



August 4, 2003

Dr. William S. Stokes  
Director, NICEATM  
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](mailto:niceatm@niehs.nih.gov).

Re: Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) on August 12-13, 2003  
Issues Pertaining to Validation of Genetically Modified Mouse Models

Dear Dr. Stokes,

The American Chemistry Council supports the discussion by Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) of the many complex issues involved in introducing and using Genetically Modified Mouse Models (“transgenic models”) in NTP's cancer hazard identification program. For over three decades the American Chemistry Council (ACC or the “Council”) and its member companies have played an active role in both screening and testing chemical substances and in the development of alternative toxicity test methods.<sup>1</sup> The Council supports NTP's research and testing efforts, and in particular encourages the use of more mechanistic data in hazard and risk assessments.

### **I. Need for Validation**

We endorse NTP's efforts to develop and validate alternative methods. We recognize that the NTP has expended significant effort to evaluate a number of transgenic models, both independently and as part of the ILSI/HESI collaborative program on alternative models for carcinogenicity assessment. ACC strongly supports and encourages such efforts and agrees that the potential benefits stated by NTP, i.e., a reduction in the time required for testing, a reduction in the number of animals used, and the potential for greater mechanistic insight for the responses observed in assays used for cancer hazard identification, are desirable.

We are supportive of use of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) to review and evaluate the transgenic models under

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<sup>1</sup> The American Chemistry Council represents the leading companies engaged in the business of chemistry. ACC members apply the science of chemistry to make innovative products and services that make people's lives better, healthier and safer. ACC is committed to improved environmental, health and safety performance through health and environmental research and product testing. Responsible Care, and common sense advocacy designed to address major public policy issues. The business of chemistry is a \$460 billion enterprise and a key element of the nation's economy. It is the nation's largest exporter, accounting for ten cents out of every dollar in U.S. exports. Chemistry companies invest more in research and development than any other business sector. Safety and security have always been primary concerns of ACC members, and they have intensified their efforts, working closely with government agencies to improve security and to defend against any threat to the nation's critical infrastructure.



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consideration by NTP. ICCVAM, created by the Director of NIEHS and housed in NTP, is the organization formally charged with conducting validation reviews of new, revised or alternative test methods. As envisioned by Congress in both Public Law 103-43 (1993) and Public Law 106-545 (2000), ICCVAM, composed of representatives from 15 Federal regulatory and research agencies, is to be the focal point to coordinate cross-agency issues relating to development, validation, acceptance, and national/international harmonization of new and alternative toxicological test methods.

The ICCVAM Authorization Act of 2000 (42 U.S.C. 2851) operates to ensure that any new or revised acute or chronic toxicity test method, including animal test methods and alternatives, is determined to be valid for proposed use prior to an Agency requiring, recommending, or encouraging the application of such test method. To date, critical scientific issues regarding the relevance, reliability, and appropriate use of the transgenic models have not yet been formally addressed by ICCVAM. Since the use of transgenic models for carcinogen identification may have widespread impact across various health and environmental programs in a multitude of Federal agencies, we believe that in the spirit of the ICCVAM statute, NTP should sponsor and support an ICCVAM evaluation of the transgenic mouse model test methods. In fact, we would be concerned that the omission of such a review would be inconsistent with the ICCVAM Authorization Act of 2000.

Further, because the NTP's strategy for use of transgenic models is extremely vague regarding how the transgenic models will be used in the chemical carcinogen screening process, an ICCVAM validation review would be beneficial. If the transgenic models are intended to supplement or replace or serve as an alternative method for a two-year carcinogenicity bioassay(s), then, as required by the ICCVAM validation criteria, "sufficient data should be provided to permit a comparison of the proposed substitute test with that of the test it is designed to replace." This is of particular importance because of the current lack of consensus on whether or not any of these models are a suitable replacement(s) for a two-year carcinogenicity bioassay. Greater scientific consensus is desired to assure that results of the NTP studies involving transgenic models meet the standards for mutual acceptance of testing data and serve to promote the reduction, refinement and replacement of animal tests with scientifically valid alternatives.

We strongly support a formal and systematic evaluation by ICCVAM before NTP uses these assays as part of the NTP's testing program for carcinogen identification. This systematic evaluation, or validation review, should cover each model proposed for use by NTP, and focus on clearly indicating: 1) mechanistic relevance to carcinogenesis, 2) test method reliability, 3) criteria for appropriate use, and 4) strengths, limitations, and uncertainties in data interpretation.

## **II. Criteria and Language to Interpret and Describe Results**

Despite the considerable efforts by NTP, the ILSI/HESI collaborative on alternative methods, there remains a considerable divergence of opinion, even controversy, about particular models and how data from individual models should be interpreted -- including those models under evaluation by NTP: the Tg.AC, p53(+/-) and rasH2 models. We support NTP's efforts to seek

input from external experts on level of evidence language and criteria. To that end, we see such activities as the February 21, 2003 NTP sponsored transgenic workshop as a productive start, but we think there is a need for more effort in this area.

