DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

National Institutes of Health
National Institute of
Environmental Health Sciences
P. O. Box 12233
Research Triangle Park, NC 27709

November 30, 2001

Maurice Zeeman, Ph.D.
U.S. National Coordinator
OECD Test Guidelines Program
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Room 6308, ICC Building, 7403M
Washington, D.C. 20460-0001

Dear Dr. Zeeman:

Thank you for forwarding the OECD document, "Draft Guidance Document on the Development, Validation and Regulatory Acceptance of New and Updated Internationally Acceptable Test Methods in Hazard Assessment," [T & A No. 34 (9-01).pdf] which you distributed to interested stakeholders on September 21, 2001. You stated that this document will be discussed at the OECD Conference on Validation and Regulatory Acceptance to be held in Stockholm, Sweden, March 6-8, 2002.

This OECD document raises many important issues and questions concerning current principles and processes for the validation of toxicity testing methods. These issues are of considerable interest to the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), which evaluates the scientific validity of proposed toxicity test methods. ICCVAM is composed of representatives from 15 U. S. government regulatory and research agencies.

ICCVAM is pleased to provide comments and recommendations (Enclosure) to assist you in discussions on this document at the upcoming meeting. Most importantly, ICCVAM recommends that an international workshop should be convened to address the issues in the OECD document, and that the workshop should be hosted by the U.S. ICCVAM also urges completion of the OECD Guidance Document on Validation that was initiated in 1998. ICCVAM would be pleased to assist with organization of the workshop and efforts necessary to complete the Guidance Document.

Please feel free to contact me if you have any questions, or if I can be of further assistance.

Sincerely,

/s/

William S. Stokes, D.V.M., Diplomate ACLAM
Co-Chair, ICCVAM

Enclosure

cc:
Dr. Richard N. Hill, EPA, Co-chair, ICCVAM
Dr. Leonard M. Schechtman, Co-Chair Elect, ICCVAM
ICCVAM Agency representatives

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

Comments on the *Draft Guidance Document on the Development, Validation, and Regulatory Acceptance of New and Updated Internationally Acceptable Test Methods forHazard Assessment*

Comments from the ICCVAM are provided below for the OECD document, *Draft Guidance Document on the Development, Validation, and Regulatory Acceptance of New and Updated Internationally Acceptable Test Methods forHazard Assessment* [September, 2001] (hereafter, GD).

1. ICCVAM is pleased that the OECD has responded to the recommendation from the 1996 Solna Workshop on Harmonization of Validation and Acceptance Criteria for Alternative Toxicological Methods that a Guidance Document should be prepared which incorporated the Workshop Report (para. 77, OECD, 1996).

2. A draft ICCVAM report on Validation and Regulatory Acceptance of Toxicoloigical Test Methods from a 1995 ICCVAM International Workshop was used as a key background document for the Solna Workshop. A final version of this report was prepared on behalf of the 15 participating ICCVAM agencies and published in 1997 (ICCVAM, 1997) after considering contributions from interested stakeholders in government, industry, public interest groups, and the international community. ICCVAM is pleased that the 1997 Report is listed as a reference for the GD. ICCVAM recommends that the contents of this Report be considered for inclusion in the appropriate sections of the GD, and as further elucidated in comments that follow.

3. ICCVAM previously submitted comments in response to an OECD document entitled *Validation Issues: Current Practices And Issues For Consideration* [Document ENV/JM/TG(2001)5], which are attached as Appendix A. ICCVAM requests that these comments be considered as formal comments relevant to the draft GD. ICCVAM also requests that the ICCVAM comments in Appendix A be distributed to participants prior to the upcoming OECD Conference on Validation and Regulatory Acceptance of New and Updated Test Methods in Hazard Assessment (hereafter, Conference) and that they be considered in revisions to the draft GD.

4. ICCVAM also submitted comments for consideration by the Steering Committee in planning the upcoming Conference, which are attached as Appendix B. These comments discuss issues relevant to both the Conference and the final GD. ICCVAM hereby submits these comments as additional formal comments relevant to this GD. ICCVAM also requests that the ICCVAM comments in Appendix B be distributed to participants prior to the upcoming Conference and be considered in revisions to the draft GD.

5. ICCVAM has evaluated the validation status of several new and revised test methods for various purposes, including the murine Local Lymph Node Assay (LLNA), Corrositex™, the revised Up-and-Down Procedure (OECD TG425), the Frog Embryo Teratogenesis Assay—*Xenopus* (FETAX), and *in vitro* methods for assessing acute systemic toxicity. Each of these evaluations has been followed by publication of a report characterizing the usefulness and limitations of the test method (see References below). These test methods provide examples of various ways to validate test methods, and how that validation will vary with the intended use of the method. These examples should be discussed in the GD, as well as other examples of test methods that have been evaluated using established validation and acceptance criteria.

