

Transgenic Mouse Models: Their Role in Carcinogen Identification

**John B. Pritchard, John E. French, Barbara J. Davis, and
Joseph K. Haseman**

doi:10.1289/ehp.5778 (available at <http://dx.doi.org/>)

Online 30 October 2002



Transgenic Mouse Models: Their Role in Carcinogen Identification

John B. Pritchard¹, John E. French², Barbara J. Davis³, and Joseph K. Haseman⁴

Laboratory of Pharmacology and Chemistry¹ and Laboratory of Experimental Carcinogenesis
and Mutagenesis², Environmental Toxicology Program

Laboratory of Women's Health³, and the Biostatistics Branch⁴,
Environmental Biology and Medicine Program

**Division of Intramural Research
National Institute of Environmental Health Sciences
Research Triangle Park, NC 27709**

Address Correspondence to:

Dr. John B. Pritchard
Laboratory of Pharmacology and Chemistry
National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, NC, 27709

Phone: (919) 541-4054
Fax: (919) 541-5737
pritchard@niehs.nih.gov

Running Head: Transgenic Models**Abbreviations (Text):**

NTP	=	National Toxicology Program
MAPKK	=	mitogen-activated protein kinase kinase
Trp53+/-	=	Trp53 heterozygous null allele (+/-) mouse
Tg.AC	=	Tg.AC (<i>v-Ha-ras</i>) mouse
<i>rasH2</i>	=	hemizygous for the human <i>c-Ha-ras</i> transgene
ILSI	=	International Life Sciences Institute
ROC	=	Report on Carcinogens
IARC	=	International Agency for Research on Cancer

Abbreviations (Tables):

+	=	positive
-	=	negative
±	=	equivocal
f	=	feed
g	=	gavage
d	=	dermal
ip	=	intraperitoneal injection
i	=	inhalation
sc	=	subcutaneous
wb	=	whole body routes of exposure
nt	=	not tested or no published record
sal	=	<i>salmonella</i> mutagenicity assay
mn	=	<i>in vivo</i> micronuclei genotoxicity assay

Keywords:

carcinogen
 hazard
 identification
 human
 mouse
 model
 mutagenic
 non-mutagenic
 transgenic

Abstract:

This report examines existing data on the use of transgenic mouse models for identification of human carcinogens. It focuses on the three most extensively studied of these mice – Trp53+/-, Tg.AC, and RasH2 – and compares their performance with the traditional 2-year rodent bioassay. Data on a total of 99 chemicals were evaluated. Using the IARC/ROC calls for the carcinogenicity of these chemicals to humans as the standard for comparison, a variety of potential testing strategies were evaluated ranging from individual transgenic models to combinations of these 3 models with each other and with traditional rodent assays. The individual transgenic models made the “correct” calls (positive for carcinogens; negative for noncarcinogens) for 77-81% of the chemicals, with an increase to as much as 88 % using combined strategies (e.g., Trp53+/- for genotoxic chemicals and RasH2 for all chemicals). For comparison, identical analysis of chemicals in this data set that were tested in the 2-year, 2-species rodent bioassay yielded “correct” calls for 69 % of the chemicals. Although the transgenic models had a high percentage of correct calls, they did miss a number of known or probable human carcinogens; whereas, the bioassay missed none of these chemicals. Therefore, “mixed” strategies using transgenic models and the rat bioassay were also evaluated. These strategies yielded ~85 % correct calls, missed no carcinogens, and cut the number of positive calls for human non-carcinogens in half. Overall, the transgenic models performed well, but important issues of validation and standardization need further attention to permit their regulatory acceptance and use in human risk assessment.

Introduction:

The National Toxicology Program (NTP) is charged with the responsibility for evaluating the toxicity and carcinogenicity of environmental agents, developing and validating improved testing methods, and strengthening the science base in toxicology. A variety of endpoints are used to assess the systemic toxicity of environmental chemicals, but the mainstay of the chemical carcinogenesis effort has been the 2-year rodent bioassay. This highly standardized method has been widely adopted throughout the world. However, like any other approach it has its strengths and weaknesses. In particular, the 2-year assay is expensive, both in resources and time required and in the numbers of animals needed. Thus, the advent of transgenic and gene knockout technology in the early 1980's and increasing knowledge of the mechanisms involved in carcinogenesis, led a number of investigators to examine whether faster, less costly, and more predictive models might be developed. NIEHS has been actively involved in this effort for more than a decade and several model systems utilizing transgenic and knockout models have been investigated (Bucher 1998;Eastin, et al. 1998;Tennant 1993;Tennant, et al. 1995).

Transgenic models have a number of potential advantages for use in carcinogen identification programs. For example, because tumors arise more quickly in the genetically engineered models, the assays can be more rapid. For the studies reviewed here, the assay length was 24-26 weeks, significantly shorter than the standard 2-year rodent bioassay. Transgenic models may also provide the opportunity to reduce animal numbers used in testing. Shorter assays using fewer animals could also reduce the overall cost of testing programs. However, proprietary issues and the limited availability of some models may impact cost savings. Furthermore, with appropriate model selection, it may become possible to more accurately predict the human response, contributing directly to the ease and effectiveness of risk assessment and regulatory decisions.

Finally, by virtue of the specific genetic modification(s) in transgenic models, it should be possible to gain additional insights into the mechanisms involved in tumor induction and development. Such insights would facilitate identification of important mechanisms participating in the tumor response and chemical features associated with carcinogenesis.

Although they have great promise, transgenic models also have actual or potential limitations for use in a carcinogen identification effort. For example, many current transgenic models (including those evaluated here) have mutations in only one pathway that may, or may not, be relevant to human cancer processes for a given chemical. In addition, the specific gene defect may influence tumor development and type, increasing the difficulty of modeling the human response.

Likewise, the strain (genetic) background can influence tumor type, incidence, and location.

Thus, short-term, gene-specific transgenic assays may lose biological information obtained in longer-term bioassays, *e.g.*, multiple target organ effects and/or interactions of time and age that are important in chemical carcinogenicity. These issues do not preclude the use of transgenic models, but they must certainly be considered in their development and selection, and in interpretation of data obtained using transgenic models.

Given the potential and the limitations of the transgenic models, the goals of the current assessment are to (1) review progress in this field of research, (2) determine if the models reviewed show sufficient merit for use in a carcinogen identification program, and (3) identify research needs and knowledge gaps that should be addressed to increase the effectiveness of transgenic models.

Review of Research Progress:

Many transgenic models are available for various investigational uses. However, three transgenic models have been most widely used for carcinogen identification: Trp53+/-, Tg.AC, and RasH2. These three models were selected for this assessment because they have the extensive data set needed for this analysis. Their selection does not indicate that they are deemed superior *a priori* to other transgenic models.

Extensive recent reviews of these three models have been published (17-24) and only their main features are briefly reviewed here. They were developed based on dysregulation of either the Trp53 tumor suppressor gene or the *ras*-protooncogene, both of which are critical to cancer development and represent the two main classes of human cancer genes. The p53 protein suppresses cancer in humans and rodents and is mutated or dysfunctional in more than 50 % of all cancers (Donehower, et al. 1992;Hollstein, et al. 1991;Weinberg 1991a). As a transcription factor, p53 regulates the activity of a variety of genes involved in cell cycle arrest, apoptosis, anti-angiogenesis, differentiation, DNA repair, and genomic stability (el-Deiry 1998;Prives and Hall 1999). The *ras* protooncogene protein (H-, K, and N-*ras* isoforms) is integral to cell proliferation through signaling by growth factors and noxious agents (chemicals, UV radiation, etc.) that act via the mitogen-activated protein kinase kinase (MAPKK) pathway (Campbell, et al. 1998;Gupta, et al. 2000;Pruitt and Der 2001). Activation and dysregulation of *ras* through mutations at specific sites within the gene are often observed in both human and rodent cancers (Bos 1989;Hruban, et al. 1993;Vogelstein, et al. 1990;Yunis, et al. 1989). In addition, increased expression of oncogenic *ras* protein is often seen during tumorigenesis by aneuploidy of the *ras* bearing chromosomes, which may be analogous to over-expression of induced transgenic *ras* protein. Overall, *ras* is over-expressed in well over 50 % of all cancers.

The *Trp53* heterozygous null allele (+/-) mouse: This model uses B6129 N5 mice heterozygous for a wild type *Trp53* tumor suppressor gene and a null allele that is not transcribed or translated (Donehower, et al. 1992;Harvey, et al. 1993). These *Trp53* heterozygotes (+/-) have a low spontaneous tumor incidence up to 9 months of age, but have increased spontaneous tumor rates thereafter with approximately 50 % survival at 18 months. Exposure to positive control and test agents between 7 and 33 weeks of age is relatively free of the development of spontaneous tumors, thus allowing a clear distinction between induced and sporadically occurring tumors that may confound long term chronic cancer bioassays (Haseman and Elwell 1996;Karstadt and Haseman 1997). It appears to be particularly useful as an *in vivo* test for mutagenic carcinogens (Donehower, et al. 1992;Eastin, et al. 1998;Harvey, et al. 1993;Kemp, et al. 1993;Kemp, et al. 1994;Tennant, et al. 1995). In human cancers, where mutations have been found in up to 50 % of all tumors (Greenblatt, et al. 1994;Hollstein, et al. 1991), point mutations or deletions in one allele of the *Trp53* gene that create a heterozygous allelic state are usually accompanied by loss of the normal allele (loss of heterozygosity or LOH) (Weinberg 1991b). Since *Trp53* +/- mice only carry one copy (germ line) of the gene, these mice were expected, according to the Knudson *et al.* two-hit hypothesis (Knudson 1996;Knudson, et al. 1975), to show a shorter latency period for tumors induced by genotoxic agents. However, there is evidence that the acceleration of tumorigenesis in *Trp53* +/- mice may be due to a gene dosage effect and a haploinsufficient phenotype such that a second (p53 LOH) event is not required (French, et al. 2001;Venkatachalam, et al. 1998).

The Tg.AC (*v-Ha-ras*) mouse: The Tg.AC transgenic mouse model provides a reporter phenotype (skin papillomas) in response to either genotoxic or non-genotoxic carcinogens, including tumor promoters (Spalding, et al. 1999;Spalding, et al. 1993;Tennant, et al. 1999).

Tg.AC mice are hemizygous for a mutant *v-Ha-ras* transgene. The model was developed by Leder et al. (Leder, et al. 1990), with an inducible β -globin promoter driving the expression of a mutated *v-Ha-ras* oncogene and is regarded as a genetically initiated model. With the exception of the bone marrow, constitutive expression of the transgene cannot be detected in adult tissues. The transgene is transcriptionally silent until activated by full-thickness wounding, UV irradiation, or specific chemical exposure (Cannon, et al. 1997; Trempus, et al. 1998). Topical application of carcinogens to the shaved dorsal surface of Tg.AC mice induces epidermal squamous cell papillomas or carcinomas, a reporter phenotype that defines the activity of the chemical. The oral route of administration can also generate tumor responses in the skin of Tg.AC mice and in addition lead to squamous cell papillomas and/or carcinomas of the forestomach. To date, the appearance of either spontaneous or induced tumors has been shown to require activation of transgene expression. However, the mechanism of response by the Tg.AC model to chemical carcinogens is not yet understood.

The rasH2 mouse: The rasH2 mouse is hemizygous for the human *c-Ha-ras* transgene under control of its endogenous promoter and enhancer sequences. It was developed by Saitoh *et al.* (Saitoh, et al. 1990) in CB6F1 mice to evaluate the association of chemically induced transgene expression and tumor induction (Katsuki, et al. 1991; Yamamoto, et al. 1996; Yamamoto, et al. 1998a). The transgene encodes a prototype c-H-ras gene product, p21 that does not induce transformation in NIH3T3 cells. Approximately 3 copies of the human transgene were integrated into the mouse genome in a tandem array through pronuclear injection (Suemizu, et al. 2002). Expression of the transgenic protein is observed in normal tissues and increased approximately 2-fold in chemically induced tumors (Maruyama, et al. 2001). Mutation of the endogenous mouse ras genes or of the transgene is infrequent and unpredictable (Katsuki, et al.

