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BACKGROUND FOR FIFRA SCIENTIFIC ADVISORY PANEL ON REGULATORY USE OF *IN VITRO* SYSTEMS

Executive Summary

Many *in vitro* and *ex vivo* methods have been developed or are under development to reduce or replace animal usage in toxicity tests. Across United States federal agencies, the Interagency Coordinating Committee for Validation of Alternative Methods (ICCVAM) typically provides for review and assessment of the status of validity of test systems, including Proprietary Test Methods (Refs. 1, 2). Thereafter, the Environmental Protection Agency determines if data generated using the new methods are acceptable for its regulatory mandate. A process is needed to provide assurance that an *in vitro* system continues to perform over time in a manner that is consistent with the test system as it was originally validated. If a validated Proprietary Test Method is of interest to the Agency for regulatory testing, the Agency writes a generic test guideline which spells out Performance Standards that must be met by any specific test system falling under it. Consistent with its goal of obtaining scientifically sound test data for hazard and risk assessment of pesticides and toxic chemicals, the Agency is exploring what changes in current policies and procedures may be needed to facilitate the acceptance of data developed using *in vitro* alternatives.

The Agency is working with national and international organizations to encourage development of policies and "standards" for scientific practice to assure quality in implementation of *in vitro* methods performed as alternatives to animal testing for regulatory purposes.

The Agency will draw on Performance Standards when writing generic guidelines for Proprietary Test Methods; identify quality control measures for *in vitro* methods in Office of Prevention, Pesticides, and Toxic Substances test guidelines (to the extent feasible - since companies may declare some quality control measures to be confidential); and identify appropriate controls, data reporting elements, and benchmarks in test guidelines so that the potential risks of pesticides and other chemicals can be reviewed and reliably assessed.

The Office of Prevention, Pesticides, and Toxic Substances intends to draw on existing Good Laboratory Practice regulations to ensure that *in vitro* tests used for regulatory purposes are reproducible, credible, and acceptable. Manufacturers and testing laboratories will be able to refer to Office of Prevention, Pesticides, and Toxic Substances test guidelines and ICCVAM essential test method components specified by ICCVAM for quality control measures for use under Good Laboratory Practice regulations. In addition, the Agency is considering the utility of technical guidance to use when laboratories performing *in vitro studies* as alternatives to animal use for regulatory purposes are audited under Good Laboratory Practice.

BACKGROUND FOR FIFRA SCIENTIFIC ADVISORY PANEL ON REGULATORY USE OF *IN VITRO* SYSTEMS

I. INTRODUCTION: REGULATORY USE OF *IN VITRO* ALTERNATIVE TEST METHODS

Background:

Historically, *in vivo* tests in laboratory animals have formed the foundation of hazard and risk assessment at the Environmental Protection Agency (EPA). The development of sound testing procedures for good science in regulation includes incorporation of the latest scientific advances, including, whenever practical, validated and accepted non-animal methods such as cell and tissue cultures, bioconstructs or microarrays, as testing alternatives to animal testing.

Many such *in vitro* methods have been developed or are under development to reduce or replace animal usage in toxicity tests. Any *ex vivo* tissue, bioconstruct or cultured cell has a limited number of ways to respond to a chemical stimulus, whereas intact animals have many other potentialities. Consistent with its goal of obtaining scientifically sound test data for hazard and risk assessment of pesticides and toxic chemicals, the Agency is exploring what changes in current policies and procedures may be needed to facilitate the acceptance and use of data developed using *in vitro* alternatives. Some *in vitro* methods are developed by sponsors for commercial marketing as Proprietary Test Methods (PTMs). Some components and principles of such tests may not always be divulged to the public. In other cases, sponsors may develop *in vitro* methods and disclose their test design and the scientific principles of the test; these may or may not be PTMs.

