APPENDIX D2

ICCVAM CONSIDERATION OF PUBLIC COMMENTS RECEIVED IN RESPONSE TO FEDERAL REGISTER NOTICES

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In response to eight *Federal Register* (*FR*) notices that were released between June 2000 and July 2006, 298 public comments were received. Comments received in response to the *FR* notices and/or were related to those *FR* notices can be obtained on CD ROM upon request to The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) by mail, fax, or email (NICEATM, NIEHS, P.O. Box 12233, MD EC-17, Research Triangle Park, NC 27709, (phone) 919-541-2384, (fax) 919-541-0947, (email) niceatm@niehs.nih.gov). The following sections, delineated by *FR* notice, provide a brief discussion of the public comments received in response to three of the published *FR* notices.

1.0 Public Comments Received in Response to *FR* Notice Released on March 22, 2005 (Volume 70, Number 54; pages 14473-14474)

NICEATM, in an *FR* notice (70 *FR* 54:14473-14474, March 22, 2005) requested nominations of scientific experts for consideration as part of an independent peer review panel to evaluate the validation status of two *in vitro* cytotoxicity assays for estimating *in vivo* oral toxicity. One comment was received in response to this request and stated that animal testing should be stopped and more accurate and humane methods should be used.

The ICCVAM appreciates the comment received. It should be noted that ICCVAM does not determine whether a test method is acceptable for use by U.S. Federal agencies or the international regulatory community. ICCVAM develops and forwards recommendations on the usefulness and limitations of the proposed test methods to each U.S. Federal agency for its review. Based on their specific statutory mandates, each U.S. Federal agency will consider ICCVAM's recommendations and then make a determination as to the acceptability of the test methods.

2.0 Public Comments Received in Response to *FR* Notice Released on March 21, 2006 (Volume 71, Number 54; pages 14229-14231)

NICEATM, in an *FR* notice (71 *FR* 54:14229-14231, March 21, 2006) requested comments on (1) the draft BRD being forwarded to the Scientific Peer Review Panel, (2) the draft ICCVAM test method recommendations, (3) draft test method protocols, and (4) draft performance standards. In response to this *FR* notice, 297 comments were received.

Of the comments received, 296 comments stated that there was a consensus at the workshop in 2000 (*In Vitro* Methods for Assessing Acute Systemic Toxicity) that cell-based methods could be used immediately to reduce the number of animals killed and could potentially be validated as replacements to current acute systemic toxicity test methods, given the proper funding and effort. However, the comments stated that announcement for the Peer Review Panel meeting scheduled for 2006 did not mention the potential of using these cell-based methods as potential replacement methods.

ICCVAM considered all the recommendations from the 2000 workshop in developing its own recommendations for activities (ICCVAM 2001a). The ICCVAM recommendations were forwarded to U.S. Federal agencies, along with the workshop report (ICCVAM 2001a)

and the *Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity* (ICCVAM 2001b). Consistent with the workshop recommendations, ICCVAM recommended that the near-term focus for validation should be on characterizing the usefulness of two standardized *in vitro* assays using rodent and human cells in predicting acute toxicity with a broader range of chemicals than had been previously tested . Therefore, the current evaluation focused on the use of these two *in vitro* methods for estimating starting doses for acute oral systemic toxicity tests.

Of the comments received, 23 stated that it was time to refine and implement non-animal, cell-based methods to replace current systemic acute toxicity test method protocols. ICCVAM appreciates the comments received. It should be noted that ICCVAM does not determine whether a test method is acceptable for use by U.S. Federal agencies or the international regulatory community. ICCVAM develops and forwards recommendations on the usefulness and limitations of the proposed test methods to each U.S. Federal agency for its review. Based on their specific statutory mandates, each U.S. Federal agency considers ICCVAM's recommendations and then determines the acceptability of the test methods.

