

APPENDIX D: ICCVAM VALIDATION AND REGULATORY ACCEPTANCE CRITERIA¹

Validation Criteria

For a new or modified test method to be considered validated for regulatory risk assessment purposes, it generally should meet the following criteria (the extent to which these criteria are met will vary with the method and its proposed use). However, there needs to be flexibility in assessing a test method given its purpose and the supporting database. Because test methods can be designed and used for different purposes by different organizations and for different categories of substances, the determination of whether a specific test method is considered by an agency to be useful for a specific purpose must be made on a case-by-case basis. Validation of a test method is a prerequisite for it to be considered for regulatory acceptance.

- The scientific and regulatory rationale for the test method, including a clear statement of its proposed use, should be available.
- The relationship of the test method's endpoint(s) to the biologic effect of interest must be described. Although the relationship may be mechanistic or correlative, tests with biologic relevance to the toxic process being evaluated are preferred.
- A detailed protocol for the test method must be available and should include a description of the materials needed; a description of what is measured and how it is measured; acceptable test method performance criteria (e.g., positive and negative control responses); a description of how data will be analyzed; a list of the species for which the test results are applicable; and a description of the known limitations of the test, including a description of the classes of materials that the test can and cannot accurately assess.
- The extent of within-test variability and the reproducibility of the test method within and among laboratories must have been demonstrated. Data must be provided describing the level of intra- and inter-laboratory reproducibility and how it varies over time. The degree to which biological variability affects this test reproducibility should be addressed.
- The test method's performance must have been demonstrated using reference chemicals or test agents representative of the types of substances to which the test method will be applied, including both known positive and known negative agents. Unless it is hazardous to do so, chemicals or test agents should be tested under code to exclude bias.
- Sufficient data should be provided to permit a comparison of the performance of a proposed substitute test with that of the test it is designed to replace. Performance should be evaluated

¹NIEHS. 1997. Validation and regulatory acceptance of toxicological methods: A report of the *ad hoc* Interagency Coordinating Committee on the Validation of Alternative Methods. NIH Publication No. 97-3981. NIEHS, Research Triangle Park, NC.

in relation to existing relevant toxicity testing data and relevant toxicity information from the species of concern. Reference data from the comparable traditional test method should be available and of acceptable quality.

- The limitations of the method must be described; for example, *in vitro* or other nonanimal test methods may not replicate all of the metabolic processes relevant to chemical toxicity that occur *in vivo*.
- Ideally, all data supporting the validity of a test method should be obtained and reported in accordance with Good Laboratory Practices (GLPs). Aspects of data collection not performed according to GLPs must be fully described, along with their potential impacts.
- All data supporting the assessment of the validity of the test method must be available for review.
- Detailed protocols should be readily available and in the public domain.
- The method(s) and results should be published or submitted for publication in an independent, peer-reviewed publication.
- The methodology and results should have been subjected to independent scientific review.

Regulatory Acceptance Criteria

Validated test methods are not automatically accepted by regulatory agencies; they need to fit into the regulatory structure. Flexibility is essential in determining the acceptability of methods to ensure that appropriate scientific information is considered in regulatory risk assessment. A test method proposed for regulatory acceptance generally should be supported by the attributes listed below:

- The test method should have undergone independent scientific peer review by disinterested persons who are experts in the field, knowledgeable of the test method, and financially (and otherwise) unencumbered by the outcome of the evaluation.
- There should be a detailed test method protocol with standard operating procedures (SOPs), a list of operating characteristics, and criteria for judging test performance and results.
- Data generated by the test method should adequately measure or predict the endpoint of interest and demonstrate a linkage between the new test method and an existing test method or between the new test method and effects in the target species.
- There should be adequate test method data for chemicals and products representative of those administered by the regulatory program or agency and for which the test is proposed.

- The test method should generate data useful for risk assessment purposes (i.e., for hazard identification, dose-response assessment, or exposure assessment). Such test methods may be useful alone or as part of a battery or tiered approach.
- The specific strengths and limitations of the test method must be clearly identified and described.
- The test method must be robust (relatively insensitive to minor changes in protocol) and transferable among properly equipped and staffed laboratories.
- The test method should be time and cost effective.
- The test method should be one that can be harmonized with similar testing requirements of other agencies and international groups.
- The test method should be suitable for international acceptance.
- The test method must provide adequate consideration for the reduction, refinement, and replacement of animal use.