Normally, when new *in vitro* test methods are developed, systematic laboratory studies are performed with a set of Reference Chemicals. These chemicals should illustrate the range of responses of the test system and be representative of the chemical classes for which the test is expected to be used. The chemicals are tested in the new *in vitro* test and compared to the existing standard or traditional animal test or human data and experience. Optimally, there should also be *in vitro* results from testing of the same materials in several different laboratories for both the *in vitro* and the *in vivo* test method (Ref. 1). Validation assesses the new test system's ability to predict the intact organism for the toxicological effect of interest. In the course of its validation, the test's reliability is assessed in terms of intra- and inter-laboratory variability. Across United States federal agencies, the Interagency Coordinating Committee for Validation of Alternative Methods (ICCVAM) typically provides for review and assessment of the status of validity of test systems, including PTMs (Refs. 1,2). Thereafter, the Agency determines if data generated using the new methods are acceptable for its regulatory mandate.

Quality and Consistency:

In vitro alternatives to animal testing pose unique issues regarding quality and performance. Such *in vitro* systems also must be maintained appropriately to ensure their quality and integrity, e.g., sterility, viability, and responsiveness. Therefore, a process is needed to provide assurance that an *in vitro* system continues to perform over time in a manner that is consistent with that of the test system as it was originally validated. Consistency of performance of the *in vitro* system is also needed with any change in ingredients or the manufacturing practices for proprietary test systems, or procedural variations among the laboratories performing the test.

Performance Standards:

Under law, United States agencies are limited in endorsement of validated PTMs unless generic guidelines and Performance Standards are developed for each method (Ref. 3). Performance Standards include a description of the essential structural and procedural elements of the test method and the levels of accuracy and reliability that the test method should achieve when evaluated using chemicals selected from among the Reference Chemicals which were used to demonstrate the acceptable performance of the validated PTM. In addition, Performance Standards for each *in vitro* test system can be used to ensure that the assay system and the laboratory are performing as expected and in accordance with performance of the *in vitro* system as originally validated.

Quality Framework for Test Data Submitted to the Agency:

Quality control requirements for assays performed to fulfill regulatory requirements are included in the Agency's Good Laboratory Practice regulations (GLP)(Ref. 4). The Office of Pesticide Programs intends to draw on currently promulgated GLP regulations as well as good scientific practices established by various scientific disciplines relevant to *in vitro* systems to ensure that *in vitro* tests used for regulatory purposes are reproducible, reliable, credible and acceptable. The Agency is also considering developing special guidance for its quality control auditors to use with *in vitro* tests submitted for regulatory purposes

The Office of Prevention, Pesticides, and Toxic Substances (OPPTS) has long standing practices for quality assurance of test data submitted to the Agency for use under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The Office of Pesticide Programs generally bases it regulatory decisions under FIFRA and the Food, Drug and Cosmetics Act on guideline studies which conform to GLP regulations. OPPTS test guidelines spell out important quality control and data reporting elements. These must be documented for thorough review by the Agency. The Office of Pesticide Programs has also specified acceptance criteria for use with studies which might have preceded GLP or may have been performed for other regulatory authorities. The Agency has a comprehensive quality assurance program to ensure the quality of data which supports our regulatory decisions (Ref. 5). OPPTS has historically acted consistently with the thrust of this program due to its longstanding use of quality assurance practices.

Anticipating the use of *in vitro* methods as alternatives to animal studies, the Agency plans to standardize data reporting elements for cell cultures, microarrays, and other *in vitro* or *in silico* methods. Efforts are already in place in other fora to identify appropriate data elements for such studies, e.g., the report of European Center for Validation of Alternative Methods (ECVAM) Good Cell Culture Practice Task Force (Ref. 6) ; and the Minimum Information About a Microarray Experiment - MIAME for Toxicogenomics (MIAME-Tox) report by the Microarray Gene Expression Data Society (Ref. 7). The Agency is following this work and will use such efforts as a starting point for developing its own policies. In addition to identifying data reporting elements, the Office of Prevention, Pesticides and Toxic Substances will identify all appropriate quality control steps for laboratories to implement when using *in vitro* tests as alternatives to animal testing.