6. The participants of the 1996 Solna Report (para. 77) recommended that the GD provide further elucidation on the validation and regulatory acceptance criteria and principles, and that the validation process should be described in more detail. ICCVAM is concerned that the draft GD does not comply with that recommendation and that the draft GD differs substantially from the 1996 Solna Report, especially in regard to content and organization. In order to adhere to the intent and spirit of the 1996 Solna Report, and to provide a more useful document, ICCVAM recommends that the GD include all of the relevant contents of the Solna Report. ICCVAM further recommends that the GD be reorganized as follows:

   a. Title: The title is highly confusing. The intent of the document is NOT to address test method development, as suggested in the current document. ICCVAM recommends the following title, which is more in keeping with the original intent and title of the 1996 Solna Report: " Guidance Document on the Validation and Acceptance of New and Updated Test Methods for Hazard Assessment."

   b. Organization: The current organization is confusing. The purpose of the GD, as originally envisioned, was to provide further practical guidance on the principles and criteria for validation and regulatory acceptance, and on the process of validation. In the 1996 Solna Report, there is a separate Section on each of these topics. However, the draft GD combines the discussion of validation principles and criteria with an extensive discussion of a specific validation process. ICCVAM therefore strongly recommends that the document be reorganized with the following chapters:

      i. Validation Criteria:
      - This chapter should further discuss the basis for each of the validation criteria, and how to adequately address each of the validation criteria for different test method types and purposes, including validation of test method revisions.
      - This chapter should expand on the corresponding paragraphs in the 1996 Solna Report (para. 18-21,23-26).

      ii. Acceptance Criteria:

- This chapter should further discuss the basis for each of the acceptance criteria, and what information is necessary to adequately address each of the validation criteria/principles for different test method types and purposes.
- This chapter should expand on the corresponding paragraphs in the 1996 Solna Report (para. 18-20, 22-28).
- This chapter should provide specific details on what information must be provided in order to evaluate the validity of a proposed test method for regulatory use.

iii. Management of the Validation Process:

- This chapter should further elaborate on practical aspects of managing the process necessary to evaluate the validity of new and revised test methods.
- It should incorporate and build on the Validation Process Chapter in the 1996 Solna Report (para. 29-61).
- It should include examples of various approaches that have been used to conduct validation studies. This should include the validation processes used by ECVAM, and validation processes used for test methods recently reviewed and recommended by ICCVAM.

iv. Independent Peer Review Evaluation and Regulatory Acceptance Processes:

- This chapter should provide practical guidance for independent peer review evaluation and regulatory acceptance processes for new and revised test methods.

7. ICCVAM has found that the greatest obstacle to the timely consideration of new test methods is the failure of test sponsors to provide adequate information and data necessary to evaluate the extent to which established validation and acceptance criteria have been addressed. In order to provide useful guidance for test sponsors, ICCVAM developed standard guidelines to assist sponsors in organizing the information necessary for a complete submission ("Evaluation of the Validation Status of Toxicological Methods: General Guidelines for Submissions to ICCVAM," Rev. October 1999. NIH Publication 99-4496). This document, originally based on guidelines developed by the Interagency Regulatory Alternatives Group (IRAG), has been updated based on experience gained by ICCVAM during several test method evaluations in the past 5 years. Information outlined in the Submissions Guidelines is critical to understanding both the usefulness and the limitations of test method, and the extent to which the results of the test method can be relied upon to protect human health and the environment. ICCVAM strongly recommends that these Submissions Guidelines be followed, and the lack of information in any given area be evaluated in accord with the use of the test method. While the procedures for achieving regulatory acceptance of a method will vary by country, adoption of new test methods can be facilitated by providing sufficient documentation supporting and evaluating its validity. Harmonization and standardization of necessary documentation would greatly facilitate the endorsement process between countries and national

organizations. ICCVAM, therefore, proposes that its Submissions Guidelines be appended to the GD to serve as a basis for harmonized/standardized guidance on the information content of a complete submission package.

The only relevant guidance on test method submissions in the draft GD (paragraph 111) consists of one sentence, which is vague and general. In order to facilitate consideration of new test methods, ICCVAM strongly recommends the GD incorporate the standard submission format outlined in Appendix A of NIH Pub. 99-4496.

8. It is important to recognize the considerable progress that has been made during the past 15 years on the development of nationally and internationally harmonized criteria and processes for the validation and regulatory acceptance of new test methods. Many national and regional organizations have contributed to this progress, including ICCVAM in the U.S., the European Centre for the Validation of Alternative Methods (ECVAM) in Italy, the Johns Hopkins University Center for Alternatives to Animal Testing (CAAT) in the U.S., the Fund for the Replacement of Animals in Medical Experiments (FRAME) in Europe, the Center for Documentation and Validation of Alternatives to Animal Experiments (ZEBET) in Germany, the European Research Group for Alternatives to Animal Testing (ERGATT), the National Centre for Alternatives (NCA) in the Netherlands, the Swiss Institute for Alternatives to Animal Testing (SIAT), and the OECD. Furthermore, many countries, including the United States, have established organizations and processes to coordinate validation and acceptance activities and these organizations have, in a relatively brief time period, implemented effective processes for such activities. Reference to and descriptions of these experiences and processes is conspicuously absent in the current draft GD. In fact, the draft GD fails to acknowledge the existence of these organizations as well as their processes, experiences, and progress. This information should be incorporated where appropriate throughout the GD, together with proper acknowledgement and credit given to these organizations.

9. "Flexibility" is mentioned in the draft GD. However, the GD should attempt to (a) define "flexibility" in the context of the GD and in the context of validation processes, (b) explain the pros and cons of such flexibility with respect to the validation of test methods, and (c) provide examples of where and how flexibility has been or might be applied to the application of validation and acceptance criteria. Since the ultimate goal of toxicological testing is to protect human health and the environmental, flexibility must be delimited as to its allowable range, and must be adequately justified and have a sound scientific basis. ICCVAM believes the acceptance of alternative test methods should be based on an adequate demonstration that the test method is reliable and performs well for its expressed purpose. The use of test methods that are not adequately validated for their intended purpose could produce wasteful, time-consuming, erroneous, confusing, and even conflicting test results. Such poor results would ultimately undermine the use and regulatory acceptance of such test methods, and cause a reluctance on the part of regulatory

bodies to employ such unreliable methods as replacements for or refinements of existing methods.

