September 10, 2002

Dr. Mary Wolfe
NTP Board Executive Secretary
NIEHS, P.O. Box 12233, MD A3-07
Research Triangle Park, NC 27709

Re: NTP Draft Strategy for Using Genetically Altered Animals in Carcinogen Identification

Dear Dr Wolfe:

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. The Council supports NTP's research and testing efforts, and in particular encourages the use of more mechanistic data in hazard and risk assessments. As NTP moves forward with development of its strategy to investigate the use of transgenic animal models as alternatives to the traditional 2-year bioassays for carcinogenic potential, the Council requests that NTP review and consider our comments and suggestions.

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. The 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. However, we are concerned that NTP's draft strategy proposes to implement the use of transgenic animal models prematurely, prior to a formal evaluation of the validity of these newly developed models (DRAFT The Use of Genetically Altered Animals in Carcinogen Identification by the National Toxicology Program, NTP June 1, 2002). We believe that a systematic evaluation should be undertaken before using 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.

The Interagency Coordinating Committee on the Validation of Alternative Methods (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. The ICCVAM Authorization Act of 2000 (42 U.S.C. 2851) dictates that any new or revised acute or chronic toxicity test method, including animal test methods and alternatives, must be 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. We believe that NTP, through ICCVAM, is obligated to evaluate the transgenic mouse model test methods in a formal validation program if it intends to routinely evaluate data from such assays as part of its carcinogen screening program. The omission of such a review is a departure from NTP's previously stated intention that its studies of the transgenic models "…will contribute to an NTP evaluation of genetically engineered mouse models by the Interagency Coordinating Committee on the Validation of Alternative Methods that is scheduled for 2001" (**http://ntp-server.niehs.nih.gov/Main_Pages/transgen/tg_summary.html**). In fact, even though the date is past, this is still the position articulated by NTP on its web site.

One of the essential concerns surrounding the transgenic models is the topic of the first item on the list of validation criteria laid out under ICCVAM which states that "the scientific and regulatory rationale for the test method, including a clear statement of its proposed use, should be available" (http://iccvam.niehs.nih.gov/docs/docs.htm#general). As you are aware, there is considerable divergence of opinion, even controversy, about particular models and how data from individual models should be interpreted. To this point, the NTP's draft strategy is extremely vague regarding which transgenic models will be used in the chemical carcinogen screening process outlined in Figure 2 of the draft strategy and how NTP will go about making decisions on which model(s) are appropriate for specific compounds. In one possible interpretation of Figure 2, the transgenic data could be used alone rather than in an overall weight of the evidence approach. This lack of clarity illustrates the need for a clear statement of the proposed use of the transgenic models.

Clearly, we are concerned about the intended use of the transgenic models, as supplements to, or replacement of, the two-year carcinogenicity study. This appears to be what NTP is proposing, in both Figure 2 and the text of the draft NTP strategy. If the transgenic models are intended to replace 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. For example, the ICH guidelines indicate that the requirements for carcinogenicity testing may be met by conducting a two-year study in one species, i.e., the rat, plus one other type of in vivo study which may be either a study using a transgenic model or a two-year carcinogenicity study in a second rodent species, i.e., the mouse (www.fda.gov/cder/guidance/1854fnl.pdf). However, in its statement on the results of the

ILSI/HESI program, the UK Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment recently concluded that "none of the models used in the ILSI/HESI Alternative Cancer Test programme were suitable as a replacement for the mouse carcinogenicity bioassay (the primary purpose for the development of these models) and that further research should look to identify models with a greater relevance to mechanisms of carcinogenicity in humans" (http://www.doh.gov.uk/coc/ilsihesiact.htm).

The mechanistic relevance of some of these models is a central scientific and regulatory issue. While some of the transgenic models may have utility in providing greater mechanistic insight, there are scientific concerns about the relevance, from a mechanistic viewpoint, of other models. Another issue, concerning interpretation of the results in the Tg.AC transgenic model, is that the animals are already "initiated" by virtue of the v-Ha-*ras* gene, and consequently this assay was anticipated to respond to both mutagenic and nonmutagenic carcinogens with a papillomatous response in the skin at the site of chemical application. In the Tg.AC model, TPA is used as the positive control. However, in the traditional mouse skin tumor bioassay, TPA is a well known skin tumor promoter, and is used as a 'promoter control.' In standard mouse 2-year skin tumor bioassays, when substances have produced effects similar to, but slightly less than TPA, NTP has classified such substances as 'weak skin tumor promoters' (see for example NTP Technical Report TR 444). However, it remains to be determined how NTP would interpret a substance eliciting a response similar to TPA in the Tg.AC model in terms of level of evidence. Such uncertainties need to be resolved prior to NTP's use of the transgenic models as a component of its routine carcinogen screening program.

One final point relates to the regulatory implications of NTP's draft strategy. The scheme in Figure 2 of the draft strategy indicates that an NTP technical report will be developed and reviewed by the NTP Board of Scientific Counselors (BSC) following the completion of the transgenic assay(s). We believe it is inappropriate to default to the established criteria used by NTP for evaluation of the results of two-year carcinogenicity bioassay results (i.e., Clear Evidence", "Some Evidence", "Equivocal Evidence", No Evidence" and "Inadequate Study") for technical reports of studies using the transgenic models. The criteria for evaluation of results and classification of materials are inherently intertwined with regulatory decision making, and the process of technical report writing and review by the BSC could result in regulatory action. While we acknowledge that NTP itself makes no regulatory decisions, this simple statement overlooks the important role that NTP bioassays have at federal, state and international regulatory agencies. For example, if transgenic assay studies are processed and reported like the standard NTP two-year studies, regulatory requirements could be triggered by OSHA, by EPA and by certain States (i.e., California Proposition 65). Therefore, it is critical that NTP take steps to ensure that studies using the mouse transgenic models are not treated, at this time, as scientifically equivalent to the over 500 standard bioassays previously conducted and reported by the NTP.

We urge NTP to integrate the following activities into its draft strategy for using genetically altered animals in carcinogen identification:

1) NTP should schedule a formal ICCVAM validation review of all of the transgenic models proposed for use in the NTP carcinogen screening program. This 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 as well as a clearly defined proposal for interpretive criteria. This should be conducted **prior** to implementing such assays into the NTP's routine testing program.

2) NTP should develop dose selection criteria for use in design of studies and 'level of evidence' criteria for interpretation of transgenic animal studies. These criteria may be considered as part of the ICCVAM review and should first be released as a draft for public comment. The final criteria should be established **prior** to implementing such assays into the NTP's routine testing program.

We appreciate your consideration of these suggestions. If you or NTP staff have any questions, please contact Richard A. Becker, Ph.D. at 703/741-5210 or **Rick_Becker@AmericanChemistry.com**

Sincerely

Original Signed By

Sarah H. Brozena
Acting Co-Leader and Counsel
Public Health Team

cc: Carol J. Henry, Ph.D.