II. PERFORMANCE STANDARDS

Proprietary Test Methods:

United States agencies may identify proprietary *in vitro* or *ex vivo* test methods in its test guidelines if they comply with the Performance Standards. If a validated PTM is of interest to the Agency for regulatory testing, the Agency writes a generic test guideline which spells out Performance Standards that must be met by any specific test system falling under it. Performance Standards can be used in two ways. Firstly, they are applicable to the specific validated PTM, allowing that test system to be used for regulatory purposes. Secondly, the Performance Standards identify criteria that should be met by any other future PTM that would fall under the generic test guideline. This means that any other PTM that meets the Performance Standards can be identified as such.

ICCVAM has described a process and developed definitions addressing ways to handle validation of PTMs, including setting Performance Standards (Ref. 2). *Performance Standards*, based on a validated test method, provide a basis for evaluating the comparability of a proposed test method that is mechanistically- and functionally-similar, i.e. a *"me-too" test method*. The three elements of Performance Standards are (a) the *essential test method components* of the validated test method, (b) a list of recommended *Reference Chemicals* drawn from the test systems's validation data base and (c) a statistical description of the accuracy and reliability that should be achieved by the proposed test system when applied to the subset of Reference Chemicals. *Essential test method components* are descriptive structural, functional, and procedural elements of a validated test method that should be included in the protocol of a proposed, me-too test method. These include unique characteristics of the test method, critical procedural details, and certain quality control measures. Adherence to essential test method components will help to assure that a proposed test method is based on the same concepts as the corresponding validated test method. The unique characteristics of *in vitro* alternative test systems generally address the target tissue and the detection method. Critical procedural details include mode of chemical application, controls, and reporting elements.

After a test method has been accepted as valid for its purpose in fulfilling data requirements for

registration or reregistration under FIFRA, a representative subset of chemicals used during the validation process may be selected to validate "me-too" test methods. To the extent possible, this subset of Reference Chemicals should:

- Be representative of the range of responses that the validated test method is capable of measuring or predicting
- Have produced consistent results in the validated test method and in the reference test method and/or the species of interest
- Reflect the accuracy of the validated test method
- Have well-defined chemical structures
- Be readily available
- Not be associated with excessive hazard or prohibitive disposal costs

This subset of Reference Chemicals represents the minimum number of chemicals that should be used to evaluate the performance of a proposed, "me-too" test method . They allow the performance, in terms of accuracy, sensitivity, specificity, false positive rates, and false negative rates, of the test method for relevant chemical classes to be determined. Here, *accuracy* means (a) The closeness of agreement between a test result and an accepted reference value and is measured as the proportion of the correct outcomes of the methods; *sensitivity* is the proportion of all positive test materials that are correctly classified as positive in a test; *specificity* is the proportion of all negative test materials that are correctly classified as negative in a test; *false positive rate* is the proportion of all negative test materials that are falsely identified as positive and; *false negative rate* is the proportion of all positive test materials that are falsely identified as negative.

A "me-too" test system may be manufactured or marketed by a different company from the original test system sponsor. The "me-too" system is mechanistically and functionally similar to the original validated system, and is not just intended to be a different system for measuring the same endpoint. For example, two test systems using human skin models for assessing dermal corrosion, discussed in the section below, are EPISKIN™ and EpiDerm™ . EpiDerm is a "me-too" test method for EPISKIN and was validated against a subset of the Reference Chemicals used to validate EPISKIN (Ref. 8). Manufacturers may use essential test method components to demonstrate that the "me-too" method is mechanistically similar to a validated PTM. Comparable performance of the "me-too" method for the subset of Reference Chemicals can demonstrate its functional similarity without the need for a full validation.

Performance Standards required for PTMs approved for use under FIFRA can also be part of the foundation for quality control of PTMs. (See section III). Performance Standards should be designed to allow manufacturers or applicants to demonstrate to the Agency that the test kit as marketed, whether a "me-too" or a PTM product, performs as scientifically validated.