10. The proposal for validating alternative tests methods (Chapter IV) outlines a procedure that is extremely detailed and costly both in time and in resources. The OECD also proposes that it take a leading role in coordinating the validation process. While the process outlined and the issues addressed are very thorough and should indeed result in assays that are completely validated, it is unlikely that this cumbersome approach will yield very many validated assays within any reasonable amount of time and at a reasonable cost. In fact, most of the assays currently in use have not received a fraction of the "validation" that is proposed for new assays. ICCVAM recommends that the GD clearly state that this is only one approach to validation and that other proven successful approaches already exist. As mentioned in ICCVAM's other comments (above), validation processes used by other organizations, such as ECVAM, ICCVAM, and ZEBET, should also be described.

11. There is one particularly disturbing aspect to the whole validation process described in Chapter IV of the GD. Most of the process involves technical issues of reproducibility and correlation with other currently used assays. This is very short sighted. The GD should convey that the most important aspect of any test system should be a thorough understanding of its underlying biology and its mechanistic relevance. Short-term tests are generally developed to measure a single biological effect. In the past, extensive effort has been spent trying to determine how well assays that measure single biological effects correlate with health outcomes that result from multiple biological effects. Because of this inherent limitation associated with such short-term tests, it was inevitable that the multi-million dollar, two-decades long effort to develop short-term genotoxicity tests to predict rodent cancer outcomes would fall short of expectations. Hopefully the lessons learned will keep similar approaches from being used for validating alternative assays in the future. The GD should include a discussion about the importance of understanding the mechanistic relevance of test models, and include a discussion of the limitations and usefulness of genotoxicity tests that were garnered as a result of those extensive validation studies.

12. The upcoming OECD Conference should be open to ALL interested individuals who can contribute relevant expertise. It is important that these discussions include individuals who have experience with efforts to harmonize/standardize protocols and to develop guidance for assay conduct and data interpretation. It is also important that people who develop assays and who have the primary expertise with the new assays be involved in the validation process. In addition, it is essential that representatives from regulatory authorities who can articulate regulatory acceptance and implementation requirements and processes also be included. Unless this happens, we will not be moving forward in an effective way to fully utilize the potential of alternative assays.

13. The Chapter on Validation Process describes the process OECD has put into place during the past two years to coordinate validation of endocrine disruptor related test

methods. This includes use of the term " Validation Management Group" as the organization that should conduct and manage validation studies. This terminology, established by OECD for its activities, suggests that OECD's proposed GD also confers methods validation authority to OECD. OECD has admirably served the purpose of standardizing test guidelines. Studies conducted in accordance with the standardized guidelines can be used by all regulatory authorities to the extent the method achieves their regulatory needs. The newly self-designated OECD role of serving as both a validation authority and, to some degree, a regulatory acceptance authority, could have significant consequences in light of the treaty obligations requiring mutual acceptance of data from OECD accepted methods. This role also raises a potential appearance of a conflict of interest where one organization assumes responsibility for validation, independent assessment and regulatory acceptance. This contrasts sharply with recently enacted law in the U. S. that establishes ICCVAM as the U.S. validation authority (ICCVAM Authorization Act of 2000, U.S. Public Law 106-545). This law also establishes an evaluation process with clear lines of separation for validation study conduct, validation status evaluation and regulatory acceptance. It is therefore strongly advised that caution should be taken not to invoke policy in the GD that may foster trade barriers as some products are regulated under different regulatory mandates internationally. Some mandates require the use of validated alternative assays and others require the use of non-alternative (classical) assays. Such a centralized OECD regulatory acceptance authority could further dilute the ability to delineate the acceptable criteria of test method validation according to chemical use. For example, OECD test guidelines makes no attempt to discriminate the usefulness and limitations of methods for use in testing substances in completely different applications, such as pharmaceuticals, environmental contaminants, or food additives. This was a key element in acceptance of LLNA. Some representatives of ICCVAM member Federal agencies therefore oppose the current draft GD proposed by OECD unless it is revised to clearly state that there is no intent to establish its authority as a formal international methods validation organization, and that it does not intend to expand its current role beyond a methods standardization authority.

14. The Draft GD provides a recommended generalized framework for the development and validation of hazard assessment test methods prior to acceptance by international regulatory agencies. The guidance is an extension of the criteria developed at the Solna workshop. Although the validation scheme elaborated by the document is generally a useful framework, it is unlikely that the large majority of the tests used by some agencies to comply with statutory responsibilities will or could be validated under the procedure outlined. In spite of the statement in paragraph 27, "The guidance in this document has been developed to be sufficiently flexible so that it can be used for any type of test, regardless of whether it is an *in vitro* or *in vivo* test, or a screening test or a definitive test," the guidance is not directly applicable to the hazard/risk assessment test methods that are commonly used in some agencies, such as those dealing with evaluation of biologics safety and efficacy. These test methods are necessarily diverse due to the diverse nature of biologics and the need to evaluate risk in the context of a risk/benefit ratio that is specific for a particular disease and clinical condition. As a result of these inherent fluidities, these specific methods are

not suitable for generalized validation to facilitate widespread use. Paragraph 104 of the document recognizes that "regulatory authorities may still have additional questions on the test beyond its established reliability and relevance, which could affect its regulatory acceptance." It appears this wording anticipates that many of the methods validated within the framework will not be relevant to agencies that have specific, focused regulatory concerns.

15. The purpose and perspective of the GD is not clearly stated. If this is supposed to be a document that generally discusses validation (of tests for potential use in OECD Test Guidelines, see Items 4 & 5), it appears to speaks too much from the perspective of OECD itself performing the validations rather than from the perspective of a general guideline for performing validation-related activities by various groups, sponsors, or organizations.

