NTP Workshop on Transgenics

On February 21, 2003, as part of an ongoing process to evaluate the utility of genetically modified animal models, the NTP hosted a workshop, "Genetically Modified Rodent Models for Cancer Hazard Identification: Selecting Substances for Study and Interpreting and Communicating Results" in Washington, DC. Approximately 100 persons with both national and international representation attended, including invited staff from NTP-participating regulatory and science agencies, members of various NTP and NIEHS external advisory committees, representatives from animal welfare groups, foreign governments, and the pharmaceutical, chemical, and academic communities. Many of these persons have participated in other efforts to evaluate the utility of genetically modified mouse models (Robinson and MacDonald, 2001).

The goal of this workshop was two-fold:

1) To solicit comment on a proposed process for selection of appropriate nominated substances to undergo cancer hazard evaluation in genetically modified or "transgenic" models
2) To solicit comment on issues related to the proper interpretation of results from genetically altered mouse cancer models, the implications of these findings for public health decisions, and the most appropriate interpretive language to describe the results of such studies to the scientific/regulatory communities and the public.

The workshop opened with plenary talks outlining the current understanding of biology of the tumor responses to carcinogens exhibited by three commonly used genetically modified mouse (GMM) models - Tg.AC, the p 53+/- and the Hras2, followed by a short history of the use of GMM models within the NIEHS and specifically in the NTP cancer bioassay program. The workshop attendees then split into two breakout groups to consider the issues posed above and ultimately reconvened to discuss the separate deliberations in an afternoon plenary session.

Since the early 1990s, scientists at the NIEHS/NTP have been working to develop rodent cancer screening models using GMM models. The NTP has conducted a number of assays on chemicals for which no long-term cancer bioassays exist using two widely studied models, the Tg.AC and p53 (+/-).

The workshop attendees commented favorably on a proposal to actively solicit nominations of substances to be studied for carcinogenic potential in GMM models during all phases of the NTP nomination review process (http://ntp-server.niehs.nih.gov/NomPage/noms.html). They recommended that the NTP gather as much information as possible about the rationale for a study early in the process to allow adequate evaluation of the request. They agreed that the NTP staff scientist and the study design teams should have responsibility for selecting the appropriate model(s) and designing the study protocols to be used.

Both breakout groups addressed the issues for the second goal through consideration of hypothetical case studies. These case studies were examples in the p53 +/-, the Tg.AC, and the Hras2 models of tumor responses of varying strengths, from none to strong, and of dose-related increases in benign and malignant tumors. The examples were designed to stimulate discussions that might reveal the current level of acceptance of these models for cancer hazard identification. The NTP also hoped to gain input about what types/level of pathologic response in the GMM models would be needed in order to apply the same categories for defining strength of evidence to the results as are currently used for 2-year bioassays using traditional rodent models.

Initial discussions revealed that neither breakout group willingly accepted the premise that results of studies with GMM models are equivalent to the results from a traditional 2-year rodent assay. Because these models possess oncogenes or disabled tumor suppressor functions, there was reluctance to accept that a positive outcome indicated that the responsible chemical is a "carcinogen." Suggestions on how to convey this lowered state of confidence took several directions. One breakout group described positive findings with the p53+/- and the Hras2 models as indicating a "neoplastic" or "tumorigenic" response, although the slight majority favored use of the term "carcinogenic activity." This latter term is currently used to characterize a positive tumor finding in the 2-year NTP rodent bioassay. The other breakout group also preferred "carcinogenic activity" to "neoplastic response," but placed a condition on the use of this term. This group requested that a preamble statement be added to reports of studies with GMM models indicating that the results should not be accorded the same weight of evidence as a standard 2-year rodent cancer bioassay.

When considering the best terminology to describe the strength of response in the GMM models, the majority of the workshop participants accepted the terminology used in the 2-year bioassay, i.e., "clear evidence," "some evidence," "equivocal evidence," or "no evidence." There was a suggestion that a tumorigenic response sufficient to achieve a call of "clear evidence" in a 2-year study, might only deserve a call of "some evidence" in a GMM model because of concerns outlined earlier; however, this suggestion did not receive widespread support.

Both breakout groups struggled to find appropriate language to describe findings with the Tg.AC model. The primary tumorigenic endpoint in this model is papilloma development in the skin, and its assay developers have frequently termed Tg.AC a "reporter phenotype" (Tennant *et al.*, 2001). Papillomas arise

transgene that appears to be inserted in an inducible form in skin, forestomach and bone marrow. In contrast to the opinions for p53+/- and rasH2, the majority in both breakout groups was uncomfortable with the term "carcinogenicity activity," for describing the model's response, even when the observed response is malignant skin neoplasms. A minority felt that "carcinogenic activity" is an appropriate descriptor in this case. Suggestions for how to describe a positive papilloma response ranged from "tumor promoter response" or "neoplastic response," to the very nonspecific "biological activity," reflecting the opinion that activation of the zeta-globin gene, whether a discriminator for carcinogens or not, cannot be characterized as a cancer response.

One of the major topics of discussion at the International Life Sciences Institute/HESI workshop the previous day concerned proposals to alter the designs of the assays with GMM models to improve their sensitivity to detect carcinogens. Pritchard *et al.* (2003) raised concerns about this issue and showed that failures of the GMM models to provide a correct classification of substances that are *known* or *reasonably anticipated to be human carcinogens* are more likely to stem from false negatives rather than false positives. Because of lingering doubts about the true meaning of negative results in these models, some of the case studies examined by the breakout groups included situations where no tumor response occurred. The NTP included these cases to determine whether the attendees felt that the results would be best described as showing "no evidence" of a tumor response under the conditions of the study, or as studies that should be considered "inadequate" to demonstrate a lack of "carcinogenic activity." T breakout groups showed little support for calling studies with negative findings in GMM models "inadequate" studies, rather the attendees seemed comfortable with the call of "no evidence" of carcinogenic activity/ neoplastic response/biological activity, as the case may be, as long as the study duration is clearly stated and the conclusion clearly reflects the assay conditions.

This workshop was the first of a series that the NTP plans to hold dealing with various issues related to the appropriate use of GMM models in cancer hazard identification and risk estimation. A topic on the immediate horizon is how the results from GMM models should be used in listing substances as *known* or *reasonably anticipated human carcinogens* in the NTP Report on Carcinogens.

References

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Tennant RW, Stasiewicz S, Eastin, WC, Mennear, J Spalding, JW. 2001. The Tg.AC (v-Ha-ras) transgenic mouse: Nature of the model. Toxicol. Pathol. 29 (suppl 1) 51-59.