APPENDIX A: OUTLINE FOR NOMINATIONS AND SUBMISSIONS TO ICCVAM[1]

1.0 Introduction and Rationale for the Proposed Test Method

1.1 Introduction

1.1.1 Describe the historical background for the proposed test method, from original concept to present. This should include the rationale for its development, an overview of prior development and validation activities, and, if applicable, the extent to which the proposed test method is mechanistically and functionally similar to a validated test method with established performance standards.

1.1.2 Summarize and provide the results of any peer review conducted to date and summarize any ongoing or planned reviews.

1.1.3 Clearly indicate any confidential information associated with the test method; however, the inclusion of confidential information is discouraged.

1.2 Regulatory rationale and applicability

1.2.1 Describe the current regulatory testing requirement(s) for which the proposed test method is applicable.

1.2.2 Describe the intended regulatory use(s) (e.g., screen, substitute, replacement, or adjunct) of the proposed test method and how it will be used to substitute, replace, or complement any existing regulatory testing requirement(s).

1.2.3 Where applicable, discuss the similarities and differences in the endpoint measured in the proposed test method and the currently used *in vivo* reference test method and, if appropriate, between the proposed test method and a comparable validated test method with established performance standards.

1.2.4 Describe how the proposed test method fits into the overall strategy of hazard or safety assessment. If a component of a tiered assessment process, indicate the weight that should be applied relative to other measures.

1.3 Scientific basis for the proposed test method

1.3.1 Describe the purpose and mechanistic basis of the proposed test method.

1.3.2 Describe what is known and not known about the similarities and differences of modes and mechanisms of action in the proposed test method as compared to the species of interest (e.g., humans for human health-related toxicity testing).

[1]Where the requested information is not yet available, this should be indicated. Plans for generating information that is not available should be described.

1.3.3 Describe the intended range of substances amenable to the proposed test method and/or the limits of the proposed test method according to chemical class or physicochemical factors.

2.0 Test Method Protocol Components

Note: A complete, detailed protocol should be included as an appendix to the nomination or submission.

2.1 Provide an overview of how the proposed test method is conducted. If appropriate, this would include the extent to which the protocol for the proposed test method adheres to established performance standards.

2.2 Provide a detailed description and rationale, if appropriate, for the following aspects of the proposed test method:

2.2.1 Materials, equipment, and supplies needed

2.2.2 Dose-selection procedures, including the need for any dose range-finding studies or acute toxicity data prior to conducting a study, if applicable

2.2.3 Endpoint(s) measured

2.2.4 Duration of exposure

2.2.5 Known limits of use

2.2.6 Nature of the response assessed

2.2.7 Appropriate vehicle, positive, and negative controls and the basis for their selection

2.2.8 Acceptable range of vehicle, positive and negative control responses and the basis for the acceptable ranges

2.2.9 Nature of the data to be collected and the methods used for data collection

2.2.10 Type of media in which data are stored

2.2.11 Measures of variability

2.2.12 Statistical or nonstatistical methods used to analyze the resulting data, including methods to analyze for a dose-response relationship. Justify and describe the method(s) employed.

2.2.13 Decision criteria and the basis for the prediction model used to classify a test chemical (e.g., positive, negative, or equivocal), as appropriate

2.2.14 Information and data that will be included in the study report and availability of standard forms for data collection and submission

2.3 Explain the basis for selection of the test method system. If an animal model is being used, this should include the rationale for selecting the species, strain or stock, sex, acceptable age range, diet, and other applicable parameters.

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

2.5 Describe the basis for the number of replicate and repeat experiments; provide the rationale if experiments are not replicated or repeated.

2.6 Discuss the basis for any modifications to the proposed test method protocol that were made based on results from validation studies.

2.7 If applicable, discuss any differences between the protocol for the proposed test method and that for a comparable validated test method with established performance standards.

3.0 Substances Used for Validation of the Proposed Test Method (See Appendix B)

3.1 Describe the rationale for the chemicals or products selected for use in the validation process. Include information on the suitability of the substances selected for testing, indicating any chemicals that were found to be unsuitable.