1991); suggesting that a 2-3-fold increase in *ras* protein expression is sufficient to cooperate with other carcinogen-induced changes (genetic and/or epigenetic) to predispose this mouse to development of neoplasia.

Merits of the Models:

Data Collection – To assess the potential merit of the three transgenic models in a research and testing program, we assembled available information on responses to chemical treatment in each model (Tables 1-3). The primary sources of these data were the recent publications of the International Life Sciences Institute (ILSI) Assay Working Groups for the Trp53^{+/-}, Tg.AC, and RasH2 Mouse Alternative Models (Popp 2001; Robinson and MacDonald 2001), NTP evaluations, and published independent laboratory research using alternative or conventional rodent models for carcinogen identification (For specific references see Tables 1-3). The resulting data set consists of 99 chemicals that were tested at the maximum tolerated dose (MTD) or proportional fractions of MTD as determined by toxicokinetic and range finding studies in the test strain using positive and negative controls groups and non-genetically altered coisogenic reference controls. In reviewing this literature, it was apparent that dosing routes, study duration, number of animals per group, and extent of histopathologic evaluation varied between studies and chemicals. Despite these limitations, for the purposes of this analysis, peer-reviewed published findings were accepted as reported.

Criteria for Analysis – Because the goal of the NTP carcinogenicity testing is prediction of human carcinogenicity of chemicals, the merit of the transgenic models was evaluated by determining their ability to identify human carcinogens. Classification of human carcinogens was based on evaluations by the NTP Report on Carcinogens (ROC) and the International Agency for

Research on Cancer (IARC) chemical evaluations/classifications. Both the NTP and IARC assessments are based on comprehensive evaluations of all relevant human and animal data from the published literature. The designation of an agent as a “known human carcinogen” by the IARC (Group 1) or the NTP ROC requires definitive data from human epidemiological studies, or strong mechanistic data from human systems in conjunction with similar mechanistic and cancer data from experimental animals. Less convincing evidence (*e.g.*, limited human data and/or sufficient animal data) will generally lead to the designation of the agent as a “probable (Group 2A) or “possible” (Group 2B) human carcinogen by IARC or a “reasonably anticipated” human carcinogen NTP ROC. A chemical that shows inadequate evidence of carcinogenicity in humans and animals will generally result in an IARC designation of “not classifiable” (Group 3). The NTP ROC has no equivalent and does not list such chemicals. Rodent carcinogenicity was not used as the primary targeted response in our analysis. Nevertheless, for completeness we did consider the correlation of each transgenic model with the outcomes of NCI/NTP long-term rodent tests. We also examined whether these transgenic assays were more, or less, accurate in predicting human carcinogenicity of genotoxic versus non-genotoxic chemicals, as defined by either a positive result in the Salmonella (Ames) test and/or in vivo rodent micronucleus assay.

A total of ninety-nine chemicals have been studied in one or more of these three transgenic models. For this analysis, these chemicals were divided into three groupings: **(i)** Known human carcinogens (IARC Group 1 and/or NTP ROC “known” – 14 chemicals, Table 1); **(ii)** Probable/Possible human carcinogens (IARC Groups 2A and 2B or NTP ROC “reasonably anticipated” – 32 chemicals, Table 2); and **(iii)** Chemicals with inadequate evidence of carcinogenicity (IARC Group 3, NTP bioassay negative, and/or not listed by ROC or IARC – 53 chemicals, Table 3).

Tables 1-3 identify each chemical by CAS number and give the IARC and/or the NTP ROC evaluations. For those chemicals evaluated in the NTP rodent bioassay, carcinogenicity results are given for each sex-species group (male rats, female rats, male mice, female mice).

Genotoxicity outcomes from the Salmonella (Ames) assay and the *in vivo* micronuclei assays are also given. Finally, the results of carcinogenicity testing in each of the three transgenic models are given. The route of administration is noted, as well as the published reference source. For chemicals tested more than once in the transgenic models, each result is given separately.

For each of the transgenic models and for the rodent bioassay, a chemical is designated as a carcinogen if positive (carcinogenic) effects were found in one or more of the sex-species groups. Similarly, a chemical found to be positive in either the Salmonella assay or the *in vivo* micronuclei assay is considered to be genotoxic.

Analysis of the Models -- Based on the 99 chemical database from Tables 1-3, ten possible strategies were considered for using transgenic models to identify chemicals as known or suspected human carcinogens or as noncarcinogens. For comparison, the standard two-year, two-species rodent bioassay and a modified strategy using the rat bioassay in conjunction with genotoxicity were also analyzed in an identical fashion. Thus, twelve strategies in all were considered. They are:

Strategy 1: Trp53+/- model

Strategy 2: Trp53+/- model, but only for genotoxic chemicals

Strategy 3: Tg.AC model

Strategy 4: RasH2 model

Strategy 5: Trp53+/- model for genotoxic chemicals; RasH2 model for nongenotoxic chemicals

- Strategy 6: Trp53+/- model for genotoxic chemicals; RasH2 model for all chemicals
- Strategy 7: Trp53+/- model for genotoxic chemicals; Tg.AC model for nongenotoxic chemicals
- Strategy 8: Trp53+/- model for genotoxic chemicals; Tg.AC model for all chemicals
- Strategy 9: NTP Bioassay
- Strategy 10: NTP Rat Bioassay plus the Tg.AC model for nongenotoxic chemicals or the Trp53+/- model for genotoxic chemicals
- Strategy 11: NTP Rat Bioassay plus the RasH2 model for nongenotoxic chemicals or the Trp53+/- model for genotoxic chemicals
- Strategy 12: NTP Rat Bioassay plus genotoxicity

When evaluating strategies that were conditional on genotoxicity (Strategies 5-8, 10-11), the following conventions were established: (i) a chemical was considered genotoxic if either the Salmonella or in vivo micronuclei assays were positive; (ii) a chemical was considered non-genotoxic only if both assays were negative; and (iii) when a chemical's genotoxicity could not be determined definitively (*i.e.*, negative in one assay and not tested in the other), the chemical was excluded from the analysis, unless the genotoxicity status of the chemical had no impact on the transgenic mouse result (*i.e.*, both transgenic models were positive or both were negative).

A valid transgenic rodent model should successfully identify (test positive) the IARC/NTP known or suspected human carcinogens listed in Tables 1 and 2. Likewise, such a model should identify as noncarcinogens (test negative) those chemicals in Table 3 that were shown in NTP long-term bioassays to be negative. While many of the remaining chemicals in Table 3 were positive in a long term rodent bioassay, these results were not considered by the IARC and/or NTP ROC to be sufficiently convincing to merit the categorization of the chemical as a known, possible, probable, or reasonably-anticipated human carcinogen. For these chemicals, it is

uncertain if the response of the transgenic models should be positive or negative as carcinogens. Thus, our initial analysis (Table 4) included only those Group 3 chemicals with negative results in the NTP rodent bioassay. Table 5 examines the same data set as Table 4, but considers each IARC/ROC classification separately to insure that pooling carcinogen groups in these analyses did not lose important distinctions between assay responses to strong or weak carcinogens.

In addition, as summarized in Table 6, we have conducted a second analysis in which all chemicals in Table 3 are regarded as human noncarcinogens, *i.e.*, we have assumed, for the sake of direct comparison between transgenic and traditional NTP bioassays, that more extensive testing of these chemicals would confirm their lack of human carcinogenicity. This assumption permits exactly the same criteria to be applied to all strategies, transgenic and traditional alike. Finally, although human carcinogenicity was used as the targeted response in our analysis, a similar analysis was conducted in which the transgenic assay responses were compared with the results of the NTP bioassay (Table 7).

Results and Discussion:

Scope of analysis — Before discussing the analysis itself, it is critical to reiterate the precise limitations and assumptions implicit in our analysis. First, this evaluation was limited to those chemicals with definitive published transgenic results available at the time of our analysis. We recognize that this is a dynamic field of research. Thus, additional transgenic studies will become available over time, and it is possible that some chemicals listed in Tables 1-3 could be reclassified after consideration of such new data. However, we suggest that the analyses for these 99 chemicals are sufficiently robust that the addition, subtraction, and/or re-assignment of chemicals will not alter the conclusions, provided that uniform criteria are applied.

Second, optimal protocol designs for specific transgenic animal cancer bioassays have not been identified and validated. Thus, the study designs that form the basis of this evaluation may differ from each other with regard to study duration, sample sizes, dose selection strategy, number of doses, tissues examined, methods of statistical analysis, historical controls, and the use of positive and negative controls.

Third, we made no interpretative decisions ourselves in regard to study results. For assessments of possible human cancer risk, we relied upon the authoritative judgments of the IARC and the NTP Report on Carcinogens. Likewise, we accepted the study authors' interpretations of the data. However, there was uniformity of study design and interpretation for a sizable number of the studies involved in the ILSI Alternatives to Carcinogenicity consortium. It was beyond the scope of this research analysis to reevaluate and reinterpret each individual study.

Fourth, we recognize and acknowledge that a "positive" transgenic study may reflect a wide range of carcinogenic responses, with some positive results being limited to a marginal increase in a single tumor type in a single sex-species group, while others reflect striking multi-site, multi-sex, carcinogenic effects. While future refinements in statistical evaluation may permit sub-classification and rank order documentation for the various "positive" transgenic responses, we have not attempted to do so at this stage in the development of transgenic rodent bioassays.

Finally, we recognize that certain chemicals listed in Table 3 may ultimately be shown to be "known" or "suspected" human carcinogens, especially those with positive rodent bioassay results. However, our current state of knowledge does not permit a higher classification of these chemicals. As noted below, the frequency of positive transgenic results for Table 3 chemicals was essentially the same for those chemicals that were evaluated by the IARC (and assigned to Category 3) and those that were not yet evaluated and are thus unclassified. This suggests that there are few, if any, important human carcinogens among the "unclassified" chemicals in Table 3.

Performance of strategies _ The overall performance of each transgenic strategy is summarized in Table 4. With the caveat that data on all chemicals were not available for each model and thus, that the subset of chemicals actually tested in each model may influence the specific outcomes reported, each of the three transgenic mouse models predicted human carcinogenesis for 77-81 % of the chemicals studied in that model, ranging from 77 % for the Trp53+/-, 78 % for the Tg.AC, and 81 % for the RasH2. Use of the Trp53+/- for only genotoxic chemicals increased its predictiveness to 84 %. The combined strategies that use more than one transgenic model (Strategies 5-8; as defined above) were somewhat more predictive, ranging from 78-88 %.

The best strategy (Trp53+/- for genotoxic chemicals and RasH2 for all chemicals; Strategy 6) correctly predicted the human outcome for 88 % of the agents (Table 4). Strategy 8 (Trp53+/- for genotoxic chemicals and Tg.AC for all chemicals) was only slightly less predictive (85 %).

Our initial analysis (Table 4) defined the targeted population of “human carcinogens” as the pool of chemicals from Tables 1 and 2, in which IARC classifications ranged from 1 to 2B. A further breakdown of these chemicals is given in Table 5. Note that **(i)** the transgenic models (considered collectively) are more apt to be positive for the more certain human carcinogens (IARC Categories 1 and 2A) than for the less certain human carcinogens (Category 2B); **(ii)** there is a striking difference in the proportion of positive transgenic responses between the 1/2A/2B chemicals and the Category 3 chemicals or those not evaluated; and **(iii)** the IARC Category 3 chemicals and those not evaluated show a similar rate of overall transgenic responses – indicating that most of the unclassified chemicals listed in Table 3 may be human noncarcinogens.

Our initial analysis (Table 4) was somewhat restrictive, in that it defined human noncarcinogens as being only those chemicals from Table 3 with negative NCI/NTP rodent bioassay results. However, Table 5 suggests that it is reasonable to expand this classification and regard all Table 3 chemicals as human noncarcinogens. This analysis is summarized in Table 6, which allows more direct comparison of the performance of the transgenic models with the traditional NTP two-species bioassay, transgenic and traditional testing strategies each show strengths and weakness. Importantly, these strengths and weaknesses differ. For the transgenic models, particularly the RasH2 and the Trp53+/-, there are relatively few positive findings for noncarcinogens (*i.e.*, Group 3 chemicals, either known negatives or chemicals unlisted by

IARC/ROC, that gave evidence of carcinogenicity in the assay). In fact, as shown in Table 4, RasH2 and Trp53+/- have no positive results for noncarcinogens if those Group 3 chemicals that lack a negative rat and mouse bioassay are eliminated from the analysis (in effect, eliminating those chemicals with greater uncertainty as to their carcinogenic potential). The Tg.AC model was more prone to this type of error than the other two transgenic models reviewed (Tables 4 and 6). The combined transgenic strategies (Strategies 5-8) did not improve predictability.