16. There is too little focus on the type of validation-activities presented in Item IV.4.d. "Validation through available data" might be closer to the case for some methods that will be brought forth for validation (and seems closer to what occurred for the LLNA in the U.S.), rather than the type of validation where new data are produced with a proposed method in a round-robin type of effort.

17. Items 35, 48: The criteria for determining whether a particular protocol is a good potential candidate for supporting the fairly structured prediction model should be clarified.

18. Item 57: The criteria for chemical selection such that the needs of various regulatory bodies would be represented should be clarified.

19. Items 81-83: The concept of "lower level of assurance" is stated to relate to the appropriateness of the test for a specific purpose (Item 81), but the discussion in (Item 83) implies that the concept is related to test performance characteristics. The meaning of and basis for determining "level of assurance" should be clarified.

20. Para. 15. The term " test's position" in a testing program is confusing. This is simply referring to the proposed use of a test, and should be changed to reflect this.

21. Para. 95. The type of philosophical conflict of interest that is to be avoided is unclear. This should be clearly defined, or deleted.

22. Para. 29. Scientists involved in planning and designing a validation study may in fact include scientists from the developing company. If appropriate provisions are made for coding of chemicals, strict adherence to GLPs, etc., then such individuals should not necessarily be excluded from these functions. However, these individuals

should clearly not serve as independent peer reviewers in view of the perceived conflict of interest.

23. Para. 89. Validation through available data. This paragraph is extremely misleading. If published literature provides information and data that adequately addresses established validation and acceptance criteria, then this could in fact serve as a means to support the validity of a test method. However, this would still require that there be sufficient data collected to demonstrate the reliability and relevance of a standardized protocol.

References

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APPENDIX A

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Comments on the OECD Test Guidelines Document:

May 8, 2001

Validation Issues: Current Practices and Issues for Consideration
[OECD Document ENV/JM/TG(2001)5]

Summary
ICCVAM comments are provided on the OECD document, entitled *Validation Issues: Current Practices And Issues For Consideration* [Document ENV/JM/TG(2001)5] (hereafter, OECD Validation Issues Document). ICCVAM formulated general recommendations, as well as responses to the questions posed in paragraph 26 of the OECD Validation Issues Document.

The ICCVAM general recommendations are:

- An international workshop with opportunity for broad stakeholder participation should be convened and charged with addressing the issues raised in the OECD Validation Issues Document. ICCVAM would be pleased to assist with organizing this workshop. If the workshop is not open to the public, member countries should be given sufficient time to query interested stakeholders for their views on proposed workshop documents and issues.
  [Note to the U.S. Coordinator: All interested U.S. stakeholders should have the opportunity to participate in a public workshop on these issues. If an OECD international workshop is convened which is not open for public participation, then U.S. stakeholders should have the opportunity to contribute to the development of a formal U.S. position prior to the OECD workshop.]
- Completion of the OECD Guidance Document on Validation, initiated by OECD in 1998, should receive high priority; ICCVAM would be pleased to assist in this effort.

Background

This paper provides comments from ICCVAM on the OECD document entitled *Validation Issues: Current Practices And Issues For Consideration* [Document ENV/JM/TG(2001)5]. The document was distributed on April 20th by the U.S. National Coordinator (NC) for the OECD Test Guidelines Programme, Dr. Maurice Zeeman. The issues in the document will be discussed at the 13th Meeting of the Working Group of the National Coordinators of the OECD Test Guidelines Programme, to be held on May 30-June 1, 2001 in Paris. Comments are provided by ICCVAM to assist the U.S. NC.

OECD Requested Action to National Coordinators

In the April 20th transmittal from OECD, the NCs were requested to:

i) take note of the document and discuss the current approaches and concerns; and

ii) recommend efficient ways leading to the acceptance of scientifically sound and adequate test methods with the available resources.

The OECD document provides a brief overview of validation principles and current approaches, and a discussion of what may be "*conflicting priorities taking into account both animal welfare and human health and environmental safety concern*." In paragraph 26, the OECD document states that " *this document is not an attempt to solve the issue of the increasing need to spend resources on validation of new and revised test methods, but rather a thought-starter to facilitate an open discussion and exchange of views*". Further, '*The discussion may or may not result in recommendations, possibly to the Joint Meeting, for resolving the "contradiction in terms" to develop scientifically sound Test Guidelines in a short period of time with limited resources.*

ICCVAM Comments and Recommendations

ICCVAM comments are organized into general recommendations, followed by specific recommendations or responses that address the nine questions in paragraph 26 of the OECD Validation Issues Document.

General Comments

The issues and questions raised in the OECD Validation Issues Document are extraordinarily complex. The general premise is that the current approaches for validating and gaining acceptance of new test methods are expensive and time-consuming. On this basis, the OECD asks NCs to "recommend efficient ways leading to the acceptance of scientifically sound and adequate test methods with the available resources." The apparent goal of this OECD effort is the identification and/or development of a relatively quick, inexpensive, and scientifically credible process for the validation and acceptance of new alternative test methods. ICCVAM agrees that this is a laudable goal, provided that the process remains scientifically credible.

