and testing information.

ICCVAM previously evaluated another *in vitro* method for determining corrosivity, Corrositex® (ICCVAM, 1999), and recommended that it could be used in a similar manner as recommended for EPISKIN™, EpiDerm™ (EPI-200), and Rat Skin TER. Corrositex® is also approved by the U.S. Department of Transportation for identifying the three United Nations packing group classifications for certain chemical classes (ICCVAM, 1999; U.S. DOT, 2000). The ICCVAM report on Corrositex® is available at http://iccvam.niehs.nih.gov/docs/reports/corprrep.pdf.

Animal Welfare Considerations

ICCVAM concludes that each of the three *in vitro* corrosivity methods sufficiently incorporates, where scientifically feasible and applicable, the 3Rs of animal use alternatives (refinement, reduction, and replacement). When EpiDerm™ (EPI-200) and EPISKIN™ are used as part of an integrated testing strategy for irritation/corrosion, there is replacement of animals because positive *in vitro* results usually eliminate the need for animal testing. There is a reduction in animal use with negative *in vitro* results because only one positive animal may be needed to identify an *in vitro* false negative as a corrosive chemical. Compared to the rabbit corrosivity test, the Rat Skin TER assay reduces the number of animals used because skin from one rat may be used to test up to five chemicals. Similar to EpiDerm™ (EPI-200) and EPISKIN™, use of the Rat Skin TER assay as part of the

integrated testing strategy for irritation/ corrosion reduces and refines the use of animals when negative *in vitro* results are obtained.