4.0 SUBMISSION GUIDELINES FOR PROPOSED TEST METHODS

4.1 Introduction and Rationale for the Proposed Test Method

The sponsor should use this section to introduce the proposed test method and describe its regulatory and scientific rationale. A description must be provided of how the proposed test method can be used in the context of current or anticipated regulatory applications (e.g., as a screen in a tiered testing strategy, as an adjunct test to provide mechanistic information, as a substitute or replacement for an existing test method). The mechanistic basis of the proposed test method and the context in which it will be used to measure or predict the toxicological activity of a test material or substance should be discussed, as well as what is known and not known about the similarities and differences of modes and mechanisms of action in the test system compared to the species of interest (e.g., humans for human health-related toxicity testing). If applicable, the extent to which the proposed test method meets the performance standards of a mechanistically and functionally similar validated and accepted test method should be addressed. The sponsor should indicate the relevant classes of chemicals and products that can and cannot be evaluated using the proposed test method and any known limitations. Finally, the sponsor should indicate where and how the proposed test method might be included in the overall safety or hazard assessment process. In particular, if the proposed test method is part of tiered or battery approaches, the weight given to the new method relative to other tests in the tier or battery should be addressed.

4.2 Test Method Protocol Components

The sponsor should explain and describe the basis for decisions on critical functional, structural, and procedural elements of the test method protocol (a complete, detailed protocol for the proposed test method should be provided in an appendix to the submission). This would include the extent to which the protocol for the proposed test method is similar to the protocol of a validated mechanistically and functionally similar test method for which performance standards exist. The basis for any protocol modifications made during the validation of the proposed test method should be discussed. The technical parameters of the proposed test method (e.g., vehicles, exposure time), the nature of the response evaluated, and the basis for proposed concurrent controls should be described. Concurrent controls (negative, solvent, and positive), as appropriate, provide a basis for experiment-to-experiment comparisons and are usually part of the acceptance criteria for a given experiment. The acceptable ranges for the control responses and historical data used to establish the acceptable range should be included.

The nature of the data to be collected, the methods used for data collection, the type of media in which data are stored, measures of variability, the statistical or nonstatistical methods used to analyze and evaluate the data (including methods used to analyze for a dose-response relationship), and the decision criteria (and their rationale) used to classify the response as positive or negative, if applicable, must be described. The procedure for dose selection and the number of animals required, if any, for dose selection and the actual test should be stated. Both the statistical and nonstatistical methods used for data evaluation should be justified. Any confidential information associated with the proposed test method should be indicated clearly; however, the inclusion of confidential information is discouraged.

The number of replicate and/or repeat experiments needed to ensure an adequate study must be provided, and the basis for the design should be described. If replicate or repeat experiments are not part of the proposed test method protocol, a rationale for their exclusion must be provided.

The basis for selection of the proposed test method system must be provided. If an animal model is used, the rationale for selecting the species, strain or stock, sex, acceptable age range, diet, frequency of dosing, the number of doses, and other applicable protocol components should be included.

If the test method employs proprietary components, the procedures used to ensure their integrity (in terms of reliability and accuracy) from "lot-to-lot" and over time should be described. Also, procedures that the user may employ to verify the integrity of the proprietary components should be described.

4.3 Substances Used for Validation of the Proposed Test Method

The rationale for the numbers and types of substances tested during the validation process should be described. The specific chemical or formulation names and relevant chemical and product classes for the substances tested must be specified. A test method may be more effective for the evaluation of certain classes of chemicals. In addition, not all data sets will be homogeneous for a given chemical characteristic (e.g., water solubility). In such cases, it may be useful to separate the data set into smaller, more uniform subsets for data analysis. To the extent possible, the following information should be provided for each test substance:

- Chemical Abstracts Service Registry Number (CASRN)
- Physical and chemical characteristics
- Concentrations tested
- Purity
- Source
- Stability of the test substance in the test medium