ICCVAM concludes that it is impossible to adequately evaluate the OECD's concerns about current validation criteria and processes and to develop sound and meaningful recommendations in the short time provided before the May 30th NC meeting and the June 13-15 32nd Joint Meeting. It is important to recognize the considerable progress that has been made during the past 15 years on the development of nationally and

internationally harmonized criteria and processes for the validation and regulatory acceptance of new test methods. Many organizations have contributed to this progress, including ICCVAM, the European Centre for the Validation of Alternative Methods (ECVAM), the Center for Alternatives to Animal Testing (CAAT), the Fund for the Replacement of Animals in Medical Experiments (FRAME), the Center for Documentation and Validation of Alternatives to Animal Experiments (ZEBET), the European Research Group for Alternatives to Animal Testing (ERGATT), the National Centre for Alternatives (NCA) in the Netherlands, the Swiss Institute for Alternatives to Animal Testing (SIAT), and OECD. Furthermore, many countries, including the United States, have established organizations and processes to coordinate validation and acceptance activities and these organizations have, in a relatively brief time period, implemented effective processes for such activities. Efforts to change existing validation and acceptance criteria and processes should therefore proceed carefully and deliberately, and only after careful review of existing procedures.

General Recommendations

1. ICCVAM recommends that the issues and questions identified in the OECD document should be the focus of an international workshop. ICCVAM strongly urges the OECD to organize an open public international workshop for the express purpose of developing a coherent and scientifically defensible plan that addresses both the theory and practice of test method validations. The United States should offer to host such a workshop, which should include participation by appropriate experts from existing organizations responsible for the validation and evaluation of alternative test methods, as well as the users of such assays. If participation in the OECD workshop is limited, then ICCVAM recommends that member countries have sufficient time, prior to the OECD workshop, to organize their positions on identified issues. Within the U.S., this should involve a workshop for all interested stakeholders prior to any closed OECD workshop.

2. To assist with resolution of issues at the workshop, ICCVAM strongly recommends that OECD give high priority to completion of the OECD Guidance Document on Validation that was initiated in 1998. This Guidance Document was to incorporate the Final Report of the OECD Workshop on Harmonization of Validation and Acceptance Criteria for Alternative Toxicological Test Methods (ENV/MC/CHEM/TG(96)9, the "Solna Report"). Availability of this document would provide helpful, internationally harmonized guidance on the very issues raised by OECD regarding validation and regulatory acceptance criteria and processes.

Specific Responses and Recommendations to Issues for Discussion and Consideration by the National Coordinators (Paragraph 26)

The OECD Validation Issues Document concludes with a series of nine sets of questions that the Secretariat states may be helpful for the NC discussions. ICCVAM considered these questions, and provides the following responses and recommendations.

Paragraph 26, Section i:

"In 1992 National Co-ordinators agreed that: any "*proposed test should have undergone a critical appraisal concerning its scientific justification, its sensitivity and its reproducibility, including, where feasible and relevant, a comparative study supporting the validity of the test*". (Quoted from paragraph 22 of the Guidance Document for the Development of OECD Guidelines for Testing of Chemicals, Environment Monograph No. 76, 1993, which is currently being revised). Today, this generally phrased requirement has evolved into detailed submission guidelines and validation approaches, recommended by leading centers of expertise in validation".

Question 1. "How closely should these validation approaches and submission guidelines be followed?"

ICCVAM Response: ICCVAM and ECVAM, two centers with expertise in validation, have developed guidance on validation processes, including criteria for test method validation and acceptance. These are criteria that a new or revised toxicological test method should generally meet in order to be considered "validated" for risk assessment purposes. The guidance states that the extent to which the criteria should be met will vary with the method and its proposed use. The guidance further states that flexibility is necessary in assessing a method given its purpose and supporting database, and to ensure that appropriate scientific information is considered in regulatory risk assessment. Submission guidelines were developed to assist test method developers in providing the information and data necessary to determine the extent to which the established validation criteria have been addressed. Such information is critical to understand both the usefulness and the limitations of the test method, and the extent to which the results of the test method can be relied upon to protect human and environmental health. Thus, ICCVAM concludes that these validation approaches and submission guidelines should be followed and the lack of information in any given area be evaluated in accord with the use of the test method.

Question 2. "Would significant deviation of these recommendations lead to non-acceptance of the proposed test in Member countries?"

ICCVAM Response: The ICCVAM/ECVAM validation and acceptance criteria and processes were developed to facilitate the regulatory acceptance of new test methods. Therefore, deviation from these guidelines without justification may in some cases lead to non-acceptance or delays in acceptance. A more detailed response to this question would require further elaboration as to what constitutes "significant" compared to "non-significant" deviations. Significant deviations might be that the test method is not reliable or has not been adequately tested for its reliability, or that it's performance compared to the currently accepted test is inadequate or has not been adequately evaluated. In such a situation, it is difficult to comprehend why any organization would "accept" the alternative test as valid for its intended purpose. Whether a less significant deviation (e.g., the lack of a formal pre-validation step, the lack of strict adherence to

GLP, or the use of un-coded chemicals only) would lead to non-acceptance would need to be addressed on a test method-by-test method basis.

Paragraph 26, Section ii:

Question 1. "Are the Solna validation and regulatory acceptance criteria for newand revised tests, agreed in 1996, still valid?"

ICCVAM Response: The Solna validation and regulatory acceptance criteria are essentially the same as the ICCVAM and ECVAM criteria. These criteria have been successfully used by ICCVAM since 1998 to evaluate the validation status of several new and revised test methods, including the murine Local Lymph Node Assay (LLNA), Corrositex™, the revised Up-and-Down Procedure (OECD TG425), the Frog Embryo Teratogenesis Assay—*Xenopus* (FETAX), and *in vitro* methods for assessing acute systemic toxicity. Thus, the experience of ICCVAM supports the usefulness of the Solna validation and regulatory acceptance criteria, and they remain comprehensive and valid. Again, the extent to which new test methods should meet these criteria will vary with their nature and anticipated use.