A more frequent shortcoming of the transgenic models (including, those strategies using multiple transgenic models) was the number of negative tests for known or suspected human carcinogens, *i.e.*, those listed in Tables 1 and 2 (Tables 4 and 6). For example, even the most predictive combination (the combined results of Trp53+/- for genotoxic chemicals plus Tg.AC for nongenotoxic chemicals; Strategy 7) still had 6 negative results for IARC/NTP known carcinogens among the total of 49 chemicals tested in both (Table 6).

In contrast, the NTP two-species bioassay identified all IARC/NTP known/probable human carcinogens (Tables 1 and 2). Thus, as shown in Table 6 (Strategy 9), among the 58 chemicals evaluated in the NTP bioassay, there were no negative results for known human carcinogens. However, this is not without a downside in the form of numerous positive findings for chemicals that are considered to be noncarcinogens in humans (Table 3). In this data set, there were 18 positive assay results for IARC/ROC noncarcinogens among a total of 58 chemicals tested, or 31 % (Table 6). Certainly, there is a cost of this type of error as well, specifically unneeded regulation and/or additional testing. It is this propensity for positive findings for chemicals considered to be human noncarcinogens that yielded the surprisingly low 69 % concordance between the standard NTP bioassay and human cancer – surprising because many of the ROC

and IARC calls are based in large part on animal data and the NTP bioassay in particular. In fact, all three transgenic models had a modestly higher concordance with human carcinogens (Tables 1 and 2) than the rodent 2-year bioassay (Trp53+/- 81 %, RasH2 75 %, and Tg.AC 74 %; Table 6). Of course, this difference is also reflected in the modest success (54-78 %) of the transgenic models as predictors of the bioassay response (Table 7).

It should be emphasized that it is possible that many of the 18 NTP rodent carcinogens labeled in our analysis as “Positive for Noncarcinogens” (Table 6, Strategy 9) may ultimately prove to be actual human carcinogens, as additional data becomes available. However, at this time the positive rodent data are not sufficiently compelling for the IARC or the NTP ROC to consider these chemicals to be known, probable, possible, or reasonably anticipated human carcinogens. In those rare cases where the IARC and ROC disagreed (*e.g.*, DEHP) we used the most recent call. Moreover, these 18 chemicals collectively were positive in only 27 % (8/30) of the three transgenic assays evaluated, as compared with 66 % (29/44) positive transgenic assays conducted on the 24 known/probable carcinogens. This difference strongly suggests that the transgenic assays are selectively identifying the trans-species carcinogens.

Since both transgenic models and the bioassay have strengths and weakness in correctly identifying carcinogenic chemicals, we examined the performance of composite strategies using both transgenic and conventional rodent models to determine if such a strategy might capitalize on the strengths of both types of models. Strategies 10 and 11 address this possibility (Table 6). Strategy 10 (rat bioassay for all chemicals plus the Trp53+/- model for genotoxic agents or the Tg.AC for non-genotoxic chemicals) provided an improvement in performance. Overall concordance increased to 84 % versus the 69 % of the bioassay itself. More importantly,

negative results for known carcinogens were completely eliminated and positive findings for noncarcinogens were reduced to 16 % (9/57) versus the 31 % (18/58) for the bioassay. A similar strategy (Strategy 11) substituting RasH2 for Tg.AC gave very similar results, with an overall concordance of 85 % (44/52), or just 15 % (8/52) with positive results for noncarcinogens.

For those chemicals evaluated in both the NTP bioassay and the transgenic models, the substitution of the transgenic models (Strategy 10: Trp53+/- for genotoxic chemicals; the Tg.AC for non-genotoxic chemicals) for the B6C3F1 mouse used in the standard bioassay resulted in a net reduction of four positive findings. Four chemicals (coconut oil diethanolamine, diethanolamine, N-methyloacrylamide and methylphenidate) were negative in the transgenic models and the NTP rat bioassay. In the B6C3F1 mouse, the first two of these chemicals produced liver tumors (both sexes) and kidney adenoma (males only). N-methyloacrylamide produced tumors of the Harderian gland, liver, lung, and ovary. Methylphenidate produced liver tumors only. None of these chemicals has been classified as a known/probable human carcinogen by the IARC or the NTP ROC (Tables 1-3).

Historically, genotoxicity has proven to be an important clue as to the likely carcinogenesis of chemicals (Ashby and Tennant 1991;Shelby 1988). In addition, as shown in Table 4, it increases the predictiveness of Trp53+/- model. Thus, to provide a more complete assessment of possible testing strategies, we compared an additional strategy (#12, Table 6) that consists of substitution of genotoxicity data for the transgenic models to be used in concert with the rat bioassay (Strategies 10 and 11, Table 6). Strategy 12 does, like the bioassay itself, avoid negative results for known carcinogens. It also has modest concordance with human carcinogenesis 67 % (44 of

66), but it has 22 positive results for noncarcinogens out of 66 chemicals (33 %). A number of the other strategies do better.

Conclusions _ Given the complementary strengths demonstrated by the transgenic models and the 2-year rodent bioassay as presented above and summarized in Table 6, it appears that a strategy employing both types of models would have advantages over either alone. Thus, Strategies 10 and 11 that employ the standard rat bioassay in conjunction with Trp53+/- for genotoxic chemicals and Tg.AC or RasH2 for non-genotoxic chemicals are promising, based on their performance with these 99 chemicals.

Research Needs:

This analysis demonstrates that transgenic models have the potential to play an important role in identification of potential human carcinogens. However, several research needs and data gaps remain to be addressed to insure that the use of transgenic models has been adequately evaluated and that protocols have been optimized or standardized for such use, critical requirements for the regulatory acceptance of transgenic model data and its use in human risk assessment.

Validation of study design – The study design for each transgenic model must be rigorously evaluated and optimized for the testing paradigm employed (*e.g.*, toxicity, mutagenicity, or carcinogenicity). Therefore, additional research will be required for each model evaluated and used in the NTP testing program. As mentioned previously, the testing strategies, animal numbers, duration of dosing, extent of pathology and interpretation of results varied among the studies evaluated. In particular, an optimal design for transgenic models has not yet been identified that clearly eliminates the potential for false negatives in carcinogen identification. Two possible strategies for increasing the power of the study (thereby reducing the negative results for known human carcinogens) are to increase the sample size beyond the 15 animals per group commonly used and/or to increase the duration of the study to allow more time for tumors to develop. The performance of the transgenics under these different conditions should be thoroughly investigated and standardized. A perhaps less obvious possibility would be to compile a rigorous historical control database for the various transgenic models and to make use of this information in "weight-of evidence" decisions. Many of the tissues in the transgenic mouse models have a low spontaneous tumor incidence. Thus, the occurrence of two or three of these tumors in a dosed group in a given study, although perhaps not statistically significant when tested against the concurrent controls, may nevertheless be significant when the low

historical control incidence is taken into account. For example, three of the seven negative results for known/suspected carcinogens associated with the RasH2 model (cyclosporin A, melphalan, and 1,4-dioxane) produced tumor effects that were considered equivocal. Had it been possible to consider these tumor responses in the context of a large historical control database, certain of these borderline cases might have been regarded as biologically significant, thereby reducing the number of incorrect findings.

Improve understanding of chemical outcomes _ One problem in our analysis was in identifying a rational basis to explain discordant results. For example, the most significant shortcoming of a combined (transgenic plus rat bioassay) strategy was not the negative results for known carcinogens, but rather the apparent number of positive chemicals in the rat bioassay that are not listed as known or reasonably anticipated to be human carcinogens (*e.g.*, the 10 of the 59 chemicals for Strategy 11; Table 6). How might this be improved? First, it might be possible to design additional studies to investigate whether or not these are truly noncarcinogenic chemicals. As discussed above, the targeted response in our investigation is imperfect, as it represents a scientific judgment by IARC and/or the NTP ROC regarding potential carcinogenicity based on available data. In many cases, the existing data are insufficient for a definitive decision to be made. Additional research could reduce the number of positive results for supposed noncarcinogens simply by revealing that certain of these chemicals are in fact carcinogens. Other options that might be considered to reduce this type of error include a rat transgenic model (if done in a manner that did not yield negative results for known carcinogens) or improvements in the design of the rat bioassay itself.

Development of chemical database to validate transgenics _ The data set summarized in Tables 1-3 may provide an important resource if appropriate statistical considerations could be developed to allow selection of an informative subset of chemicals for evaluation of new models and/or modification of current protocols. Such a set of chemicals that represents a spectrum of mechanisms or modes of action consistent with human carcinogenesis would not only be valuable in the context of the models discussed above, but would lend themselves to the evaluation and validation of any new model, transgenic or otherwise.

Development of Models The current analysis examined the Trp53^{+/-}, Tg.AC, and RasH2 transgenic models because these models had the most complete data sets available. Other models are also under evaluation at the NIEHS/NTP (p16^{Ink4a} and p19^{Arf} deficient mice) or elsewhere (*XPA-Trp53* deficient mice). A new generation of transgenic models is also currently being developed (Berns 2001), such as one incorporating a point mutation in k-Ras (Johnson, et al. 2001), or models subject to premature aging or having telomere dysfunction (Artandi and DePinho 2000; Rudolph, et al. 2001). If the NTP incorporates transgenic models into routine testing, it must necessarily include a strong research program aimed at developing the transgenic models appropriate for chemical carcinogenesis investigation and identification of carcinogens of the greatest presumptive risk to humans. As our analysis shows, the best strategy for testing may be combining different transgenic models depending on their particular attributes and utility. Thus, the NTP should actively develop such an arsenal of models. Likewise, site specific or mechanism-specific models could be developed and used with great impact in both basic research and carcinogen identification. The NTP could also develop or support research to evaluate transgenic rats or in assessment of possible refinements in the 2-year rat bioassay.

References Cited

- Albert RE, French JE, Maronpot R, Spalding J, Tennant R.1996. Mechanism of skin tumorigenesis by contact sensitizers: the effect of the corticosteroid fluocinolone acetonide on inflammation and tumor induction by 2,4 dinitro-1-fluorobenzene in the skin of the TG.AC (v-Ha-ras) mouse. *Environ Health Perspect* 104:1062-1068.
- Artandi SE, DePinho RA.2000. Mice without telomerase: what can they teach us about human cancer? *Nat Med* 6:852-855.
- Ashby J, Tennant RW.1991. Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP. *Mutat Res* 257:229-306.
- Battershill JM, Fielder RJ.1998. Mouse-specific carcinogens: an assessment of hazard and significance for validation of short-term carcinogenicity bioassays in transgenic mice. *Hum Exp Toxicol* 17:193-205.
- Berns A.2001. Cancer. Improved mouse models. *Nature* 410:1043-1044.
- Blanchard KT, Ball DJ, Holden HE, Furst SM, Stoltz JH, Stoll RE.1998. Dermal carcinogenicity in transgenic mice: relative responsiveness of male and female hemizygous and homozygous Tg.AC mice to 12-O-tetradecanoylphorbol 13-acetate (TPA) and benzene. *Toxicol Pathol* 26:541-547.
- Blanchard KT, Barthel C, French JE, Holden HE, Moretz R, Pack FD, Tennant RW, Stoll RE.1999. Transponder-induced sarcoma in the heterozygous p53^{+/-} mouse. *Toxicol Pathol* 27:519-527.
- Bos JL.1989. ras oncogenes in human cancer: a review. *Cancer Res* 49:4682-4689.
- Bucher JR.1998. Update on national toxicology program (NTP) assays with genetically altered or "transgenic" mice. *Environ Health Perspect* 106:619-621.
- Campbell SL, Khosravi-Far R, Rossman KL, Clark GJ, Der CJ.1998. Increasing complexity of Ras signaling. *Oncogene* 17:1395-1413.
- Cannon RE, Spalding JW, Trempus CS, Szczesniak CJ, Virgil KM, Humble MC, Tennant RW.1997. Kinetics of wound-induced v-Ha-ras transgene expression and papilloma development in transgenic Tg.AC mice. *Mol Carcinog* 20:108-114.
- Carmichael NG, Debruyne EL, Bigot-Lasserre D.2000. The p53 heterozygous knockout mouse as a model for chemical carcinogenesis in vascular tissue. *Environ Health Perspect* 108:61-65.
- Delker D, Yano B, Gollapudi B.1999. Transgene expression in the liver and kidney of Tg.AC mice following tissue injury. *Toxicological Sciences* 50:90-97.

- Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery CAJ, Butel JS, Bradley A.1992. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumors. *Nature* 356:215-221.
- Dunnick JK, Hardisty JF, Herbert RA, Seely JC, Furedi-Machacek EM, Foley JF, Lacks GD, Stasiewicz S, French JE.1997. Phenolphthalein induces thymic lymphomas accompanied by loss of the p53 wild type allele in heterozygous p53-deficient (+/-) mice. *Toxicol Pathol* 25:533-540.
- Eastin WC, Haseman JK, Mahler JF, Bucher JR.1998. The National Toxicology Program evaluation of genetically altered mice as predictive models for identifying carcinogens. *Toxicol Pathol* 26:461-473.
- Eastin WC, Mennear JH, Tennant RW, Stoll RE, Branstetter DG, Bucher JR, McCullough B, Binder RL, Spalding JW, Mahler JF.2001. Tg.AC Genetically Altered Mouse Assay Working Group Overview of Available Data. *Toxicologic Pathology* 29:60-80.
- el-Deiry WS.1998. Regulation of p53 downstream genes. *Semin Cancer Biol* 8:345-357.
- Finch GL, March TH, Hahn FF, Barr EB, Belinsky SA, Hoover MD, Lechner JF, Nikula KJ, Hobbs CH.1998. Carcinogenic responses of transgenic heterozygous p53 knockout mice to inhaled ²³⁹PuO₂ or metallic beryllium. *Toxicol Pathol* 26:484-491.
- French J. Inhalation exposure of p53 deficient and Tg.AC (v-Ha-ras) mice to chloroprene., 2001.
- French JE, Lacks GD, Trempus C, Dunnick JK, Mahler J, Tice RR, Tennant RW.2001. Loss of heterozygosity frequency at the Trp53 locus in p53 heterozygous (+/-) mice is carcinogen and tissue dependent. *Carcinogenesis* 21:101-108.
- Germolic D, Spalding J, Boorman GA, Wilmer J, Yoshida K, Simenova P, Bruccoleri A, Kayama F, Gaido K, Tennant R, Burlison F, Dong W, Lang R, Luster M.1997. Arsenic can mediate skin neoplasia by chronic stimulation of keratinocyte-derived growth factors. *Mutation Research* 386:209-218.
- Greenblatt MS, Bennett WP, Hollstein M, Harris CC.1994. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res* 54:4855-4878.
- Griesemer RA, Cueto J, C. 1980. Toward a classification scheme for degrees of experimental evidence for the carcinogenicity of chemicals for animals. In: *In Molecular And Cellular Aspects Of Carcinogen Screening Tests*, vol IARC Sci. Publ. No. 27 (R. Montesano HB, L. Tomatis, ed). Lyon.
- Gupta S, Plattner R, Der CJ, Stanbridge EJ.2000. Dissection of Ras-dependent signaling pathways controlling aggressive tumor growth of human fibrosarcoma cells: evidence for a potential novel pathway. *Mol Cell Biol* 20:9294-9306.

- Harvey M, McArthur MJ, Montgomery CAJ, Butel JS, Bradley A, Donehower LA.1993. Spontaneous and carcinogen-induced tumorigenesis in p53-deficient mice. *Nat. Genet.* 5:225-229.
- Haseman JK, Elwell MR.1996. Evaluation of false positive and false negative outcomes in NTP long-term rodent carcinogenicity studies. *Risk Anal* 16:813-820.
- Hollstein M, Sidransky D, Vogelstein B, Harris CC.1991. p53 mutations in human cancers. *Science* 253:49-53.
- Hruban RH, van Mansfeld AD, Offerhaus GJ, van Weering DH, Allison DC, Goodman SN, Kensler TW, Bose KK, Cameron JL, Bos JL.1993. K-ras oncogene activation in adenocarcinoma of the human pancreas. A study of 82 carcinomas using a combination of mutant-enriched polymerase chain reaction analysis and allele-specific oligonucleotide hybridization. *Am J Pathol* 143:545-554.
- IARC Agent Page. City: IARC. Available:
http://193.51.164.11/cgi/iHound/Chem/iH_Chem_Frames.html [5 May 2002].
- Jiang W, Ananthaswamy HN, Muller HK, Kripke ML.1999. p53 protects against skin cancer induction by UV-B radiation. *Oncogene* 18:4247-4253.
- Johnson L, Mercer K, Greenbaum D, Bronson RT, Crowley D, Tuveson DA, Jacks T.2001. Somatic activation of the K-ras oncogene causes early onset lung cancer in mice. *Nature* 410:1111-1116.
- Karstadt M, Haseman JK.1997. Effect of discounting certain tumor types/sites on evaluations of carcinogenicity in laboratory animals. *Am J Ind Med* 31:485-494.
- Katsuki M, Ando K, Saitoh A, Doi S, Kimura M, Takahashi R, Hasegawa T, Yokoyama M, Nomura T, Izawa M, et al.1991. Chemically induced tumors in transgenic mice carrying prototype human c-Ha-ras genes. *Princess Takamatsu Symp* 22:249-257.
- Kemp CJ, Donehower LA, Bradley A, Balmain A.1993. Reduction of p53 gene dosage does not increase initiation or promotion but enhances malignant progression of chemically induced skin tumors. *Cell* 74:813-822.
- Kemp CJ, Wheldon T, Balmain A.1994. p53-deficient mice are extremely susceptible to radiation-induced tumorigenesis. *Nature Genetics* 8:66-69.
- Knudson AG.1996. Hereditary cancer: two hits revisited. *J Cancer Res Clin Oncol* 122:135-140.
- Knudson AG, Jr., Hethcote HW, Brown BW.1975. Mutation and childhood cancer: a probabilistic model for the incidence of retinoblastoma. *Proc Natl Acad Sci U S A* 72:5116-5120.
- Koujitani T, Yasuhara K, Usui T, Nomura T, Onodera H, Takagi H, Hirose M, Mitsumori K.2000. Lack of susceptibility of transgenic mice carrying the human c-Ha-ras proto-

oncogene (rasH2 mice) to phenolphthalein in a 6-month carcinogenicity study. *Cancer Lett* 152:211-216.

Leder A, Kuo A, Cardiff RD, Sinn E, Leder P.1990. *v-Ha-ras* transgene abrogates the initiation step in mouse skin tumorigenesis: Effects of phorbol esters and retinoic acid. *Proc. Natl. Acad. Sci. USA* 87:9178-9182.

Maronpot RR, Mitsumori K, Mann P, Takaoka M, Yamamoto S, Usui T, Okamiya H, Nishikawa S, Nomura T.2000. Interlaboratory comparison of the CB6F1-Tg rasH2 rapid carcinogenicity testing model. *Toxicology* 146:149-159.

Marsella JM, Liu BL, Vaslet CA, Kane AB.1997. Susceptibility of p53-deficient mice to induction of mesothelioma by crocidolite asbestos fibers. *Environ Health Perspect* 105:1069-1072.

Martin K, Trempus C, Saulnier M, Kari F, Barrett J, French J.2001. N-acetyl-L-cysteine (NAC) reduces benzo[a]pyrene-induced tumor incidence overall but increases the fraction of malignant skin tumors in *Trp53* haploinsufficient Tg.AC (*v-Ha-ras*) mice. *Carcinogenesis* 22:1373-1378.

Maruyama C, Tomisawa M, Wakana S, Yamazaki H, Kijima H, Suemizu H, Ohnishi Y, Urano K, Hioki K, Usui T, Nakamura M, Tsuchida T, Mitsumori K, Nomura T, Tamaoki N, Ueyama Y.2001. Overexpression of human H-ras transgene is responsible for tumors induced by chemical carcinogens in mice. *Oncol Rep* 8:233-237.

McCormick DL, Ryan BM, Findlay JC, Gauger JR, Johnson TR, Morrissey RL, Boorman GA.1998. Exposure to 60 Hz magnetic fields and risk of lymphoma in PIM transgenic and TSG-p53 (p53 knockout) mice. *Carcinogenesis* 19:1649-1653.

Mitsumori K, Onodera H, Shimo T, Yasuhara K, Takagi H, Koujitani T, Hirose M, Maruyama C, Wakana S.2000. Rapid induction of uterine tumors with p53 point mutations in heterozygous p53-deficient CBA mice given a single intraperitoneal administration of N-ethyl-N-nitrosourea. *Carcinogenesis* 21:1039-1042.

Mori I, Yasuhara K, Hayashi SM, Nonoyama T, Nomura T, Mitsumori K.2000. Carcinogen dose-dependent variation in the transgene mutation spectrum in urethane-induced lung tumors in transgenic mice carrying the human prototype c-Ha-ras gene. *Cancer Lett* 153:199-209.

Nagao M.1999. A new approach to risk estimation of food-borne carcinogens--heterocyclic amines--based on molecular information. *Mutat Res* 431:3-12.

NCI/NTP. 1978a. Bioassay of 1,1,2-Trichloroethane for Possible Carcinogenicity (CAS No. 79-00-5) Technical TR-74. Bethesda, MD: DHHS/NIH/NCI.

NCI/NTP. 1978b. Bioassay of 1,4-Dioxane for Possible Carcinogenicity (CAS No. 123-91-1) Technical TR-80. Bethesda, MD: DHHS/NIH/NCI.

- NCI/NTP. 1978c. Bioassay of 4,4'-Thiodianiline for Possible Carcinogenicity (CAS No. 139-65-1) Technical TR-47. Bethesda, MD: DHHS/NIH/NCI.
- NCI/NTP. 1978d. Bioassay of Cupferron for Possible Carcinogenicity (CAS No. 135-20-6) Technical TR-100. Bethesda, MD: DHHS/NIH/NCI.
- NCI/NTP. 1978e. Bioassay of p-Anisidine Hydrochloride for Possible Carcinogenicity (CAS No. 20265-97-8) Technical TR-116. Bethesda, MD: US DHHS/NIH/NCI.
- NCI/NTP. 1978f. Bioassay of Thio-TEPA for Possible Carcinogenicity (CAS No. 52-24-4) Technical TR-58. Bethesda, MD: DHHS/NIH/NCI.
- NCI/NTP. 1978g. Bioassays of Dieldrin for Possible Carcinogenicity (CAS No. 60-57-1) Technical TR-21 and 22.
- NCI/NTP. 1979a. Bioassay of 2,4-Diaminotoluene for Possible Carcinogenicity (CAS No. 95-80-7) Technical TR-162. Bethesda, MD: US DHHS/NCI/NIH.
- NCI/NTP. 1979b. Bioassay of 4-Nitro-o-phenylenediamine for Possible Carcinogenicity (CAS No. 99-56-9) Technical TR-180. Bethesda, MD: US DHHS/NIH/NCI.
- NCI/NTP. 1979c. Bioassay of 6-Nitrobenzimidazole for Possible Carcinogenicity (CAS No. 94-52-0) Technical TR-117. Bethesda, MD: DHHS/NIH/NCI.
- NCI/NTP. 1979d. Bioassay of Procarbazine for Possible Carcinogenicity (CAS No. 366-70-1) Technical TR-19. Bethesda, MD: DHHS/NIH/NCI.
- NCI/NTP. 1979e. Bioassay of Sulfisoxazole for Possible Carcinogenicity (CAS No. 127-69-5) Technical TR-138. Bethesda, MD: DHHS/NIH/NCI.
- NCI/NTP. 1980. Bioassay of Phenol for Possible Carcinogenicity (CAS No. 108-95-2) Technical TR-203. Bethesda, MD: DHHS/NIH/NCI. NCI/NTP. 1982a. Bioassay of Reserpine for Possible Carcinogenicity (CAS No. 50-55-5) Technical TR-193. Bethesda, MD: US DHHS/NIH/NCI/NTP.
- NCI/NTP. 1982b. Carcinogenesis Bioassay of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (CAS No. 1746-01-6) in Osborne-Mendel Rats and B6C3F1 Mice (Gavage Study) Technical TR-209: US DHHS/National Cancer Institute/National Toxicology Program.
- NCI/NTP. 1982c. Carcinogenesis Bioassay of D-Mannitol (CAS No. 69-65-8) in F344/N Rats and B6C3F1 Mice (Feed Study) Technical TR-236. Research Triangle Park, NC 27709: US DHHS/NIH/NIEHS.
- NCI/NTP. 19778. Bioassay of 5-Nitro-o-toluidine for Possible Carcinogenicity (CAS No. 99-55-8) Technical TR-107. Bethesda, MD: DHHS/NIH/NCI.
- NTP. 1982. Carcinogenesis Bioassay of Di(2-ethylhexyl)phthalate (CAS No. 117-81-7) in F344 Rats and B6C3F1 Mice (Feed Studies) Technical TR-217. Research Triangle Park, NC 27709: US DHHS/NIH/NIEHS.