3.2 Discuss the rationale for the number of substances that were tested.

3.3 Describe the chemicals or products evaluated. For each chemical or product, include the following information:

3.3.1 Chemical or product name, or if a mixture, provide information on all components

3.3.2 CASRN

3.3.3 Chemical and product class

3.3.4 Physical/chemical characteristics (e.g., water and lipid solubility, pH, pKa, etc.). Any characteristics thought or known to impact test method accuracy and/or reliability should be clearly described

3.3.5 Stability of the test substance in test medium

3.3.6 Concentrations tested

3.3.7 Purity, including the presence and identity of contaminants and stabilizing additives

3.3.8 Supplier or source

3.4 Describe the coding procedures used in the validation studies.

3.5 For proposed test methods that are mechanistically and functionally similar to a validated test method with established performance standards, discuss the extent to which the recommended reference chemicals were tested in the proposed test method. In situations where a listed reference chemical was 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 mechanistically and functionally similar validated test method. If 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.

4.0 *In Vivo* Reference Data Used for an Assessment of the Accuracy of the Proposed Test Method

4.1 Provide a clear description of the protocol(s) used to generate data from the *in vivo* reference test method. If a specific guideline has been followed, it should be provided. Any deviations should be indicated, including the rationale for the deviation.

4.2 Provide the *in vivo* reference test method data used to assess the accuracy of the proposed test method. Individual human and/or animal reference test data, if available, should be provided. Provide the source of the reference data, including the literature citation for published data, or the laboratory study director and year generated for unpublished data.

4.3 If not included in the submission, indicate if original records are available for the *in vivo* reference test method data.

4.4 Indicate the quality of the *in vivo* reference test method data, including the extent of GLP compliance and any use of coded chemicals.

4.5 Discuss the availability and use of relevant toxicity information from the species of interest (e.g., human studies and reported toxicity from accidental or occupational exposure for human health-related toxicity testing).

4.6 Discuss what is known or not known about the accuracy and reliability of the *in vivo* reference test method.

5.0 Test Method Data and Results

5.1 Describe the proposed test method protocol used to generate each submitted set of data. Any differences from the proposed test method protocol should be described, and a rationale or explanation for the difference provided. Any protocol modifications made during the development process and their impact should be clearly stated for each data set.

5.2 Provide all data obtained to evaluate the accuracy and reliability of the proposed test method. This should include copies of original data from individual animals and/or individual samples, as well as derived data. The laboratory's summary judgment regarding the outcome of each test should be provided. The submission should include data (and explanations) from all studies, whether successful or not.

5.3 Describe the statistical approach used to evaluate the data resulting from studies conducted with the proposed test method.

5.4 Provide a summary, in graphic or tabular form, of the results. The suggested tabular format for providing data for use in an assessment of accuracy is provided in **Appendix B**.

5.5 For each set of data, indicate whether coded chemicals were tested, whether experiments were conducted without knowledge of the chemicals being tested, and the extent to which experiments followed GLP guidelines.

5.6 Indicate the "lot-to-lot" consistency of the test substances, the time frame of the various studies, and the laboratory in which the study or studies were conducted. A coded designation for each laboratory is acceptable.

5.7 Indicate the availability of any data not submitted for external audit, if requested.

6.0 Test Method Accuracy

6.1 Describe the accuracy (e.g., concordance, sensitivity, specificity, positive and negative predictivity, false positive and negative rates) of the proposed test method compared with the reference test method. Explain how discordant results in the same or multiple laboratories from the proposed test were considered when calculating accuracy.

6.2 Discuss results that are discordant with results from the *in vivo* reference method.

6.3 Discuss the accuracy of the proposed test method compared to data or recognized toxicity from the species of interest (e.g., humans for human health-related toxicity testing), where such data or toxicity classification are available. This is essential when the method is measuring or predicting an endpoint for which there is no preexisting method. In instances where the proposed test method was discordant from the *in vivo* reference test method, describe the frequency of correct predictions of each test method compared to recognized toxicity information from the species of interest.

6.4 State the strengths and limitations of the proposed test method, including those applicable to specific chemical classes or physical-chemical properties.

6.5 Describe the salient issues of data interpretation, including why specific parameters were selected for inclusion.

6.6 In cases where the proposed test method is mechanistically and functionally similar to a validated test method with established performance standards, the results obtained with both test methods should be compared with each other and with the *in vivo* reference test method and/or toxicity information from the species of interest.

7.0 Test Method Reliability (Repeatability/Reproducibility)

7.1 Discuss the selection rationale for the substances used to evaluate the reliability (intralaboratory repeatability and intra- and interlaboratory reproducibility) of the proposed test method as well as the extent to which the chosen set of substances represents the range of possible test outcomes.