Question 2. "Can they [Solna validation and regulatory acceptance criteria] also be applied to expensive long-term animal studies such as 2-year chronic carcinogenicity studies?"

ICCVAM Response: The existing 2-year chronic carcinogenicity studies do not need to be prospectively validated as their usefulness and limitations have been empirically characterized during decades of use. However, a new long-term animal test that utilizes an endpoint not previously demonstrated to be relevant or reliable would likely need "appropriate" validation. The validation process should be based on proven scientific principles, such as that described in the ICCVAM Report on Validation (http://iccvam.niehs.nih.gov/validate.pdf). The extent to which they must be followed would depend on the nature and purpose of the new long-term animal test and the degree to which the test components have been adequately evaluated.

Paragraph 26, Section iii:

"Are current guidelines and recommendations for validation study designs and evaluation of validation studies predominantly based on the understanding that the (new) test has a detailed description of the procedure to be followed (as in a protocol or standard operating procedure), or do they apply in the same way to OECD Test Guideline proposals which are more flexible and often provide a selection of choices (e.g., species, fasting/non-fasting), allow ranges (e.g., humidity, temperature), and/or intentionally lack detail (e.g., histo-pathological observations)?"

ICCVAM Response: ICCVAM believes very strongly that the validation process requires the use of specific protocols. Scientific validation is a prerequisite for

regulatory acceptance. It is only after a specific protocol has been validated for its expressed purpose that guidelines can be written that incorporate flexibility, based on the demonstrated limits of the test method. Protocol variations during validation should be evaluated for their effects on test method reliability and performance. Flexibility in guideline formulation should be allowed where such flexibility has been determined to not adversely impact test method reliability or performance. It should be noted that the flexibility choices mentioned in the above question are for testing situations where it has been determined not to impact on the results (e.g., animal temperature/humidity ranges set for the LLNA).

Paragraph 26, Section iv:

Question 1. "Are the various existing procedures for "peer review" or "independent evaluation" as adopted by ECVAM, ICCVAM, EPA, OECD and others all equally reliable and valid?"

ICCVAM Response: The review and evaluation procedures used by these various organizations are conducted for different purposes, and therefore it is impossible to compare their usefulness. For instance, while one organization such as ICCVAM may conduct "independent scientific peer review evaluation" of the validation status of a test method, others may conduct an "independent evaluation" process for different purposes. The organizations listed have very different mandates and constituencies, and exist for different purposes. For example, ICCVAM and ECVAM are scientific organizations directed solely at determining the scientific validity of specific proposed test methods. ECVAM generally conducts validation studies whereas ICCVAM generally evaluates validation efforts conducted by others. In contrast, the U.S. EPA reviews methods for their regulatory acceptability, while OECD strives to achieve internationally harmonized test guidelines. The OECD process involves acceptance by consensus, and decisions must balance policy as well as science matters.

Question 2. "If so, would a statement of validity made by any of these centers, agencies or organisations be sufficient for the WNT to accept the test as scientifically valid?"

ICCVAM Response: Based on the response to **iv., Question 1**, not necessarily. Statements of scientific validity should be accompanied by adequate documentation supporting and evaluating the validity of the test method. Determination of acceptability will depend on the proposed application of the test method.

Paragraph 26, Section v:

"How can it be promoted that a new test (e.g., *in vitro* Phototoxicity), validated in one region (EU) and with a statement of "scientific validity" made by a recognised center (ECVAM), will also be accepted as valid by national experts and/or other centers or organisations (OECD)?"

ICCVAM Response: The procedures for achieving regulatory acceptance of a method will vary by country. However, adoption of a new test method can be facilitated by provision of documentation supporting and evaluating its validity. Harmonization and standardization of necessary documentation would greatly facilitate the endorsement process between countries and national organizations. Additionally, ECVAM has an established process for evaluating ICCVAM recommended test methods and providing recommendations to the EC, and ICCVAM has recently implemented a fast-track process for expediting the review of test methods validated by ECVAM.

Paragraph 26, Section vi:

"Would the increasing pressure from Member countries for efficient and fast development of Test Guidelines, which is based on various environmental and human health concerns, affect the validation process?"

ICCVAM Response: The ultimate goal of toxicological testing is to protect human and environmental health. Thus, ICCVAM believes that the acceptance of alternative test methods should be based on an adequate demonstration that the test method is reliable and performs well for its expressed purpose. The use of test methods that are not adequately validated for their intended purpose could produce wasteful, time-consuming, erroneous, and confusing test results.

Paragraph 26, Section vii:

Question 1. "Should an effort be made to shift (a larger part of) the work involved in (evaluation of) validation projects from centralised institutions and international organisations to a decentralised structure involving Member countries?"

ICCVAM Response: Certainly, in some situations, it would be appropriate for some member countries to become scientifically and fiscally responsible for the validation efforts connected with a particular test method. For other countries without the scientific or fiscal resources, this would not be feasible. In situations where there is international interest in a test method that will be undergoing validation, there should be the opportunity for interested countries to review and comment on the proposed validation study design and protocols. What is needed is greater recognition that the efforts to develop validated assays require adequate support from both the regulated and the regulatory communities.

Question 2. "Or would a centralised management of validation projects in recognised centers be preferable?"

ICCVAM Response: To the extent that adequate resources could be made available for such validation efforts, this would provide many advantages, including utilization of scientific expertise and established processes. With appropriate coordination and cooperation with and among interested stakeholders, this could be preferable.

Question 3. "Are sufficient budgets and laboratory capacity available in the long term for extensive validation studies?"