- NTP. 1985a. Toxicology and Carcinogenesis Studies of 2-Chloroethanol (Ethylene Chlorohydrin) (CAS No. 107-07-3) in F344/N Rats and Swiss CD-1 Mice (Dermal Studies) Technical TR-275. Research Triangle Park, NC 27709: DHHS/NIH/NIEHS.
- NTP. 1985b. Toxicology and Carcinogenesis Studies of 8-Hydroxyquinoline (CAS No. 148-24-3) in F344/N Rats and B6C3F1 Mice (Feed Studies) Technical TR-276. Research Triangle Park, NC: US DHHS/NIH/NIEHS.
- NTP. 1986a. Carcinogenesis Studies of Ethyl Acrylate (CAS No. 140-88-5) in F344/N Rats and B6C3F1 Mice (Gavage Studies) Technical TR-259. Research Triangle Park, NC 27709: US DHHS/NIH/NIEHS.
- NTP. 1986b. Toxicology and Carcinogenesis Studies of Dimethylvinyl Chloride (1-Chloro-2-Methylpropene) (CAS No. 513-37-1) in F344/N Rats and B6C3F1 Mice (Gavage Studies) Technical TR-316. Research Triangle Park, NC 27709: DHHS/NIH/NIEHS.
- NTP. 1986c. Toxicology and Carcinogenesis Studies of Xylenes (Mixed) (60% m-Xylene, 14% p-Xylene, 9% o-Xylene, and 17% Ethylbenzene) (CAS No. 1330-20-7) in F344/N Rats and B6C3F1 Mice (Gavage Studies) Technical TR-327. Research Triangle Park, NC 27709: DHHS/NIH/NIEHS.
- NTP. 1987. Toxicology and Carcinogenesis Studies of Ampicillin Trihydrate (CAS No. 7177-48-2) in F344/N Rats and B6C3F1 Mice (Gavage Studies) Technical TR-318. Research Triangle Park, NC 27709: US DHHS/NIH/NIEHS.
- NTP. 1988a. Toxicology and Carcinogenesis Studies of Crocidolite Asbestos (CAS No. 12001-28-4) In F344/N Rats (Feed Studies) Technical TR-280. Research Triangle Park, NC 27709: US DHHS/National Toxicology Program.
- NTP. 1988b. Toxicology and Carcinogenesis Studies of Rotenone (CAS No. 83-79-4) in F344/N Rats and B6C3F1 Mice (Feed Studies) Technical TR-320. Research Triangle Park, NC 27709: US DHHS/NIH/NIEHS.
- NTP. 1989a. Toxicology and Carcinogenesis Studies of 4-Vinyl-1-cyclohexene Diepoxide (CAS No. 106-87-6) in F344/N Rats and B6C3F1 Mice (Dermal Studies) Technical TR-362. Research Triangle Park, NC 27709: US DHHS/NIEHS/NIH.
- NTP. 1989b. Toxicology and Carcinogenesis Studies of N-Methylolacrylamide (CAS No. 924-42-5) in F344/N Rats and B6C3F1 Mice (Gavage Studies) Technical TR-352. Research Triangle Park, NC 27709: US DHHS/NIH/NIEHS.
- NTP. 1990a. Toxicology and Carcinogenesis Studies of d-Limonene (CAS No. 5989-27-5) in F344/N Rats and B6C3F1 Mice (Gavage Studies) Technical TR-347. Research Triangle Park, NC 27709: US DHHS/NIH/NIEHS.
- NTP. 1990b. Toxicology and Carcinogenesis Studies of Furfural (CAS No. 98-01-1) in F344/N Rats and B6C3F1 Mice (Gavage Studies) Technical TR-382. Research Triangle Park, NC 27709: DHHS/NIH/NCI.

- NTP. 1990c. Toxicology and Carcinogenesis Studies of Glycidol (CAS No. 556-52-5) In F344/N Rats and B6C3F1 Mice (Gavage Studies Technical TR-374. Research Triangle Park, NC 27709: US DHHS/NIEHS/NIH.
- NTP. 1990d. Toxicology and Carcinogenesis Studies of Mirex (1,1a,2,2,3,3a,4,5,5,5a,5b,6-Dodecachlorooctahydro-1,3,4- metheno-1H-cyclouta[cd]pentalene) (CAS No. 2385-85-5) in F344/N Rats (Feed Studies) Technical TR-313. Research Triangle Park, NC 27709: DHHS/NIH/NIEHS.
- NTP. 1992a. Toxicology and Carcinogenesis Studies of Ethylene Thiourea (CAS: 96-45-7) in F344 Rats and B6C3F1 Mice (Feed Studies) Technical TR-388. Research Triangle Park, NC 27709: DHHS/NIH/NIEHS.
- NTP. 1992b. Toxicology and Carcinogenesis Studies of Resorcinol (CAS No. 108-46-3) in F344 Rats and B6C3F1 Mice (Gavage Studies) Technical TR-403. Research Triangle Park, NC 27709: US DHHS/NIH/NIEHS.
- NTP. 1994. Toxicology and Carcinogenesis Studies of o-Benzyl-p-Chlorophenol (CAS No. 120-32-1) in F344/N Rats and B6C3F1 Mice (Gavage Studies) Technical TR-424. Research Triangle Park, NC 27709: DHHS/NIH/NIEHS. NTP. 1995a. Toxicology and Carcinogenesis Studies of Methylphenidate Hydrochloride (CAS No. 298-59-9) in F344/N Rats and B6C3F1 Mice (Feed Studies) Technical TR-439. Research Triangle Park, NC 27709: US DHHS/NIH/NIEHS.
- NTP. 1995b. Toxicology and Carcinogenesis Studies of Benzethonium Chloride (CAS No. 121-54-0) in F344/N Rats and B6C3F1 Mice (Dermal Studies) Technical TR-438. Research Triangle Park, NC 27709: DHHS/NIH/NIEHS.
- NTP. 1995c. Toxicology and carcinogenesis studies of phenolphthalein in F344/N rats and B6C3F1 mice Technical NTP Tech. Rep. Series No. 4xx. Research Triangle Park, NC 27709: US Department of Health and Human Services.
- NTP. 1996. Comparative Initiation/Promotion Skin Paint Studies of B6C3F1 Mice, Swiss (CD-1®) Mice, and SENCAR Mice Technical TR-441. Research Triangle Park, NC 27709: US DHHS/NIH/NIEHS.
- NTP. 1998a. Toxicology and Carcinogenesis Studies of 1-Chloro-2-propanol (Technical Grade) (CAS NO. 127-00-4) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies Technical TR-477. Research Triangle Park, NC 27709: US DHHS/NIH/NIEHS.
- NTP. 1998b. Toxicology and Carcinogenesis Studies of Chloroprene (CAS No. 126-99-8) in F344/N Rats and B6C3F1 Mice (Inhalation Studies) Technical TR-467. Research Triangle Park, NC 27709: US DHHS/NIH/NIEHS.
- NTP. 1999a. Toxicology and Carcinogenesis Studies of Furfuryl Alcohol (CAS No. 98-00-0) in F344/N Rats and B6C3F1 Mice (Inhalation Studies) Technical TR-482. Research Triangle Park, NC 27709: US DHHS/NIH/NIEHS.

- NTP. 1999b. Toxicology and Carcinogenesis Studies of Lauric Acid Diethanolamine Condensate (CAS NO. 120-40-1) in F344/N Rats and B6C3F1 Mice (Dermal Studies) Technical TR-480. Research Triangle Park, NC 27709: US DHHS/NIH/NIEHS.
- NTP. 1999c. Toxicology and Carcinogenesis Studies of Magnetic Field Promotion (DMBA Initiation) in Female Sprague-Dawley Rats (Whole-body Exposure/Gavage Studies) Technical TR-489. Research Triangle Park, NC 27709: DHHS/NIH/NCI.
- NTP. 1999d. Toxicology and Carcinogenesis Studies of Oleic Acid Diethanolamine Condensate (CAS No. 93-83-4) in F344/N Rats and B6C3F1 Mice (Dermal Studies) Technical TR-481. Research Triangle Park, NC 27709: US DHHS/NIH/NIEHS.
- NTP. 1999e. Toxicology and Carcinogenesis Studies of Oxymetholone (CAS NO. 434-07-1) in F344/N Rats and Toxicology Studies of Oxymetholone in B6C3F1 Mice (Gavage Studies) Technical TR-485. Research Triangle Park, NC 27709.
- NTP. 1999f. Toxicology and Carcinogenesis Studies of Pentachlorophenol (CAS NO. 87-86-5) in F344/N Rats (Feed Studies) Technical TR-483. Research Triangle Park, NC 27709: US DHHS/NIH/NIEHS.
- NTP. 1999g. Toxicology and Carcinogenesis Studies of Triethanolamine (CAS No. 102-71-6) in F344 Rats and B6C3F1 Mice (Dermal Studies) Technical TR-449. Research Triangle Park, NC 27709: DHHS/NIH/NIEHS.
- NTP. 1999h. Toxicology and Carcinogenesis Studies of Diethanolamine (CAS No. 111-42-2) in F344/N Rats and B6C3F1 Mice (Dermal Studies) Technical TR-478. Research Triangle Park, NC 27709: US DHHS/NIH/NIEHS.
- NTP. 2000. Toxicology and Carcinogenesis Studies of Pyridine (CAS No. 110-86-1) in F344/N Rats, Wistar Rats, and B6C3F1 Mice (Drinking Water Studies) Technical TR-470. Research Triangle Park, NC 27709: US DHHS/NIH/NIEHS.
- NTP. 2001. Toxicology and Carcinogenesis Studies of Coconut Oil Acid Diethanolamine Condensate (CAS No. 68603-42-9) in F344/N Rats And B6C3F1 Mice (Dermal Studies) Technical TR-479. Research Triangle Park, NC 27709: US DHHS/NIH/NIEHS. Search. City: National Toxicology Program. Available: <http://ntp-server.niehs.nih.gov/> [5 May 2002].
- NTP U. 1979. Bioassay of p-cresidine for possible carcinogenicity Technical NCI Carcinogenesis Tech. Rep. Ser. No. 142: US Department of Health, Education and Welfare.
- NTP 1986d. Toxicology and carcinogenesis studies of benzene in F344/N rats and B6C3F1 mice Technical NTP Tech. Rep. Series No. 289. Research Triangle Park, NC 27709: US Department of Health and Human Services.
- Nylander-French LA, French JE. 1998. Tripropylene glycol diacrylate but not ethyl acrylate induces skin tumors in a twenty-week short-term tumorigenesis study in Tg.AC (v-Ha-ras) mice. *Toxicol Pathol* 26:476-483.