We believe that a validation review undertaken by ICCVAM will foster greater agreement within the scientific community, and importantly, across other Federal agencies. As the February 21, 2003 workshop showed, at the present time there is clearly a lack of consensus in the scientific community on this issue. Indeed, in September 2002, the NTP's own Board of Scientific Counselors voted not to accept NTP's description of the results of transgenic Tg.AC studies. Furthermore, at the February 21, 2003 workshop, scientists from federal regulatory agencies also urged NTP to take steps to ensure that the criteria and language used by NTP include a caveat that studies of the carcinogenic potential of chemicals in transgenic mouse models are not equivalent to the results from the NTP's typical cancer tests.

Even though NTP itself makes no regulatory decisions, because the criteria for evaluation of results and classification of materials are inherently intertwined with regulatory decision making, steps must be taken to ensure that approaches to use, interpret and report transgenic animal studies meet the Federal standards required for science data quality and regulatory guidelines. For technical reports of studies using the transgenic models, we believe it would be inappropriate for NTP to default to the established criteria used for describing results of two-year carcinogenicity bioassay (i.e., "Clear Evidence", "Some Evidence", "Equivocal Evidence", "No Evidence" and "Inadequate Study" ... 'of Carcinogenicity'). The overwhelming majority of experts participating in NTP's February 21st workshop agreed that for the Tg.AC transgenic mouse model, NTP should use the descriptor "activity" in lieu of "carcinogenesis" when describing results, to make it clear that the results of a Tg.AC assay are not equivalent to the 2-year rodent bioassays, in particular in terms of relevance for assessing potential threats to human health. In addition, although consensus was not reached among the experts on the exact language for describing results of the p53(+/-) and rasH2 transgenic models, it was clear that many experts, close to or perhaps even exceeding 50%, felt that at the very least "neoplasia" should be used in lieu of "carcinogenesis."

As evidenced by the discussions at the February 21, 2003 workshop, it is a widely held perspective within the scientific community that tumor findings in transgenic models have not been shown to be equivalent to findings in traditional rodent cancer bioassays. Therefore, we believe that it would be inappropriate at this time for NTP to use the same terms or level of evidence as descriptors to describe results of transgenic model assays as NTP uses for the traditional 2-year rodent bioassays. Most importantly, the descriptor for transgenic studies must be accompanied by additional language. NTP's language in describing the results of any given transgenic assay needs to be different from, and readily distinguished from, the language used to describe standard rodent cancer bioassays. For example, we believe that the available scientific data would support level of evidence statements for transgenic mouse models along the lines of: *"There is ["clear", "equivocal", "some", "no", "inadequate"] evidence of [activity in the Tg.AC] [neoplasia in the p53(+/-) or rasH2] transgenic mouse model. The [xxx] transgenic mouse model provides qualitative information on biological potential and mode of action, but*

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*results of this assay alone cannot be used to extrapolate (either qualitatively or quantitatively) to potential hazards to humans.”*

Again, a formal ICCVAM validation review of all of the transgenic models proposed for use in the NTP or other Federal programs would achieve greater coordination and consistency in the use and interpretation of results from transgenic animal test systems across at least the 15 Federal agencies that are represented on ICCVAM. Such a review should consider all of the validation criteria described by ICCVAM, and include review of all of the data from the transgenic models validation efforts. Furthermore, this ICCVAM review should be conducted prior to implementing such assays into the NTP's routine testing program.

Critical aspects of the methods such as dose selection criteria for use in design and interpretation of transgenic animal studies, and relevance in terms of 'level of evidence' and descriptors for interpreting and reporting results of transgenic animal studies should be included as part of the ICCVAM review.

### **III. Conclusion**

In closing, we commend NTP and others for their considerable efforts to develop, standardize and validate alternative test methods. The introduction of transgenic models in testing and evaluation programs raises many complex issues that we feel can best be addressed by a formal validation review. We strongly support a formal and systematic evaluation by ICCVAM before NTP uses these assays as part the NTP's testing program for carcinogen identification. This systematic evaluation, or validation review, should cover each model proposed for use by NTP, and focus on clearly indicating: 1) mechanistic relevance to carcinogenesis, 2) test method reliability, 3) criteria for appropriate use, and 4) strengths, limitations, and uncertainties in data interpretation.

We appreciate the consideration of these suggestions by the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) as part of their deliberations in developing recommendations on the role of ICCVAM as the focal point within NIEHS and the Federal government to coordinate cross-agency issues relating to development, validation, acceptance, and national/international harmonization of new and alternative toxicological test methods. We look forward to continued progress within NIEHS and other Federal Agencies on the development, standardization and validation of alternative toxicity test methods. Please don't hesitate to contact me by e-mail (Rick\_Becker@americanchemistry.com) or by phone (703-741-5210) should you wish to discuss any of these matters in greater detail.

Sincerely,  
*Original Signed By*  
Richard A. Becker, Ph.D., DBAT  
Public Health Team

cc Dr. Mary Wolfe, NTP Executive Secretary, NTP Liaison and Scientific Review Office,  
NIEHS P.O. Box 12233, Research Triangle Park, NC 27709