ICCVAM Response: No. For example, with current resources, ECVAM can validate only a few tests concurrently and ICCVAM can evaluate the validation status of only a few test methods per year. Accelerating the process based on current approaches would require the availability of more resources. In addition, exceeding the validation and evaluation limitations of regulatory agencies increases the likelihood of acceptance of insufficiently validated test methods or protracted negotiations by OECD over what constitutes acceptable guidelines.

Paragraph 26, Section viii:

"Is there a way of accepting patented (validated) tests without requiring for the conduct of these tests equipment or material or animals from a unique source?"

ICCVAM Response: It is difficult to respond to this question without access to the specific OECD policy that states that patented and validated tests are not acceptable for test guidelines. Also, ICCVAM would need information on the process required to change such a policy. In principle, ICCVAM believes that accepting patented tests would accelerate the process by which alternative test methods are developed and validated (i.e., there would be increased financial incentive for the developer). As long as the validation status of the patented method is evaluated for its expressed purpose and the assay is not recommended over any other validated assay, acceptance of patented test methods should not be an issue. This conclusion was also made by the participants in the Solna meeting in 1996.

Paragraph 26, Section ix:

"Would it be possible for the WNT to make recommendations for efficient ways leading to the acceptance of scientifically sound and adequate test methods with the available resources in an acceptable period of time?"

ICCVAM Response: Such recommendations are possible so long as they are based on an appreciation that the ultimate goal of toxicological testing is to protect human and environmental health. Thus, ICCVAM believes that the acceptance of new test methods should be based on an adequate determination that the test method is reliable and performs well for its expressed purpose.

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APPENDIX B

OECD VALIDATION WORKSHOP: POTENTIAL ISSUES FOR STEERING GROUP DISCUSSION

ICCVAM – August 28, 2001

It is the mark of an educated mind to rest satisfied with the degree of precision which the nature of the subject permits, and not to seek an exactness where only an approximation of the truth is possible---Aristotle

Issue 1. Means of assessing test method validity

Historically, in vivo toxicological test methods underwent a standardization process to demonstrate their practical utility. The method was tested in a limited number of chemicals with different hazard potential to ensure the method was sensitive to test measures. More recently, detailed validation procedures have been articulated to demonstrate the relevance and reliability of methods.

Subissue: Test standardization

a. Is there merit in continuing test standardization for in vivo test methods?
b. If so, which tests (e.g., costly, long-term studies)?
c. Is such standardization adequate in determining the usefulness of at least some in vivo methods?
d. Is test standardization adequate for in vitro methods?

Subissue: Detailed validation

e. Are detailed validation criteria (test relevance and reliability) and process valuable tools in assessing test method validity?
f. For which tests--in vivo, in vitro, both?
g. How closely should detailed validation approaches be followed?
h. Would significant deviation from these lead to non-acceptance of the proposed test in Member countries?

Subissue: Combination

i. Is there merit in using both test standardization and detailed validation practices?
j. If so, which should be considered for each?

Issue 2. Test relevance

One part of knowing the usefulness of a test deals with test relevance. Traditionally, there are two components. One is a scientific determination--what is the meaning of the observations in the test under review to observations in the species of concern? The other is a pragmatic issue, namely--what is the relevance of test outcomes to a given regulatory program?

Subissue: Test observations to species of interest

Guidance needs to be given regarding the meaning of test observations to the species of interest regarding:

a. Nature of response
b. Health impact of responses
c. Correspondence of responses
d. Dose level
e. Dose response (when applicable)
f. Timing of response
g. Time action (when applicable)
h. Reversibility of response
i. Sex differences
j. Strain differences
k. Species differences

Subissue: Test observations to regulatory programs

l. Is the health effect of interest to the program?
m. Is the test measure of import to the program?
n. Is the test observation adequate to meet programmatic needs?

Issue 3. Test variability

One important component of a test's validity deals with the reliability of the method. The number of chemicals under test, and the number of laboratories under test are variables. In some cases, authorities simply have a different mind set as to the design of validation studies; in others, consider of cost and time are paramount.

Considering (a) in vitro methods, (b) short term in vivo methods and (c) chronic in vivo methods

i. Can guidance be provided as to the range of number of chemicals that are needed for determination of intralaboratory variability?
ii. Can guidance be provided as to the range of number of laboratories that are needed for determination of interlaboratory variability?
iii. Can guidance be provided as to the range of number of chemicals needed for determination of interlaboratory variability?

Issue 4. Submission guidelines.

In preparation for the IRAG international workshop on in vitro eye irritation methods, guidance was developed for submission of information on test methods (IRAG, 1997). ICCVAM drew upon those efforts and developed guidance for the submission of test materials to aid in the validation assessment of methods. The materials were judged to be of such importance, they have been revised as experience in employing them accumulated (ICCVAM, 1999).

a. Are submission guidelines valuable in identifying information that may be important in judging the validity of a method?
b. How closely should submission guidelines be followed?
c. Would significant deviation from these lead to non-acceptance of the proposed test in Member countries?

Issue 5. Validation criteria

Test validation has become central, determining the relevance and reliability of methods. In 1992 National Coordinators agreed that any "proposed test should have undergone a critical appraisal concerning its scientific justification, its sensitivity and its reproducibility, including, where feasible and relevant, a comparative study supporting the validity of the test" (OECD, 1993).

The generally phrased OECD requirement for test validation has been expanded. Attention to test method validation became focused with the advent of interest in in vitro tests. Many conferences were devoted to the topic, resulting in both ECVAM and ICCVAM devising criteria to assess whether a method is valid for a given use. These criteria were also agreed to at the OECD meeting in Solna in 1996.