- Ozaki K, Sukata T, Yamamoto S, Uwagawa S, Seki T, Kawasaki H, Yoshitake A, Wanibuchi H, Koide A, Mori Y, Fukushima S.1998. High susceptibility of p53(+/-) knockout mice in N-butyl-N-(4-hydroxybutyl)nitrosamine urinary bladder carcinogenesis and lack of frequent mutation in residual allele. *Cancer Res* 58:3806-3811.
- Popp JA.2001. Criteria for the Evaluation of Studies in Transgenic Models. *Toxicologic Pathology* 29 (Supplement):20-23.
- Prives C, Hall PA.1999. The p53 pathway. *J Pathol* 187:112-126.
- Pruitt K, Der CJ.2001. Ras and Rho regulation of the cell cycle and oncogenesis. *Cancer Lett* 171:1-10.
- Robinson DE, MacDonald JS.2001. Background and Framework for ILSI's Collaborative Evaluation Program on Alternative Models for Carcinogenicity Assessment. *Toxicologic Pathology* 29 (Supplement):13-19.
- Rudolph KL, Millard M, Bosenberg MW, DePinho RA.2001. Telomere dysfunction and evolution of intestinal carcinoma in mice and humans. *Nat Genet* 28:155-159.
- Sagartz JE, Curtiss SW, Bunch RT, Davila JC, Morris DL, Alden CL.1998. Phenobarbital does not promote hepatic tumorigenesis in a twenty-six-week bioassay in p53 heterozygous mice. *Toxicol Pathol* 26:492-500.
- Saitoh A, Kimura M, Takahashi R, Yokoyama M, Nomura T, Izawa M, Sekiya T, Nishimura S, Katsuki M.1990. Most tumors in transgenic mice with human c-Ha-ras gene contained somatically activated transgenes. *Oncogene* 5:1195-1200.
- Shelby MD.1988. The genetic toxicity of human carcinogens and its implications. *Mutation Research* 204:3-15.
- Spalding JW, French JE, Stasiewicz S, Furedi-Machacek M, Conner F, Tice RR, Tennant RW.2000. Responses of transgenic mouse lines p53(+/-) and Tg.AC to agents tested in conventional carcinogenicity bioassays. *Toxicol Sci* 53:213-223.
- Spalding JW, French JE, Tice RR, Furedi-Machacek M, Haseman JK, Tennant RW.1999. Development of a transgenic mouse model for carcinogenesis bioassays: evaluation of chemically induced skin tumors in Tg.AC mice. *Toxicol Sci* 49:241-254.
- Spalding JW, Momma J, Elwell MR, Tennant RW.1993. Chemical induced skin carcinogenesis in a transgenic mouse line (TG•AC) carrying a v-Ha-ras gene. *Carcinogenesis* 14:1335-1341.
- Stoll RE, Holden HE, Barthel CH, Blanchard KT.1999. Oxymetholone: III. Evaluation in the p53+/- transgenic mouse model [comment] [see comments]. *Toxicol Pathol* 27:513-518.
- Storer R, French J, Haseman J, Hajian G, LeGrand E, Long G, Mixson L, Ochoa R, Sagartz J, Soper K.2001. p53+/- Hemizygous Knockout Mouse: Overview of Available Data. *Toxicol Pathol* 29:30-50.

- Suemizu H, Muguruma K, Maruyama C, Tomisawa M, Kimura M, Hioki K, Shimozawa N, Ohnishi Y, Tamaoki N, Nomura T.2002. Transgene stability and features of rasH2 mice as an animal model for short-term carcinogenicity testing. *Mol Carcinog* 34:1-9.
- Tennant RW.1993. Stratification of rodent carcinogenicity bioassay results to reflect relative human hazard. *Mutat Res* 286:111-118.
- Tennant RW, French JE, Spalding JW.1995. Identifying chemical carcinogens and assessing potential risk in short-term bioassays using transgenic mouse models. *Environ Health Perspect* 103:942-950.
- Tennant RW, Stasiewicz S, Mennear J, French JE, Spalding JW.1999. Genetically altered mouse models for identifying carcinogens. *IARC Sci Publ*:123-150.
- Tice RR, Nylander-French LA, French JE.1997. Absence of systemic in vivo genotoxicity after dermal exposure to ethyl acrylate and tripropylene glycol diacrylate in Tg.AC (v-Ha-ras) mice. *Environ Mol Mutagen* 29:240-249.
- Trempus CS, Mahler JF, Ananthaswamy HN, Loughlin SM, French JE, Tennant RW.1998. Photocarcinogenesis and susceptibility to UV radiation in the v-Ha-ras transgenic Tg.AC mouse. *J Invest Dermatol* 111:445-451.
- Umemura T, Kodama Y, Hioki K, Inoue T, Nomura T, Kurokawa Y.1999. Susceptibility to urethane carcinogenesis of transgenic mice carrying a human prototype c-Ha-ras gene (rasH2 mice) and its modification by butylhydroxytoluene. *Cancer Lett* 145:101-106.
- Usui T, Mutai M, Hisada S, Takoaka M, Soper KA, McCullough B, Alden C.2001. CB6F1-rasH2 Mouse: Overview of Available Data. *Toxicologic Pathology* 29:90-108.
- Venkatachalam S, Shi YP, Jones SN, Vogel H, Bradley A, Pinkel D, Donehower LA.1998. Retention of wild-type p53 in tumors from p53 heterozygous mice: reduction of p53 dosage can promote cancer formation. *Embo J* 17:4657-4667.
- Vogelstein B, Civin CI, Preisinger AC, Krischer JP, Steuber P, Ravindranath Y, Weinstein H, Elfferich P, Bos J.1990. RAS gene mutations in childhood acute myeloid leukemia: a Pediatric Oncology Group study. *Genes Chromosomes Cancer* 2:159-162.
- W Lijinsky MR, BN Blackwell,.1080. Liver Tumors Induced in Rats by Oral Administration of the Antihistaminic Methapyrilene Hydrochloride. *Science* 209:817-819.
- Weinberg RA. 1991a. Oncogenes, Tumor Suppressor Genes, and Cell Transformation: Trying to put it all together. In: *Origins of Human Cancer* (Brugge J, Curran T, Harlow E, McCormick F, eds). Cold Spring Harbor:Cold Spring Harbor Laboratory Press; 1-16.
- Weinberg RA.1991b. Tumor suppressor genes. *Science* 254:1138-1146.
- Weisburger E.1977. Bioassay Program for Carcinogenic Hazards of Cancer Chemotherapeutic Agents. *Cancer* 40:1935-1949.

- Yamamoto M, Tsukamoto T, Sakai H, Shirai N, Ohgaki H, Furihata C, Donehower LA, Yoshida K, Tatematsu M. 2000. p53 knockout mice (-/-) are more susceptible than (+/-) or (+/+) mice to N-methyl-N-nitrosourea stomach carcinogenesis [In Process Citation]. *Carcinogenesis* 21:1891-1897.
- Yamamoto S, Mitsumori K, Kodama Y, Matsunuma N, Manabe S, Okamiya H, Suzuki H, Fukuda T, Sakamaki Y, Sunaga M, Nomura G, Hioki K, Wakana S, Nomura T, Hayashi Y. 1996. Rapid induction of more malignant tumors by various genotoxic carcinogens in transgenic mice harboring a human prototype c-Ha-ras gene than in control non-transgenic mice. *Carcinogenesis* 17:2455-2461.
- Yamamoto S, Urano K, Koizumi H, Wakana S, Hioki K, Mitsumori K, Kurokawa Y, Hayashi Y, Nomura T. 1998a. Validation of transgenic mice carrying the human prototype c-Ha-ras gene as a bioassay model for rapid carcinogenicity testing. *Environ Health Perspect* 106 Suppl 1:57-69.
- Yamamoto S, Urano K, Nomura T. 1998b. Validation of transgenic mice harboring the human prototype c-Ha-ras gene as a bioassay model for rapid carcinogenicity testing. *Toxicol Lett* 102-103:473-478.
- Yunis JJ, Boot AJ, Mayer MG, Bos JL. 1989. Mechanisms of ras mutation in myelodysplastic syndrome. *Oncogene* 4:609-614.

Table 1. Comparison of results from 14 known human carcinogens¹ tested in rodent NCI/NTP cancer bioassays, Salmonella (Sal) and/or in vivo micronuclei (Mn) genotoxicity assays and/or 3 transgenic mouse cancer bioassays. Individual results were found in the cited references in parenthesis or at the IARC(IARC 2002) or the US NTP database(NTP 2002). NCI/NTP peer-reviewed conclusions are reported for male rat, female rat, male mouse; or female mouse, respectively. Results from transgenic models are presented as the summary conclusion for each route of exposure using one or both sexes of the strain used.

Agent	CAS No.	IARC	NTP ROC	NCI/NTP Bioassays	Genotoxicity (Sal; Mn)	p53+/-	Tg.AC	RasH2
Benzene	71-43-2	1	Known	+;+;+;+ g(NTP 1986d)	-;+	+ g; + g (French, et al. 2001;Storer, et al. 2001)	+ d; + g (Blanchard, et al. 1998;Spalding, et al. 1999)	+ g (Yamamoto, et al. 1998b)
Cyclophosphamide	6055-19-2	1	Known	+;+;+;+ip (Weisburger 1977)	+;+	+ g (Storer, et al. 2001)	±d; +g (Eastin, et al. 2001)	± g;+ g; + g (Usui, et al. 2001;Yamamoto, et al. 1998b)
Melphalan	148-82-3	1	Known	+;+;+;+ ip (Weisburger 1977)	+;+	+ ip;+ ip (Eastin, et al. 1998;Storer, et al. 2001)	±d; +g (Eastin, et al. 1998;Eastin, et al. 2001)	± ip(Yamamoto, et al. 1998b)
Cyclosporin A	79217-60-0	1	Known	nt	-;-	- g;+ f;+f (Eastin, et al. 1998;Storer, et al. 2001)	+d; ± f (Eastin, et al. 1998;Eastin, et al. 2001)	± g (Maronpot, et al. 2000;Usui, et al. 2001;Yamamoto, et al. 1998a)
Diethylstilbestrol	56-53-1	1	Known	nt	-;nt	- sc ;+ f (Eastin, et al. 1998;Storer, et al. 2001)	+d; -g (Eastin, et al. 1998;Eastin, et al. 2001)	+ f (Usui, et al. 2001)
Estradiol, 17-β	50-28-2	1	Reasonable	nt	-;-	± g; - g (Storer, et al. 2001)	+d; -g ² (Eastin, et al. 2001)	- g (Usui, et al. 2001)
TCDD ³	1746-01-6	1	Known	+;+;+;+ f (NCI/NTP 1982b)	-;nt	- g (Eastin, et al. 1998)	+ d (Eastin, et al. 1998)	nt
UVR (312-450 nM)	NA	1	Known	nt	+;+	+ d (Jiang, et al. 1999)	+ d (Trempus, et al. 1998)	nt
Asbestos fibers	1332-21-4	1	Known	-;-;nt;nt d (NTP 1988a)	nt;-	+ ip (Marsella, et al. 1997)	nt	nt

¹ As identified by the International Agency for Research on Cancer (IARC) and/or the NTP 9th Report on Carcinogens, revised January 2001.

² Both dermal and gavage studies in the Tg.AC mice employed ethinyl estradiol (CAS No. 57-63-6), a synthetic form of estradiol, 17β.

³ 2,3,7,8-Tetrachlorodibenzo-para-dioxin

Agent	CAS No.	IARC	NTP ROC	NCI/NTP Bioassays	Genotoxicity (Sal; Mn)	p53+/-	Tg.AC	RasH2
Beryllium	7440-41-7	1	Known	nt	-;-	+ i (Finch, et al. 1998)	nt	nt
Plutonium ²³⁹	NA	1	Known	nt	+;+	+ i (Finch, et al. 1998)	nt	nt
Cobalt ⁶⁰ (LET)	NA	1	Known	nt	-;+	+ wb (Kemp, et al. 1994)	nt	nt
Sodium arsenate	7784-46-5	1	Known	nt	nt;nt	nt	-d (Germolic, et al. 1997)	nt
Thiotepa	52-24-4	1	Known	+;+;+;+ g (NCI/NTP 1978f)	+;nt	nt	nt	+ ip (Yamamoto, et al. 1998b)

Table 2. Comparison of results from 32 suspected human carcinogens¹ tested in rodent NCI/NTP cancer bioassays, Salmonella and/or in vivo micronuclei genotoxicity assays and/or 3 transgenic mouse bioassays. Individual results are found in the cited references in parenthesis or in the IARC(IARC 2002) or in the US NTP database(NTP 2002). NCI/NTP Peer-reviewed conclusions are reported for male F344 rat, female F344 rat, male B6C3F1 mouse; or female B6C3F1 mouse, respectively. Results from transgenic models are presented as the summary conclusion for each route of exposure using one or both sexes.