Of the comments received, two focused on the rationale for ICCVAM to not consider or implement the recommendations of the participants of the *International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity* (ICCVAM 2001a). ICCVAM notes that the participants of the workshop made the following recommendations (among others):

- *In vitro* cytotoxicity data should be used to predict starting doses for *in vivo* lethality studies.
- Test laboratories should evaluate and compare the performance of several *in vitro* cytotoxicity tests with the existing RC data.
- A prevalidation study should be initiated as soon as possible to evaluate various cell types, exposure periods, and endpoint measurements as predictors of acute toxicity. The assay, or battery of assays, determined to be the best predictor of *in vivo* lethality could then be optimized further to identify, standardize, and validate simple predictive systems for gut absorption, blood-brain barrier passage, kinetics, and metabolism.
- In the longer-term, preferably as a parallel activity, there should be a focus on the development and validation of human *in vitro* test systems for predicting human acute toxicity.
- The evaluation and ultimate acceptance of *in vitro* assays for human acute toxicity will need a larger reference database than is presently available for validation purposes.

ICCVAM considered these as well as other recommendations from the workshop in developing its own recommendations. The ICCVAM recommendations were forwarded to U.S. Federal agencies along with the workshop report and *Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity* (ICCVAM 2001b). Consistent with the workshop recommendations, ICCVAM recommended that the near-term focus for validation should be on characterizing the usefulness of two standardized *in vitro* assays using rodent and human cells in predicting acute toxicity with a broader range of chemicals than had been previously tested. The NICEATM/ECVAM validation study was

based on this recommendation and its goals and purpose are entirely consistent with the workshop recommendations. Research activities to identify appropriate *in vitro* absorption, distribution, metabolism, and excretion systems was identified as a longer-term objective. NICEATM proceeded with the validation study to establish the utility of setting the starting dose across the range of GHS hazard classification, and to establish a high quality database as a foundation for the development of other *in vitro* tests that could be used, along with *in vitro* basal cytotoxicity test methods, to improve the prediction of *in vivo* acute toxicity.

ICCVAM received a comment that the NICEATM/ECVAM validation study objectives appeared to be a mixture of partly conflicting goals (e.g., validating the RC prediction model, assessing the boundaries of applicability, and assessing the predictive capacity of LD_{50} point measures). As stated in the BRD, ICCVAM notes that the study objectives were to:

- Further standardize and optimize the *in vitro* NRU basal cytotoxicity protocols using 3T3 and NHK cells to maximize test method reliability (intralaboratory repeatability, intra- and inter-laboratory reproducibility)
- Assess the accuracy of the two standardized *in vitro* 3T3 and NHK NRU basal cytotoxicity test methods for estimating rodent oral LD_{50} values across the five United Nations (UN) GHS categories of acute oral toxicity, as well as unclassified toxicities (GHS; UN 2005)
- Estimate the reduction and refinement in animal use achievable from using the *in vitro* 3T3 and NHK NRU basal cytotoxicity test methods to identify starting doses for *in vivo* acute oral toxicity tests, assuming that no other information were available
- Develop high quality *in vivo* acute oral lethality and *in vitro* NRU cytotoxicity databases that can be used to support the investigation of other *in vitro* test methods necessary to improve the prediction of *in vivo* acute oral lethality

ICCVAM received a comment focused on the selection of the test chemicals for the validation study. The comment noted that these chemicals were not appropriate to achieve the main goal of the validation study (i.e., verification or falsification of the RC prediction model). ICCVAM appreciates the comment but notes that the verification of falsification of the RC prediction model was not a goal of this effort (see above).

ICCVAM received a comment regarding the variability of *in vitro* data obtained during Phase I and Phase II of the validation study. The comment stated that the *in vitro* test protocols were optimized, and that the necessity of this step was questionable. The comment recommended that the outcomes from this study be compared with other interlaboratory validation studies that have used the 3T3 NRU standard protocol. ICCVAM notes that the test acceptance criteria for the VC OD and placement of the cytotoxicity points were revised after it was noted that good dose-response data were obtained even in tests that failed the original criteria. Thus, to increase the test method experimental success rate, the criteria were revised. These changes did not alter the performance of the test methods.

