

# **Transgenic Mouse Models: Their Role in Carcinogen Identification**

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## 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  
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**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. However, 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  $\zeta$ -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<sup>+/-</sup>, 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<sup>Ink4a</sup> and p19<sup>Arf</sup> 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.



Table 1. Comparison of results from 14 known human carcinogens<sup>1</sup> 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. 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 <sup>2</sup> (Eastin, et al. 2001)	- g (Usui, et al. 2001)
TCDD <sup>3</sup>	1746-01-6	1	Known	++++ f (NCI/NTP 1982b)	-;nt	- g (Eastin, et al. 1998)	+ d (Eastin, et al. 1998)	nt

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

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

<sup>3</sup> 2,3,7,8-Tetrachlorodibenzo-para-dioxin

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 <sup>2</sup> (Eastin, et al. 2001)	- g (Usui, et al. 2001)
UVR (312-450 nM)	NA	1	Known	nt	++;+	+ d (Jiang, et al. 1999)	+ d (Trempeus, et al. 1998)	nt
Asbestos fibers	1332-21-4	1	Known	-;-;nt;nt d (NTP 1988a)	nt;-	+ ip (Marsella, et al. 1997)	nt	nt
Beryllium	7440-41-7	1	Known	nt	-;-	+ i (Finch, et al. 1998)	nt	nt
Plutonium <sup>239</sup>	NA	1	Known	nt	++;+	+ i (Finch, et al. 1998)	nt	nt
Cobalt <sup>60</sup> (LET)	NA	1	Known	nt	-;+	+ wb (Kemp, et al. 1994)	nt	nt

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 <sup>2</sup> (Eastin, et al. 2001)	- g (Usui, et al. 2001)
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 carcinogens<sup>1</sup> 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	+ <sup>2</sup> ;+;+;+ 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)
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 <sup>3</sup>	57-97-6	NE	Not Listed	nt;nt;+;+ d, i-p (NTP 1996)	+;+	+ d (Kemp, et al. 1993)	+ d (Spalding, et al. 1993)	nt

<sup>1</sup> “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.

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

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

Agent	CAS No.	IARC	NTP ROC	NCI/NTP Bioassays	Genotoxicity (Sal; Mn)	p53+/-	Tg.AC	RasH2
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	- <sup>4</sup> ;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)
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

<sup>4</sup> 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
Dimethylvinylchloride	513-37-1	2B	Not listed	++;+;+ g (NTP 1986b)	++;+	nt	+ d (Stoll, et al. 1999)	(Yamamoto, et al. 1998b) nt
4-Nitroquinoline N-oxide <sup>5</sup>	56-57-5	NE	Not listed	nt	++;nt	nt	nt	+sc (Yamamoto, et al. 1998b)
4-Hydroxyaminoquinoline-1-oxide <sup>5</sup>	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

<sup>5</sup> 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 53 chemicals<sup>1</sup> 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,	- d; - g	nt

<sup>1</sup> With insufficient evidence to be considered potential human carcinogens as identified by the International Agency for Research on Cancer (IARC) and/or the NTP Report on Carcinogens (9th NTP ROC, revised January 2001).

Agent	CAS No.	IARC	NTP ROC	NCI/NTP Bioassays	Genotoxicity (Sal; Mn)	p53+/-	Tg.AC	RasH2
Methylphenidate	298-59-9	NE	Not Listed	-;-;+;+ f (NTP 1995a)	-;nt	et al. 1995) -f (Tennant, et al. 1999)	(Eastin, et al. 1998) - 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)
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.	nt



Agent	CAS No.	IARC	NTP ROC	NCI/NTP Bioassays	Genotoxicity (Sal; Mn)	p53+/-	Tg.AC	RasH2
							2001)	
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	-;-;-1; 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
2-Chloroethanol	107-07-3	NE	Not Listed	-;-;-;- d (NTP 1985a)	+;-	nt	- d	nt

Agent	CAS No.	IARC	NTP ROC	NCI/NTP Bioassays	Genotoxicity (Sal; Mn)	p53+/-	Tg.AC	RasH2
Phenol	108-95-2	3	Not Listed	-;-;- dw (NCI/NTP 1980)	-;+	nt	(Spalding, et al. 1999) -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

Agent	CAS No.	IARC	NTP ROC	NCI/NTP Bioassays	Genotoxicity (Sal; Mn)	p53+/-	Tg.AC	RasH2
Benzoyl peroxide	94-36-0	3	Not Listed	+ i-p <sup>2</sup> (NTP 1996)	-;nt	nt	+ d (Spalding, et al. 1993)	nt
Ethanol <sup>3</sup>	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)
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

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

**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.*

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

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

<sup>1</sup> Two of the three that were not positive were equivocal.

<sup>2</sup> 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.*

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

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

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

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