Any characteristics thought to have direct impact on test method accuracy and/or reliability should be described. Information concerning coding of substances during validation studies should be included. In the case of mixtures, the constituents and their relative concentrations should be stated, whenever possible. A suggested spreadsheet format for listing this information is provided in **Appendix B**. Information regarding the use of coded substances and blind testing during the validation process should be included. For a proposed test method mechanistically and functionally similar to a validated test method with established performance standards, the extent to which the reference chemicals recommended in the performance standards were tested in the proposed test method should be discussed, and any deviations from this list should be justified. In situations where a listed reference chemical is unavailable, the criteria used to select a replacement chemical should be described. To the extent possible, when compared to the original reference chemical, the replacement chemical should be from the same chemical/product class and produce similar effects in the *in vivo* reference test method. In addition, if applicable, the replacement chemical should have been tested in the comparable validated test method. Also where applicable, the rationale for

adding additional chemicals and the adequacy of data from the *in vivo* reference test method or the species of interest should be provided.

4.4 *In Vivo* Reference Data Used to Assess the Accuracy of the Proposed Test Method

If the proposed test method is intended to replace or substitute for an existing *in vivo* reference test method, then a comparison of data between the proposed test method and the *in vivo* reference test method is necessary. The submission should include:

- Comparative data for the same substances tested using the *in vivo* reference test method and, where available, from human studies. If possible, individual animal and human data should be provided.
- The criteria used to select the *in vivo* reference test method (or human) data
- The source of the *in vivo* reference test method data (e.g., the literature citation for published information, the laboratory study director, the year generated for unpublished data)
- A description of the protocol(s) employed to generate the *in vivo* reference test method or human data. Any modifications to the *in vivo* reference test method protocol(s) should be stated clearly for each data set, along with a discussion of the potential impact of these modifications on the assessment of the accuracy of the proposed test method.
- A description of the quality of the *in vivo* reference test method data, including the extent of Good Laboratory Practices (GLP) compliance (7-12) and the use of coded test chemicals
- The availability of original study data for the *in vivo* reference test method studies
- A summary of the availability and use of other, relevant toxicity information from the species of interest (e.g., data from human studies, accidental exposures for human health-related toxicity test methods, results of postmarketing surveillance)

4.5 Test Method Data and Results

The data generated by testing chemicals and substances using the proposed test method protocol are provided here. Any protocol modifications made during the development process and their impact should be stated clearly for each data set. All data, both original and derived, should be submitted, along with each laboratory's summary judgment regarding the outcome of each study. The submission should include data (and explanations) from all studies, whether successful or not. The statistical approach used to evaluate the data should be described and justified.

It is also important to describe the "lot-to-lot" consistency of the test chemicals, the time frame of the various studies, and the laboratory(ies) in which the studies were conducted. A coded designation for each laboratory involved in an interlaboratory evaluation of test method reliability and accuracy is acceptable. Any original data not submitted should be available for review, if requested. Presenting all available data, including data from published sources, is essential for an adequate scientific assessment of the proposed test method.

Results should be presented in graph or tabular form for easy comparison of results from the reference test methods with those from the proposed test method. A suggested tabular format for presenting the results is provided in **Appendix B**.

4.6 Test Method Accuracy

The sponsor should describe the accuracy of the proposed test method with respect to its ability to measure or predict the effect of interest. The accuracy (i.e., sensitivity, specificity, positive and negative predictivity, false positive and negative rates) of the proposed test method should be compared to that obtained for the in vivo reference test method currently accepted by regulatory agencies and to data or recognized toxicity information from the species of interest (e.g., humans for human-health-related toxicity testing). In instances where the proposed test method is measuring or predicting an endpoint for which there is no pre-existing test method, the frequency of correct predictions should be compared to relevant information from the species of interest. In cases where the proposed test method is mechanistically and functionally similar to a validated test method with established performance standards, the accuracy of both test methods should be compared. When the results obtained using the proposed test method is discordant from that obtained using the comparable validated test method, the frequency of correct predictions of each test method compared to recognized toxicity information from the species of interest should be presented. The basis for any discordance in results for the following comparisons should be discussed.