Regarding the variability of the *in vitro* data, this comment appears to refer to the difference between the 3T3 NRU and NHK NRU IC_{50} values since no such variation occurred across laboratories for the same cell type. ICCVAM notes that it should not be a surprise that, for

some chemicals, large variation exists for IC_{50} results obtained using different cell lines even when using very similar test protocols. Such data are important for characterizing which cell line(s) may be optimal for *in vitro* cytotoxicity testing and for identifying chemicals that may require additional evaluation.

ICCVAM received a comment regarding the variability of the *in vivo* reference data. The comment noted that there had been extensive efforts by ICCVAM to obtain multiple *in vivo* LD_{50} values per test chemical. The comment noted that while most validation studies assess the variability of the *in vivo* data to analyze the performance of the alternative methods, this type of analysis was not present in the BRD. ICCVAM appreciates the comments and notes that the BRD analyzed the variation of *in vivo* data in Section 4 (ICCVAM 2006). Table 4-2 in the BRD provides the ratio of the maximum to the minimum acceptable LD_{50} for each chemical (ICCVAM 2006).

ICCVAM received a comment stating that the evaluation of the two *in vitro* assays was highly biased by the unbalanced selection of chemicals used in the validation study. The commenter stated that all calculations (e.g., the contingency tables for prediction of the GHS classes) were influenced by the bias in the chemical selection, so that even the strength of the prediction model (correct prediction of the absence of toxicity) was lost. The commenter stated that a thorough discussion of the influence of chemical selection on the study outcome should be included.

ICCVAM agrees with the comment that the selection of chemicals and their fit to the regression being evaluated affects the accuracy of GHS category predictions. Even though the selection of chemicals and their fit to the regressions affects the accuracy of GHS category predictions, the analyses provide a valid comparison of the test methods to one another and of the regressions to one another.

One comment stated that the results of the current study should be correlated to the results and information obtained from previous studies. ICCVAM agrees and notes that Section 9 of the BRD provides a literature review of studies most relevant to the NICEATM/ECVAM validation study. The literature review addresses (a) the use of *in vitro* NRU cytotoxicity test methods for correlations with rodent lethality and other toxicities and (b) the use of *in vitro* basal cytotoxicity to predict starting doses for acute oral lethality assays.

ICCVAM received a comment related to (a) the draft ICCVAM recommendation proposing that the RC should be revised and (b) the draft minimum performance standards. ICCVAM appreciates the comment received and notes that the proposed revisions were based on a variety of factors, were independent of each other, and are justified based on the breadth of the RC database. Furthermore, ICCVAM notes that the draft performance standards take into account the technical aspects of the test methods and proposes reference substances compatible with the RC regression after excluding substances without rat LD_{50} data and those with known mechanisms of action that are not expected to be active in the 3T3 and NHK cell cultures.

3.0 Public Comments Received in Response to *FR* Notice Released on July 11, 2006 (Volume 71, Number 132; pages 39122-39123)

NICEATM, in an *FR* notice (71 *FR* 132:39122-39123, Jul 11, 2006) requested comments on the Panel's conclusions on the draft ICCVAM test method recommendations. In response to this *FR* notice, one comment was received.

The comment stated that there was concern that despite near unanimous agreement at the 2000 workshop that the cell-based methods could be used immediately to set the starting dose for oral toxicity tests and that given appropriate effort and funding these method could be validated as a replacement measure, there has been little progress on the issue. There was concern that the Peer Panel Report did not require the use of the *in vitro* methods to estimate a starting dose, due to the understandable contention that significant information may already be available on the chemical or its class. The commentor stated that companies should be encouraged to use the non-animal methods to obtain another level of comfort with using and reading data generated by them. The comment stated that, based on the available scientific evidence, the Peer Panel Report should address expedient steps to replace lethal dose animal tests at the extremes of toxicity.

ICCVAM appreciates the comments provided. ICCVAM notes that the Peer Panel Report contains the conclusions of the Peer Review Panel and the document would not be edited by ICCVAM. However, the Peer Panel Report and all the comments received in response to the published *FR* notices were considered by ICCVAM during the development of the ICCVAM Test Method Evaluation Report.

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