Subissue: General applicability of validation criteria.

a. Are the Solna, ECVAM, and ICCVAM validation criteria still generally applicable to new and revised test guidelines?

Subissue: Specific applicability of validation criteria.

b. Are the Solna criteria specifically applicable to in vitro and to in vivo test methods?
c. Are they applicable in chronic in vivo tests?

Issue 6. Regulatory acceptance criteria

At the OECD meeting in Solna in 1996, criteria for the regulatory acceptance of test methods were devised. They drew upon similar materials developed through ECVAM**??** and ICCVAM

a. Are the Solna regulatory acceptance criteria applicable to new and revised tests?
b. Should they be amended or replaced for any subclass of tests?

Issue 6. What test is validated?

Traditionally, OECD test guidelines are flexible, allowing for some case specific latitude in the conduct of the test (provide a selection of choices (e.g., species, fasting/non-fasting), allow ranges (e.g., humidity, temperature), and/or intentionally lack detail (e.g., histopathological observations). It has been noted that some test validation activities have suffered because of differences in protocols used by different investigators. Without control of test parameters, estimates of variability can be artificially inflated. This has led some to propose that a specific protocol needs to be used in validation studies.

a. Is there merit in validating a specific test protocol?
b. Are validation efforts compromised if a specific protocol is not adhered to?
c. Can a general test guideline be adequately validated, with strict specification of test method design?
d. Would it be advisable to validate a specific protocol, but then write a more generic test guideline?

Issue 7. Peer review or peer involvement?

Two methods have been traditionally used to evaluate the scientific underpinnings of new test methods: peer review is independent evaluation by persons uninvolved with the method under review; peer involvement is evaluation of the method by parties involved with the method (e.g., industry, government public interest). OECD uses a peer involvement process, where test method users from member countries evaluate the strengths and weaknesses of the method; methods have been nominated for OECD review by member countries. ECVAM has a science board that oversees the conduct and review of validation studies; activities are carried out in closed sessions. The U.S. EPA endocrine program plans to use an independent public advisory committee to assess test method performance in public sessions. Finally, ICCVAM forms independent peer review panels that have no association with the development or validation of the methods; business is conducted in meetings open to public input.

Subissue: Comparability of processes

a. What are the pros and cons (e.g., scientific rigor, policy analysis) for the processes used by different authorities?
b. Is there an optimal process?

Subissue: Sufficiency of processes

c. Do the different processes yield similar decisions as to the adequacy of tests (e.g., standardization, reliability, relevance)? *Consider using case studies of dermal corrosion (Corrositex, TER, Episkin and Epiderm*).
d. Should a statement of validity of a method by one of the above authorities be considered adequate for all other authorities?
e. Is there an optimal process for assessing validity of test methods?
g. Should member countries or regional authorities use the same type of review process before sending a method to OECD for consideration?

Subissue: Transparency of process

Authorities vary in the degree of openness of proceedings and decisionmaking Some are essentially closed processes, open to but a few parties. At the other end, regulatory deliberations in the US must be open to public input. In these cases, processes are totally open, where meetings are conducted in open fora, and the public has opportunity to input deliberations. OECD is increasingly being lobbied by various parties (e.g., animal welfare groups) to have access to meetings.

What degree of access should the public have during

a. test method development?
b. prevalidation study design and evaluation?
c. validation study design?
d. review of test method validation status?
e. acceptability of test methods by authorities (e.g., OECD, member country regulatory bodies?

Issue 8. Conflict of interest

Authorities vary in the need to avoid conflict of interest. European requirements do not seem as rigorous as those that apply to government activities in the US, where even the perception of conflict must be avoided.

a. Should the same people design and evaluate validation studies?
b. Should test sponsors be part of the evaluation process?
c. Should test evaluators be involved with regulatory acceptance?

Issue 9. Timing of review process

There is accentuating interest for methods to move more quickly through the OECD test guideline approval process. Some methods that have undergone validation by an authority are still subject to a deliberative OECD review. Only the Local Lymph Node Assay has moved through OECD within a single review cycle, but there had been

significant peer review of the test's validity and there was a test method implementation workshop to coalesce different views on the test.

a. Will it be possible to devise a process that will quickly move methods through OECD?
b. Would such an abbreviated process in any way jeopardize assurance that the method is scientifically sound?
c. Can methods be devised to assess methods while keeping budgetary considerations limited?

Issue 10. Who should validate

Test methods have been developed and its scientific attributes evaluated by different authorities, including Member countries, OECD, and local and regional authorities. Every authority has limited resources, which necessitates setting priorities for the methods in the queue. Such limitations impede an overall speedy development and validation framework.

a. Should we continue the test method development process that has been in use at OECD?
b. Should test development become
    i. centralized to a limited number of authorities?
    ii. spread out among countries and other authorities?
c. Should any authority bring forward a new test be required to have standardized or validated the method before sending it to OECD?
d. What is the best way to ensure development of budgets that are adequate to meet the desires of Member countries to develop OECD guidelines?

Issue 11. Patented tests

Patented test are becoming more and more common: commercial test kits are available to screen for health hazards; transgenic strains are being developed; gene chip and other new informatic methods are being assembled. Valid patented tests have been judged to be acceptable locally (U.S. transport, pharmaceuticals, pesticides and industrial chemicals) and regionally (EU). Current OECD policy necessitates that such methods are described generally without attribution to the patented method.

a. Should OECD continue to describe methods generally?
b. Should OECD establish a process to amend its policy so that patented methods can be cited as test guidelines?