Much of the activity to develop non-animal methods for toxicological evaluation has been and is expected to be at the behest of commercial sponsors. United States agencies need Performance Standards for all new PTMs. Use of Performance Standards is also desirable for other *in vitro* methods as well. Availability of Performance Standards can be expected to facilitate the development of "me-too" test methods while setting standards for the accuracy and reliability of the original test method and any mechanistically and functionally similar methods.

Performance Standards for TER, Corrositex, and EPISKIN/EpiDerm:

ICCVAM previously evaluated and recommended four validated test methods for assessing the dermal corrosivity hazard potential of chemicals: Corrositex®, EPISKIN™, EpiDerm™ (EPI-200), and the rat skin transcutaneous electrical resistance (TER Assay (Refs. 8,9). Corrositex uses a biological gel supported by an inert semipermeable membrane to determine the ability of a corrosive chemical or mixture to pass through, by diffusion and/or destruction/erosion and elicit a color change in an underlying liquid indicator. EPISKIN uses a three-dimensional human skin model composed of human collagen and human keratinocytes and utilizes cell viability as the measured endpoint. EpiDerm is mechanistically and functionally related to EPISKIN and consists of a three-dimensional human skin model utilizing cultured epidermal keratinocytes, with cell viability being the endpoint. The TER assay uses disks of *ex vivo* rat skin to which the application of corrosive material produces a loss of normal stratum corneum integrity and barrier function as measured by a reduction of the inherent transcutaneous electrical resistance below a predetermined threshold level. The Agency requested that ICCVAM establish Performance Standards for the three proprietary dermal corrosivity test methods. In response, the ICCVAM Dermal Corrosion Interagency Working Group drafted proposed Performance Standards based on the validated test methods for these three types of *in vitro* dermal corrosivity assays: membrane barrier test methods, human skin model system test methods, and skin TER test methods (Refs. 10, 11, 12). The ICCVAM Performance Standards include the essential test method components for each assay and the subset of Reference Chemicals to be used to demonstrate comparability of performance. The essential test method components can be used as a basis for generic guidelines. ICCVAM is expected to finalize the Performance Standards for these corrosivity assays in November 2003. EPA has an opportunity to provide comments on these draft Performance Standards and generic guidelines including consideration of Scientific Advisory Panel recommendations.

OPPTS will revise its 870.2500 test guideline for Dermal Irritation to incorporate the three validated *in vitro* methods for corrosivity, drawing upon the ICCVAM Performance Standards for the three assays.

New test methods proposed for use as "me-too's" based on each of these corrosivity assays must meet the Performance Standards. A range of corrosive substances is included in each list of Reference Chemicals. Other test methods meeting the Performance Standards should have reliability and performance that are equivalent to or better than that of each validated method.

III. ENSURING QUALITY OF *IN VITRO* ALTERNATIVE TEST METHODS

Special Considerations for *In Vitro* Assays:

Testing laboratories must use good scientific practices, namely appropriate calibration and standardization methodology from a variety of other technical disciplines in order to handle the elements of all assay systems. For example, prior to running an unknown chemical, chemical instrumentation should be calibrated in accordance with the manufacturer's specifications and standard samples evaluated; *in vitro* assay using cells in culture should use good cell culture practices; etc. Similarly, performance of each lot of an *in vitro* system should also be "calibrated" to assess how their response levels can be used to predict *in vivo* effects.

The use of *in vitro* systems to replace animal tests in toxicology testing calls for meticulous characterization of manufacturing processes or isolation and handling of cells in culture, tissue constructs, microchip arrays, and the like. The inherent variation of *in vitro* test systems calls for special standardization. Target tissues for *in vitro* systems must be well-characterized and identified, maintained, and handled under appropriate conditions and shown to be viable with expected responsiveness.

Cells in Culture

Cell culture test systems must be accompanied by provisions to assure that they are morphologically and physiologically correct and that they show the responsiveness to chemicals is the same as that of the validated cell culture system. Assay systems that use normal human cells (e.g., keratinocytes or hepatocytes) have special challenges in that these cells must be used as primary or early passage cultures. This often precludes comprehensive cell characterization, making the user laboratory more dependent on the supplier for cell characterization and safety data (e.g., evidence of freedom from pathogens).