Agent	CAS No.	IARC	NTP ROC	NCI/NTP Bioassays	Genotoxicity (Sal; Mn)	p53+/-	Tg.AC	RasH2
p-Cresidine	120-71-8	2B	Reasonable	+;+;+;+ f (NTP 1979)	+;-	+ f; + g (Storer, et al. 2001;Tennant, et al. 1995)	+ d (Tennant, et al. 1999)	+ f (Yamamoto, et al. 1998b)
Glycidol	556-52-5	2A	Reasonable	+;+;+;+ g (NTP 1990c)	+;+	- g (Tennant, et al. 1999)	- d; - g (Tennant, et al. 1999)	+ g (Usui, et al. 2001)
Phenolphthalein	77-09-8	2B	Reasonable	+;+;+;+ f (NTP 1995c)	-;+	+ f;+ f (Dunnick, et al. 1997)	nt	- f (Koujitani, et al. 2000)
4-Vinyl-1-cyclohexene diepoxide	106-87-6	2B	Reasonable	+;+;+;+ d (NTP 1989a)	+;+	+ d (Tennant, et al. 1995)	- d (Tennant, et al. 1999)	+ d (Yamamoto, et al. 1998b)
2,4-Diaminotolulene	95-80-7	2B	Reasonable	+;+;-;+ f (NCI/NTP 1979a)	+;-	± f (Eastin, et al. 1998)	+ d (Eastin, et al. 1998)	nt
Chloroprene	126-99-8	2B	Reasonable	+;+;+;+ I (NTP 1998b)	-;-	- i (French 2001)	- i (French 2001)	nt
Pentachlorophenol	87-86-5	2B	Not Listed	+ ² ;+;+;+ f (NTP 1999f)	-;-	- f (Spalding, et al. 2000)	+ d (Spalding, et al. 2000)	nt
Phenacetin	62-44-2	2A	Reasonable	nt	-;nt	- f; - g (Storer, et al. 2001)	-d; -f (Eastin, et al. 2001)	+ f (Yamamoto, et al. 1998b)
Phenobarbital	50-06-6	2B	Not Listed	nt	wk+;nt	- f;- f (Sagartz, et al. 1998;Storer, et al. 2001)	ia d; ia g; ia f (Eastin, et al. 2001)	-g (Usui, et al. 2001)
Chloroform	67-66-3	2B	Reasonable	+;-;+;+ w (Griesemer and Cueto 1980)	-;+	± g (Storer, et al. 2001)	- g (Delker, et al. 1999)	- g (Usui, et al. 2001)

¹ “Probable” (2A) or “possible” (2B) human or “reasonably anticipated” to be a human carcinogen as identified by the International Agency for Research on Cancer (IARC) and/or NTP Report on Carcinogens (9th NTP ROC, revised January 2001), respectively.

² Positive in 1000 ppm 1 year exposure stop study but not with 2 year exposure to technical grade pentachlorophenol (technical grade, TR349; purified, TR483)

Agent	CAS No.	IARC	NTP ROC	NCI/NTP Bioassays	Genotoxicity (Sal; Mn)	p53+/-	Tg.AC	RasH2
Benzo[a]pyrene	50-32-8	2A	Reasonable	nt	+;nt	+ d,g (Martin, et al. 2001)	+ d (Martin, et al. 2001)	nt
Dimethylnitrosamine	62-75-9	2A	Not Listed	nt	+;nt	+ w (Harvey, et al. 1993)	nt	nt
7,12-Dimethylbenzanthracene ³	57-97-6	NE	Not Listed	nt;nt;+; d, i-p (NTP 1996)	+;+	+ d (Kemp, et al. 1993)	+ d (Spalding, et al. 1993)	nt
N-ethyl-N-nitrosourea	759-73-9	2A	Not Listed	nt	+;+	+ ip (Mitsumori, et al. 2000)	nt	+ ip (Yamamoto, et al. 1998b)
2-Amino-3-methylimidazo[4,5-f]quinoline	76180-96-6	2A	Not Listed	nt	+;+	+ g (Nagao 1999)	nt	nt
N-Butyl-N-(4-hydroxybutyl) nitrosamine (BBN)	64091-91-4	2B	Not Listed	nt	nt;-	+ w (Ozaki, et al. 1998)	nt	nt
N-methyl-N-nitrosourea	684-93-5	2A	Not Listed	nt	nt;+	+ip (Yamamoto, et al. 2000)	nt	+ ip (Yamamoto, et al. 1998b)
Urethane	51-79-6 5	2B	Reasonable	nt	+;+	+ ip (Carmichael, et al. 2000)	+d (Spalding, et al. 1993)	+ ip (Mori, et al. 2000;Umemura, et al. 1999)
Oxymetholone	434-07-1	2A	Reasonable	±;+;nt;nt (NTP 1999e)	-;-	- g (Stoll, et al. 1999)	+ d (Stoll, et al. 1999)	nt
1, 2-Dimethylhydrazine	540-73-8	2A	Not listed	nt	- ⁴ ;nt	nt	nt	+ d (Yamamoto, et al. 1998b)
1,4-Dioxane	123-91-1	2B	Reasonable	+;+;+;+ w (NCI/NTP 1978b)	-;+	nt	nt	± w (Yamamoto, et al. 1998b)
Ethylene thiourea	96-45-7	2B	Reasonable	+;+;+;+ f (NTP 1992a)	-;nt	nt	nt	+f (Yamamoto, et al. 1998b)
Methylazoxymethanol acetate	592-62-1	2B	Not listed	nt	-;nt	nt	nt	+ sc (Yamamoto, et al. 1998b)

³ Reasonably anticipated to be a human carcinogen based on its use a prototypical mutagenic carcinogen used in initiation-promotion and complete carcinogenicity studies.

⁴ 1,2-dimethylhydrazine dihydrochloride (CAS No. 306-37-6) tested in *Salmonella* mutagenicity assay.

Agent	CAS No.	IARC	NTP ROC	NCI/NTP Bioassays	Genotoxicity (Sal; Mn)	p53+/-	Tg.AC	RasH2
Procarbazine	366-70-1	2A	Reasonable	+;+;+;+ ip (NCI/NTP 1979d)	+;+	nt	nt	+ip (Yamamoto, et al. 1998b)
4,4'-Thiodianiline	139-65-1	2B	Not listed	+;+;+;+ f (NCI/NTP 1978c)	+;nt	nt	nt	+f (Yamamoto, et al. 1998b)
MNNG	70-25-7	2A	Reasonable	+;+;+ d ip (NTP 1996)	+;nt	nt	nt	+g (Yamamoto, et al. 1998b)
Cupferron	135-20-6	2A	Reasonable	+;+;+;+ f (NCI/NTP 1978d)	+;nt	nt	nt	+f (Yamamoto, et al. 1998b)
N-nitrosodiethylamine	55-18-5	2A	Reasonable	nt	+;nt	nt	nt	+ip (Yamamoto, et al. 1998b)
Dimethylvinylchloride	513-37-1	2B	Not listed	+;+;+;+ g (NTP 1986b)	+;+	nt	+ d (Stoll, et al. 1999)	nt
4-Nitroquinoline N-oxide ⁵	56-57-5	NE	Not listed	nt	+;nt	nt	nt	+sc (Yamamoto, et al. 1998b)
4-Hydroxyaminoquinoline-1-oxide ⁵	4637-56-3	NE	Not listed	nt	+;nt	nt	nt	+ ip (Yamamoto, et al. 1998b)
Mirex	2385-85-5	2B	Reasonable	+;+;nt;nt f (NTP 1990d)	-;nt	nt	+d (Stoll, et al. 1999)	nt

⁵ Reasonably anticipated to be a human carcinogen based upon its use as a prototypical mutagenic carcinogen for mechanistic investigation of chemical carcinogenesis.

Table 3. Comparison of results from 52 suspected human carcinogens¹ tested in rodent NCI/NTP cancer bioassays, Salmonella and/or in vivo micronuclei genotoxicity assays and/or 3 transgenic mouse bioassays. Individual results are found in the cited references in parenthesis or in the IARC(IARC 2002) or in the US NTP database(NTP 2002). NCI/NTP Peer-reviewed conclusions are reported for male F344 rat, female F344 rat, male B6C3F1 mouse; or female B6C3F1 mouse, respectively. Results from transgenic models are presented as the summary conclusion for each route of exposure using one or both sexes.; w, water (routes of exposure); nt, not tested or no published record.

Agent	CAS No.	IARC	NTP ROC	NCI/NTP Bioassays	Genotoxicity (Sal; Mn)	p53+/-	Tg.AC	RasH2
p-Anisidine	90-04-0	3	Not Listed	±;-;- f, (NCI/NTP 1978e)	+;-	- f (Tennant, et al. 1995)	- d (Tennant, et al. 1995)	- g (Maronpot, et al. 2000)
1-Chloro-2-propanol	127-00-4	NE	Not Listed	-;-;- w (NTP 1998a)	+;nt	- g (Tennant, et al. 1999)	- d (Tennant, et al. 1999)	nt
2,6-Diaminotoluene	820-40-5	NE	Not Listed	-;-;- f (Battershill and Fielder 1998)	+;-	- f (Eastin, et al. 1998)	- d(Eastin, et al. 1998)	nt
8-Hydroxyquinoline	148-24-3	3	Not Listed	-;-;- f (NTP 1985b)	+;-	-f (Eastin, et al. 1998)	-d (Eastin, et al. 1998)	nt
Coconut oil diethanolamine	68603-42-9	NE	Not Listed	-; ±;+;+ d (NTP 2001)	-;+	- d (Spalding, et al. 2000)	- d (Spalding, et al. 2000)	nt
Diethanolamine	111-42-2	3	Not Listed	-;-;+;+ d (NTP 1999h)	-;-	nt	- d (Spalding, et al. 2000)	nt
Ethyl Acrylate	140-88-5	2B	Delisted	+;+;+;+ g (NTP 1986a)	-;-	nt	- d (Nylander-French and French 1998;Tice, et al. 1997)	+ g (Yamamoto, et al. 1998b)
Furfuryl alcohol	98-00-0	NE	Not Listed	+; ±;+;- i (NTP 1999a)	-;-	nt	- d (Spalding, et al. 2000)	nt
Lauric acid diethanolamine	120-40-1	NE	Not Listed	-;-;-+ d (NTP 1999b)	-;-	-f (Spalding, et al. 2000)	+ d (Spalding, et al. 2000)	nt
N-methyloacrylamide	924-42-5	3	Not Listed	-;-;+;+ g (NTP 1989b)	-;-	-g (Tennant, et al. 1995)	- d; - g (Eastin, et al. 1998)	nt
Methylphenidate	298-59-9	NE	Not Listed	-;-;+;+ f (NTP 1995a)	-;nt	-f (Tennant, et al. 1999)	- d (Tennant, et al. 1999)	nt
Pyridine	110-86-1	3	Not Listed	+;±;+;+ w (NTP 2000)	-;-	-g (Spalding, et al. 2000)	- d (Spalding, et al. 2000)	nt
Reserpine	50-55-5	3	Reasonable	+;-;+;+ f (NCI/NTP 1982a)	-;-	-f (Tennant, et al. 1995)	-d;-g (Tennant, et al. 1995)	- f (24)
Rotenone	83-79-4	NE	Not Listed	±;-;-; f (NTP 1988b)	-;nt	- f (Eastin, et al. 1998)	+ d;- g (Eastin, et al. 1998)	- g (Yamamoto, et al. 1998b)

¹ “Probable” (2A) or “possible” (2B) human or “reasonably anticipated” to be a human carcinogen as identified by the International Agency for Research on Cancer (IARC) and/or NTP Report on Carcinogens (9th NTP ROC, revised January 2001), respectively.