- The proposed test method and currently accepted *in vivo* reference test methods
- The proposed test method and, if applicable, the comparable validated test method with established performance standards
- The proposed test method and the accepted *in vivo* reference test method in predicting responses in the species of interest, where data from the species of interest is available

The submission should include a discussion of the strengths and limitations of the proposed test method and should describe salient issues of data interpretation.

4.7 Test Method Reliability (Repeatability/Reproducibility)

An assessment of test method reliability (repeatability and reproducibility) must be provided. This assessment should include discussion of the rationale for the selection of the substances used to evaluate intra- and interlaboratory reproducibility, and the extent to which they represent the range of possible test outcomes. Outlying values should be identified and discussed. A quantitative statistical analysis of the extent of intra- and inter-laboratory variability, such as that described in ASTM Publication Number E691-92 (13) or coefficient-of-variation analysis, should be included. Measures of central tendency and variation, should be summarized for historical control data (negative, vehicle, and positive, where applicable). In cases where the proposed test method is mechanistically and functionally similar to a validated test method with established performance standards, the reliability of the two test methods should be compared and the potential impact of any differences discussed.

4.8 Test Method Data Quality

The extent of adherence to national and international GLP guidelines (7-12) for the data presented in the submission, as well as the results of any data quality audits, should be included here. Deviations from GLP guidelines and the impact of any noncompliance detected in audits should be described. Information on the availability of laboratory notebooks and other data retained

by the sponsor(s) for external audits by ICCVAM should be stated. Unpublished data should be supported by laboratory notebooks.

4.9 Other Scientific Reports and Reviews

The submission should discuss all data from other published or unpublished studies conducted using the proposed test method. Comment should be provided on any conclusions presented in independent peer-reviewed reports or other scientific reviews of the proposed test method. The conclusions of such scientific reports or reviews should be compared to the conclusions reached in the submission. Any other ongoing or planned evaluations of the proposed test method should be described. In cases where the proposed test method is mechanistically and functionally similar to a validated test method with established performance standards, the results of studies conducted subsequent to the ICCVAM evaluation should be included, and any impact on the reliability and accuracy of the proposed test method discussed.

4.10 Animal Welfare Considerations (Refinement, Reduction, and Replacement)

A description should be included of how the proposed test method will refine, reduce, or replace animal use as compared to current methods used for the endpoint of interest. If the proposed test method requires the use of animals, the rationale for such use should be provided. A description of the sources used to determine the possible availability of alternative test methods that would refine, reduce, or replace animal use for the endpoint of interest should be provided (14, 15). The description should include, at a minimum, the databases searched, the search strategy, the search date(s), the database search results, and the rationale for not utilizing available alternative methods. The basis for determining the appropriate number of animals for the proposed test method should be described. If the test involves potential animal pain and distress, the procedures and approaches that have been incorporated to minimize and, whenever possible, to eliminate the occurrence of such pain and distress should be discussed.

4.11 Practical Considerations

The cost and time involved in conducting a study using the proposed test method should be specified and compared to the reference test method(s) and, if applicable, to the mechanistically and functionally validated test method with established performance standards. Also include the following:

- A discussion of the facilities and major fixed equipment needed to conduct the test method
- The general availability of other necessary equipment and supplies
- The required level of training, expertise, and demonstrated proficiency needed by the study personnel

4.12 References

A listing of all publications referenced in the submission should be provided.

4.13 Supporting Materials

The appendices should contain:

- A detailed protocol for the proposed test method
- Copies of all relevant publications, including those containing data from the proposed test method, the *in vivo* reference test method, and if applicable, a comparable validated test method
- All available nontransformed original data used to evaluate the validity of the proposed test method
- Suggested performance standards for consideration by NICEATM and ICCVAM, if performance standards for the proposed test method do not exist. Examples of performance standards can be located on the ICCVAM/NICEATM web site at http://iccvam.niehs.nih.gov.