Tissue Constructs

Tissue constructs, such as EPISKIN and EpiDerm, generally have a very short shelf life and are produced in "small" batches over time. These factors limit the number of replicate tissues that each user can dedicate for the quality control efforts. Thus, the user is dependent upon the manufacturer to supply many of the basic elements of cell/tissue characterization and quality control. Such data should include information on cell characterization, tests for adventitious agents, structural characteristics, and responses to positive control chemicals. The structural and functional evaluation should be performed on each lot of tissue, and these

data should be available to the user laboratory. Even with these procedures, users also need to ensure that the cells or tissues are functioning within normal limits after shipping and handling that the execution of the assay is within normal limits.

Bioconstructs

Noncellular bioconstructs for test systems are generally prepared by the testing laboratory. It is critical to assure that morphology and responsiveness is within acceptable ranges for each batch. Manufacturers of kits using bioconstructs should recommend tests for user laboratories so that performance of such bioconstructs can be assured.

Ex Vivo Tissues

Suppliers of *ex vivo* tissues may use variable sources for such tissues and differences in handling may affect tissue viability. Procedures must be available to assure viability and responsiveness of *ex vivo* systems on a regular basis.

Microassays

Currently there are wide variations in the design, data extraction and analysis for microarrays for toxicogenomic experiments. This calls for recording of sufficient information so that results can be correctly interpreted or replicated (Ref. 7).

Good Laboratory Practice Regulations:

Quality control is an essential element for any regulated study so that results of the assay can be determined to be meaningful and can be compared with data from previous studies within laboratories and from one laboratory to another. The principles of GLP have been agreed internationally to promote the quality and relevance of test data used for determining the safety of chemicals and chemical products (Ref. 13). Agency GLP regulations are concerned with the organizational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, recorded, archived and reported (Ref. 4). Their aim is to assure consistency, traceability and reproducibility of test results. Quality control procedures for studies submitted to the Agency for pesticides and industrial chemicals can be set forth in OPPTS test guidelines, and included in Performance Standards established by ICCVAM for each new test method when it reviews the status of their validations.3

GLP regulations place the ultimate responsibility with the company submitting data to the government to verify that the assay fulfills regulatory testing needs. GLP and good science responsibilities of the testing laboratories (users of *in vitro* systems) include using a pre-defined study protocol for the assay, training the technical staff and ensuring that staff skills are maintained, and employing concurrent controls, including benchmark controls, if appropriate, to monitor assay performance.

It is the Agency's assumption that the manufacturer of the assay system conducts appropriate quality control for the manufacturing process of PTMs in order to ensure that test systems maintain consistency of performance. Data for each batch of PTMs demonstrating test system performance should be available as part of the study record. If not, the testing laboratory should be able to request such quality control documentation from the manufacturer. In addition, the testing laboratory should also have its own procedures to verify performance of the test system when it is received in the laboratory.

Use of Reference Chemicals to Demonstrate Test Performance:

The set of Reference Chemicals used to validate a particular test method are well-characterized *in vivo* as well as *in vitro* in multiple laboratories. Such Reference Chemicals are expected to behave in a consistent manner and can also be used as a source of controls, benchmark chemicals, and training or calibration sets. (See definitions below)

Test guidelines generally call for use of positive and negative controls. When available, normative ranges of historical values for positive controls provide Agency reviewers a basis for assessing the way the test system performs in historical context. In addition, when reviewing results for test chemicals, Agency reviewers can gain extra confidence in their assessments by comparing results for the test chemical with those for benchmark chemicals from a similar chemical class but with various potencies. Therefore, the Agency intends to require use of concurrent controls in its guidelines for *in vitro* test systems and also recommends use of benchmark chemicals, especially for assessment of test chemicals that may show weak or negligible responses.

Laboratories using new *in vitro* methods (proprietary or otherwise) can demonstrate that the test is being performed properly by using training or calibration sets and comparing results with those found during validation trials.