Agent	CAS No.	IARC	NTP ROC	NCI/NTP Bioassays	Genotoxicity (Sal; Mn)	p53+/-	Tg.AC	RasH2
Resorcinol	108-46-3	3	Not Listed	-;-;- g (NTP 1992b)	-;+	- g (Eastin, et al. 1998)	+ d (Eastin, et al. 1998)	- g (Maronpot, et al. 2000)
Oleic acid diethanolamide	93-83-4	NE	Not Listed	-;-;- d (NTP 1999d)	-; nt	- d (Spalding, et al. 2000)	- d (Spalding, et al. 2000)	nt
Clofibrate	637-07-0	3	Not Listed	nt	-;-	- g; - g (Storer, et al. 2001)	+d (Eastin, et al. 2001)	± g; + g (Usui, et al. 2001)
Dieldrin	60-57-1	3	Not Listed	-;-;±; f (NCI/NTP 1978g)	-;nt	- f (Storer, et al. 2001)	nt	- f (Usui, et al. 2001)
Methapyrilene HCl	135-23-9	NE	Not Listed	+;+;nt;nt f (W Lijinsky 1080)	-;-	- g;-f (Storer, et al. 2001)	-d (Eastin, et al. 2001)	- g (Yamamoto, et al. 1996)
Haloperidol	52-86-8	NE	Not Listed	nt	nt;nt	- g (Storer, et al. 2001)	nt	- g (Usui, et al. 2001)
Chlorpromazine HCl	69-09-0	NE	Not Listed	nt	-;nt	- g;-g (Storer, et al. 2001)	nt	- g (Usui, et al. 2001)
Metaproterenol	586-06-1	NE	Not Listed	nt	nt;nt	- f;-f (Storer, et al. 2001)	nt	- f (24)
WY-14643	50892-23-4	NE	Not Listed	nt	nt;nt	- f (Storer, et al. 2001)	-d; ±f (Eastin, et al. 2001)	nt
Di(2-ethylhexyl)phthalate	117-81-7	3	Reasonable	+;+;+;+ f (NTP 1982)	-;-	± f (Storer, et al. 2001)	-d; -f (Eastin, et al. 2001)	+ (Usui, et al. 2001)
Sulfamethoxazole	723-46-6	3	Not Listed	nt	-;nt	- f (Storer, et al. 2001)	-d; -g (Eastin, et al. 2001)	-f (Usui, et al. 2001)
Sulfisoxazole	127-69-5	3	Not Listed	-;-;-l; f (NCI/NTP 1979e)	-;nt	- f (Storer, et al. 2001)	- d;-g (Eastin, et al. 2001)	-f (Usui, et al. 2001)
Ampicillin	7177-48-2	3	Not Listed	±;-;- f (NTP 1987)	-;nt	- g (Storer, et al. 2001)	nt	-g (Usui, et al. 2001)
D-Mannitol	69-65-8	NE	Not Listed	-;-;- f (NCI/NTP 1982c)	-;-	-f (Storer, et al. 2001)	nt	- f (24)
1,1,2-Trichloroethane	79-00-5	3	Not Listed	-;-;+;+ g (NCI/NTP 1978a)	-;-	nt	nt	+ g (Yamamoto, et al. 1998b)
Xylenes (mixed)	1330-20-7	3	Not Listed	-;-;-;- g (NTP 1986c)	-;nt	nt	nt	- g (Yamamoto, et al. 1998b)
Furfural	98-01-1	3	Not Listed	+;-;-+;+ g (NTP 1990b)	-;nt	nt	nt	+ g (Yamamoto, et al. 1998b)
5-Nitro-o-toluidine	99-55-8	3	Not Listed	-;-;+;+ f (NCI/NTP 19778)	+;nt	nt	nt	+f (Yamamoto, et al. 1998b)
Benzethonium chloride	121-54-0	NE	Not Listed	-;-;- d (NTP 1995b)	-;nt	nt	- d (Spalding, et al. 1999)	nt
<i>o</i> -Benzyl- <i>p</i> -chlorophenol	120-32-1	NE	Not Listed	-;±;+;- g (NTP 1994)	-;nt	nt	+ d (Spalding, et al. 1999)	nt

Agent	CAS No.	IARC	NTP ROC	NCI/NTP Bioassays	Genotoxicity (Sal; Mn)	p53+/-	Tg.AC	RasH2
2-Chloroethanol	107-07-3	NE	Not Listed	-;-;- d (NTP 1985a)	+;-	nt	- d (Spalding, et al. 1999)	nt
Phenol	108-95-2	3	Not Listed	-;-;- dw (NCI/NTP 1980)	-;+	nt	-d (Spalding, et al. 1999)	nt
Triethanolamine	102-71-6	3	Not Listed	±;-;ia;ia d (NTP 1999g)	-;-	nt	-d (Spalding, et al. 1999)	nt
Acetic anhydride	108-24-7	NE	Not Listed	nt	-;nt	nt	-d (Spalding, et al. 1999)	nt
2,4-dinitro-1-fluorobenzene	70-34-8	NE	Not Listed	nt	+;nt	nt	+d (Albert, et al. 1996)	nt
Diisopropylcarbodiimide	693-13-0	NE	Not Listed	in progress	-;+	in progress	+d (Spalding, et al. 1999)	nt
Dicyclohexylcarbodiimide	538-75-0	NE	Not Listed	In progress	+;+	nt	-d (Spalding, et al. 1999)	nt
Fluocinolone acetonide	67-73-2	NE	Not Listed	nt	nt;nt	nt	- d (Albert, et al. 1996)	nt
Tripropylene Glycol Diacrylate	42978-66-5	NE	Not Listed	nt	-;-	nt	+ d(Albert, et al. 1996)	nt
d-Limonene	5989-27-5	3	Not Listed	+!;-;- f (NTP 1990a)	-;nt	- g (Carmichael, et al. 2000)	nt	nt
Foreign body (transponder)	NA	NE	Not Listed	nt	-;-	+ sc (Blanchard, et al. 1999)	- sc (French 2001)	nt
Acetone	67-64-1	NE	Not Listed	nt	-;-	nt	- d (Spalding, et al. 1999;Spalding, et al. 1993)	nt
Benzoyl peroxide	94-36-0	3	Not Listed	+ i-p ² (NTP 1996)	-;nt	nt	+ d (Spalding, et al. 1993)	nt
Ethanol ³	64-17-5	NE	Not Listed	in progress	-;nt	nt	- d (Spalding, et al. 1999)	nt
Methyl ethyl ketone peroxide	1338-23-4	NE	Not Listed	in progress	+;-	nt	+ d (Spalding, et al. 1993)	nt
4-Nitro-o-phenylenediamine	99-56-9	3	Not Listed	-;-;- f (NCI/NTP 1979b)	+; inc	nt	nt	± f (Yamamoto, et al. 1998b)
6-Nitrobenzimidazole	94-52-0	NE	Not Listed	-;-;+;+ f (NCI/NTP 1979c)	+;nt	nt	nt	- f (Yamamoto, et al. 1998b)

² Results from initiation-promotion studies in B6C3F1, Swiss (CD-1), and SENCAR mice (see reference 85).

Agent	CAS No.	IARC	NTP ROC	NCI/NTP Bioassays	Genotoxicity (Sal; Mn)	p53+/-	Tg.AC	RasH2
Cholestyramine	11041-12-6	NE	Not Listed	nt	nt; nt	nt	nt	- f (Yamamoto, et al. 1998b)
60 mHz Magnetic fields	NA	NE	Not Listed	-;-;-wb (NTP 1999c)	-;-	- wb (McCormick, et al. 1998)	- wb (McCormick, et al. 1998)	nt

Table 4. Summary performance of each strategy versus likely human cancer. All chemicals in Tables 1 and 2 are included as human carcinogens, but only those chemicals in Table 3 with negative NCI/NTP bioassay results are regarded as true human noncarcinogens.

Strategy	Positive for Carcinogens	Negative for Noncarcinogens	Positive for Noncarcinogens	Negative for Carcinogens	Overall Accuracy
(1) Trp53+/-	21	12	0	10	77 % (33/43)
(2) Trp53+/- (genotoxic)	16	5	0	4	84 % (21/25)
(3) Tg.AC	17	11	2	6	78 % (28/36)
(4) RasH2	21	9	0	7	81 % (30/37)
(5) Trp53+/- (genotoxic); RasH2 (nongenotoxic)	18	7	0	7	78 % (25/32)
(6) Trp53+/- (genotoxic); RasH2 (all)	31	7	0	5	88 % (38/43)
(7) Trp53+/- (genotoxic); Tg.AC (nongenotoxic)	21	9	0	6	83 % (30/36)
(8) Trp53+/- (genotoxic); Tg.AC for all	25	8	2	4	85 % (33/39)

Definitions

Positive for Carcinogens = Positive assay results for IARC/ROC carcinogens
 Negative for Noncarcinogens = Negative assay results for IARC/ROC noncarcinogens
 Positive for Noncarcinogens = Positive assay results for IARC/ROC noncarcinogens
 Negative for Carcinogens = Negative assay results for IARC/ROC carcinogens

Table 5. Proportion of positive responses in the three transgenic models as a function of the IARC classification of these 99 chemicals.

IARC Classification	Trp53+/-	Tg.AC	RasH2	Overall
Group 1	83 % (10/12)	89 % (8/9)	57 % (4/7) ¹	79 % (22/28)
Group 2A	62 % (5/8)	50 % (2/4)	100 % (9/9)	76 % (16/21)
Group 2B²	55 % (6/11)	64 % (7/11)	69 % (9/13)	63 % (22/35)
Group 3	0 % (0/13)	21 % (3/14)	36 % (5/14)	20 % (8/41)
Not Evaluated	7 % (1/15)	29 % (7/24)	0 % (0/8)	17 % (8/47)

¹ Two of the three that were not positive were equivocal.

² Includes 7,12-dimethylbenzanthracene, 4-nitroquinoline N-oxide, and 4-hydroxyaminoquinoline-1-oxide.

Table 6. Summary of performance of each Strategy versus likely human cancer when all chemicals in Table 3 are regarded as true human non-carcinogens.

<u>Strategy</u>	Positive for Carcinogens	Negative for Noncarcinogens	Positive for Noncarcinogens	Negative for Carcinogens	Overall Accuracy
(1) Trp53+/-	21	27	1	10	81 % (48/59)
(2) Trp53+/- (genotoxic)	16	6	0	4	85 % (22/26)
(3) Tg.AC	17	29	10	6	74 % (44/62)
(4) RasH2	21	17	6	7	75 % (38/51)
(5) Trp53+/- (genotoxic); RasH2 (nongenotoxic)	18	17	1	7	81 % (35/43)
(6) Trp53+/- (genotoxic); RasH2 (all)	30	13	6	5	80 % (43/54)
(7) Trp53+/- (genotoxic); Tg.AC (nongenotoxic)	21	21	1	6	86 % (42/49)
(8) Trp53+/- (genotoxic); Tg.AC for all	25	20	10	5	75 % (45/60)
(9) NTP Rodent Bioassay	23	17	18	0	69 % (40/58)
(10) NTP Rat Bioassay; Tg.AC (nongenotoxic); Trp53+/- (genotoxic)	35	13	9	0	84 % (48/57)
(11) NTP Rat Bioassay; RasH2 (nongenotoxic); Trp53+/- (genotoxic)	33	11	8	0	85 % (44/52)
(12) NTP Rat Bioassay; genotoxicity	36	7	23	0	65 % (43/66)

Table 7. Summary performance of each strategy (vs. NTP rodent cancer results)

Strategy	Positive for Carcinogens	Negative for Noncarcinogens	Positive for Noncarcinogens	Negative for Carcinogens	Overall Accuracy
(1) Trp53+/-	7	12	0	16	54 % (19/35)
(2) Trp53+/- (genotoxic)	7	5	0	4	75 % (12/16)
(3) Tg.AC	14	10	2	14	60 % (24/40)
(4) RasH2	16	9	0	7	78 % (25/32)
(5) Trp53+/- (genotoxic); RasH2 (nongenotoxic)	9	10	0	7	73 % (19/26)
(6) Trp53+/- (genotoxic); RasH2 (all)	19	7	0	3	90 % (26/29)
(7) Trp53+/- (genotoxic); Tg.AC (nongenotoxic)	10	8	0	13	58 % (18/31)
(8) Trp53+/- (genotoxic); Tg.AC for all	15	7	2	12	61 % (22/36)