7.2 Provide analyses and conclusions reached regarding the repeatability and reproducibility of the proposed test method. Acceptable methods of analyses might include those described in ASTM E691-92 (13) or by coefficient of variation analysis.

7.3 Summarize historical positive and negative control data, including number of experiments, measures of central tendency, and variability.

7.4 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 any differences discussed.

8.0 Test Method Data Quality

8.1 State the extent of adherence to national and international GLP guidelines (7-12) for all submitted data, including that for the proposed test method, the *in vivo* reference test method, and if applicable, a comparable validated test method. Information regarding the use of coded chemicals and coded testing should be included.

8.2 Summarize the results of any data quality audits, if conducted.

8.3 Discuss the impact of deviations from GLP guidelines or any noncompliance detected in the data quality audits.

8.4 Address the availability of laboratory notebooks or other records for an independent audit. Unpublished data should be supported by laboratory notebooks.

9.0 Other Scientific Reports and Reviews

9.1 Summarize all available and relevant data from other published or unpublished studies conducted using the proposed test method.

9.2 Comment on and compare the conclusions published in independent peer-reviewed reports or other independent scientific reviews of the proposed test method. The conclusions of such scientific reports and reviews should be compared to the conclusions reached in this submission. Any ongoing evaluations of the proposed test method should be described.

9.3 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 with the validated test method subsequent to the ICCVAM evaluation should be included and any impact on the reliability and accuracy of the proposed test method should be discussed.

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

10.1 Describe how the proposed test method will refine (reduce or eliminate pain or distress), reduce, or replace animal use compared to the reference test method.

10.2 If the proposed test method requires the use of animals, the following items should be addressed:

10.2.1 Describe the rationale for the need to use animals and describe why the information provided by the proposed test method requires the use of animals (i.e., cannot be obtained using nonanimal methods).

10.2.2 Include a description of the sources used to determine the availability of alternative test methods that might further refine, reduce, or replace animal use for this testing. This should, at a minimum, include the databases searched, the search strategy used, the search date(s), a discussion of the results of the search, and the rationale for not incorporating available alternative methods.

10.2.3 Describe the basis for determining that the number of animals used is appropriate.

10.2.4 If the proposed test method involves potential animal pain and distress, discuss the methods and approaches that have been incorporated to minimize and, whenever possible, eliminate the occurrence of such pain and distress.

11.0 Practical Considerations

11.1 Discuss the following aspects of proposed test method transferability. Include an explanation of how this compares to the transferability of the *in vivo* reference test method and, if applicable, to a comparable validated test method with established performance standards.

11.1.1 Discuss the facilities and major fixed equipment needed to conduct a study using the proposed test method.

11.1.2 Discuss the general availability of other necessary equipment and supplies.

11.2 Discuss the following aspects of proposed test method training. Include an explanation of how this compares to the level of training required to conduct the *in vivo* reference test method and, if applicable, a comparable validated test method with established performance standards.

11.2.1 Discuss the required level of training and expertise needed for personnel to conduct the proposed test method.

11.2.2 Indicate any training requirements needed for personnel to demonstrate proficiency and describe any laboratory proficiency criteria that should be met.

11.3 Cost Considerations

Discuss the cost involved in conducting a study with the proposed test method. Discuss how this compares to the cost of the *in vivo* reference test method and, if applicable, with that of a comparable validated test method with established performance standards.

11.4 Time Considerations

Indicate the amount of time needed to conduct a study using the proposed test method and discuss how this compares with the *in vivo* reference test method and, if applicable, with that of a comparable validated test method with established performance standards.

12.0 References

12.1 List all publications referenced in the submission.

13.0 Supporting Materials (Appendices)

13.1 Provide the complete, detailed protocol for the proposed test method.

13.2 Provide the detailed protocol(s) used to generate reference data for this submission and any protocols used to generate validation data that differ from the proposed protocol.

13.3 Provide 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 with established performance standards.

13.4 Include all available nontransformed original data for both the proposed test method, the *in vivo* reference test method, and if applicable, a comparable validated test method with established performance standards.

13.5 If appropriate performance standards for the proposed test method do not exist, performance standards for consideration by NICEATM and ICCVAM may be proposed. Examples of established performance standards can be located on the ICCVAM/NICEATM web site at http://iccvam.niehs.nih.gov.