<u>Controls</u>:

Controls may be positive or negative. For *in vitro* studies, positive control response(s) are part of the process of demonstrating the functional integrity of target tissues, proper treatment of the cells or tissue, and proper execution of the test method. Negative control responses are often used to set the baseline of cell or tissue response against which the responses of the cells or tissues treated with the test article or positive control can be compared.

One would expect that controls address the endpoint reported in the assay and can be used to provide a measure of the performance of the assay at each run. Concurrent positive and negative controls should be used for each trial using the assay. In that way, they help to establish whether a valid trial was performed when test data for the unknown chemical are submitted to regulatory agencies. In addition, results of control trials can be compared with historical data and used for trend analysis so that any drift in the assay system can be detected.

The positive control should be able to detect over- and under- response. A trial where the control values falls outside the acceptable limits would be repeated and the data from such a trial would not be included in a regulatory submission. This avoids the inclusion of spurious data from a trial that is outside the normal limits of the assay. In some types of assays (e.g., cytotoxicity studies), the negative control is used to normalize the measure of cell viability (e.g., dye uptake) and so the acceptance criteria focus on the performance of the positive control.

Benchmark Class and Potency Chemicals:

Benchmark materials are not a substitute for positive controls, but are a valuable addition to an assay. Whereas controls provide a measure of stability of the *in vitro* assay system, the appropriate benchmark chemicals demonstrate a range of acceptable responses for each class of chemicals for which the assay is valid. Benchmark chemicals should be selected from chemicals that are well characterized for the assay, i.e., the Reference Chemicals. They differ from positive controls in that they are matched to the chemical class of the test material in each test trial and are used to set upper and/or lower limits of response against which the response of the unknown chemical may be judged. The upper limit (and lower limit if applicable) of each benchmark is set relative to acceptable responses *in vivo*.

Calibration Set:

Calibration of elements of a test system or method may be warranted for certain *in vitro* test methods. For example, the apparatus used to probe or measure the endpoint or to augment an *ex vivo* tissue or tissue construct may not be standardized. In such cases, such detection or support equipment can be calibrated using a calibration set, which is a suitable subset of the Reference Chemicals used to validate the test.

Training Set:

When laboratories begin to use a validated test method or test kit, a training set drawn from the list of Reference Chemicals for the method can be used to learn the method or refine testing techniques. Once use of the new method is established in the laboratory and instrumentation and procedures calibrated, use of controls and benchmarks with each test trial should be sufficient.

Consideration of Quality Issues by ICCVAM, ECVAM, and OECD:

Organizations in the United States and Europe have been evaluating approaches to ensure integrity and performance of new *in vitro* methods when they are proposed to fulfill regulatory test requirements as alternatives to animal testing. These actions can be expected

to help the testing laboratories (i.e., study directors, technical staff) in developing processes to ensure compliance with GLP principles and provide regulatory scientists with specific information and guidance to better assess the quality of *in vitro* studies and the authenticity of *in vitro* study results submitted to them for regulatory purposes.

In the course of executing its mission to develop and promote *in vitro* studies as alternatives for conventional animal testing, the ECVAM) has been heavily involved in considering quality control measures specifically for *in vitro* studies. In response to a recommendation by ECVAM in coordination with ICCVAM, the Organization for Economic Coorperation and Development (OECD) will develop a consensus document to interpret the principles of GLP for execution of *in vitro* assays (Ref. 14). The report of the ECVAM workshop (Ref. 15) on principles of GLP when applied to *in vitro* toxicology provides several examples of areas where specific guidance for *in vitro* studies might be incorporated into the new OECD consensus document for GLP.

ECVAM has also issued a report on Good Cell Culture Practice (GCCP) (Ref. 6). The GCCP report specifies procedures to ensure that test systems are free of any contamination or other diseases or conditions at the beginning of the study that might interfere with the outcome of the study and calls for the origin (species/tissue), source, arrival condition and maintenance requirements to be documented and confirmed at the laboratory on a regular basis. Documentation of critical cell culture parameters is expected to help regulatory authorities in the acceptance and interpretation of *in vitro* data. In addition, ECVAM in cooperation with ICCVAM is planning to convene a series of workshops to develop special technical guidance for *in vitro* studies.

References.

1. Interagency Coordinating Committee for Validation of Alternative Methods. 1997. *Validation and Regulatory Acceptance of Toxicological Methods of Test Methods*. NIH Publication 97-3981. See Chapter 2. Validation of Test Methods and Chapter 3. Regulatory Acceptance of Toxicological Test Methods.

2. Interagency Coordinating Committee for Validation of Alternative Methods. 2003. ICCVAM Guidelines for the Nomination and Submission of New, Revised and Alternative Test Methods. NIH Publication 03-4508. September 2003.

3. Environmental Protection Agency. Ethics Advisory 95-18, Use of Public Office for Private Gain - Endorsements. Based on provisions of the "Standards of Ethical Conduct for Employees of the Executive Branch" at 5 CFR 2635.702 and on provisions of 5 CFR 2635.703.

4. Environmental Protection Agency. 40 CFR 160 Good Laboratory Practice Standards (Office of Pesticides) and 40 CFR 792 Good Laboratory Practice Standards (Office of Prevention and Toxic Substances).

5. Environmental Protection Agency. Order 5360.1 A2. Policy and Program Requirements for the Mandatory Agency-Wide Quality System. May 2000.

6. Hartung, T., Balls, M., Bardouille, C., Blanck, O., Coecke, S., Gstraunthaler, G., and Lewis, D. (2003). Good Cell Culture Practice: ECVAM Good Cell Culture Practice Task Force Report 1. ATLA 30: 407-414.

7. Minimum Information About a Microarray Experiment -MIAME for Toxicogenomics(MIAME/TOX). Draft report February 12, 2003. Available at http://www.mged.org/.

8. Interagency Coordinating Committee for Validation of Alternative Methods. 2002. ICCVAM Evaluation of EPISKIN™, EpiDerm™ (EPI-200) , and the Rat Skin Transcutaneous Electrical Resistance (TER) Assay: *In Vitro* Test Methods for Assessing Dermal Corrosivity Potential of Chemicals. NIH Publication No. 02-4502. June 2002. Available at http://iccvam.niehs.nih.gov/methods/epiddocs/cwgfinal/cwgfinal.pdf

9. Interagency Coordinating Committee for Validation of Alternative Methods. 1999. Corrositex®: An *In Vitro* Test Method for Assessing Dermal Corrosivity Potential of Chemicals. NIH Publication No. 99-4495. June 1999. Available at: http://iccvam.niehs.nih.gov/docs/reports/corprrep.pdf.

10. Interagency Coordinating Committee for Validation of Alternative Methods. 2003. ICCVAM Minimum Performance Standards: In Vitro Membrane Barrier Test Systems for Skin Corrosion.

11. Interagency Coordinating Committee for Validation of Alternative Methods. 2003. ICCVAM Minimum Performance Standards: In Vitro Human Skin Model Systems for Skin Corrosion.

12. Interagency Coordinating Committee for Validation of Alternative Methods. 2003. ICCVAM Minimum Performance Standards: In Vitro Skin Transcutaneous Electrical Resistance (TER) Tests for Skin Corrosion.

13. Organization for Economic Cooperation and Development. 1981. Decision of the Council Concerning the Mutual Acceptance of Data in the Assessment of Chemicals, adopted May 12, 1981.

14. Interagency Coordinating Committee for Validation of Alternative Methods. Memorandum to OECD dated February 19, 2003. Justification for Development of An OECD Guidance Document on the Application of Good Laboratory Practice (GLP) Principles to *In Vitro* Testing.

15. Cooper-Hannan, R., Harbell, J.W., Coecke, S., Balls, M., Bowe, G., Cervinka, M.,

Clothier, R., Hermann, F., Klahn, L.K., de Lange, J., Lievsch, M and Vanparys, P. (1999). The principles of Good Laboratory Practice: Application to *in vitro* toxicology studies. The report and recommendations of ECVAM workshop 37. ATLA 27, 539-577.