The Role of Transgenic Mouse Models in Carcinogen Identification

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In this article, we examine existing data on the use of transgenic mouse models for identification of human carcinogens. We focus on the three most extensively studied of these mice, Trp53+/–, Tg/AC, and RasH2, and compare their performance with the traditional 2-year rodent bioassay. Data on 99 chemicals were evaluated. Using the International Agency for Research on Cancer/Report on Carcinogens determinations for the carcinogenicity of these chemicals to humans as the standard for comparison, we examined a variety of potential testing strategies ranging from individual transgenic models to combinations of these three models with each other and with traditional rodent assays. The individual transgenic models made the “correct” determinations (positive for carcinogens; negative for noncarcinogens) for 74–81% of the chemicals, with an increase to as much as 83% 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, two-species rodent bioassay yielded correct determinations for 69% of the chemicals. However, although the transgenic models had a high percentage of correct determinations, they did miss a number of known or probable human carcinogens, whereas the bioassay missed none of these chemicals. Therefore, we also evaluated mixed strategies using transgenic models and the rat bioassay. These strategies yielded approximately 85% correct determinations, missed no carcinogens, and cut the number of positive determinations for human noncarcinogens 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. Key words: carcinogens, hazard identification, mouse model, mutagenesis screening, transgenic models. Environ Health Perspect 111:444–454 (2003). doi:10.1289/ehp.5778 available via http://dx.doi.org/ [Online 30 October 2002]

The National Toxicology Program (NTP) is responsible for evaluating the toxicity and carcinogenicity of environmental agents, developing and validating improved testing methods, and strengthening the science base of toxicology. A variety of end points are used to assess the systemic toxicity of environmental chemicals, but the mainstay of chemical carcinogenicity testing has been the 2-year rodent bioassay. This highly standardized method has been widely adopted throughout the world. However, like any other approach, the rodent bioassay has its strengths and weaknesses. In particular, the 2-year bioassay is expensive, both in resources and time required and in the numbers of animals needed. Thus, the advent of transgenic and gene knock-out technology in the early 1980s 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. The National Institute of Environmental Health Sciences (NIEHS) has been actively involved in this effort for more than a decade, and several model systems using 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 the number of animals 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 of 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.

Review of Research Progress

Many transgenic models are available for various investigational uses, but three transgenic models have been most widely used for carcinogen identification: Trp53+/–, Tg/AC, and RasH2. We selected these three models 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, and only their main features are briefly reviewed here. They were developed based on dysregulation of either the Trp53 tumor-suppressor gene or the rasproto-oncogene, both of which are critical to cancer development and which 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.
that may arise independently and confound a cancer bioassay (Haseeman and Elwell 1996; Karstardt and Haseman 1997). The Trp53 model 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, 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). Because Trp53 +/– mice only carry one copy (germline) of the gene, these mice were expected, according to the two-hit hypothesis (Knuudson 1996; Knuudson 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 haplo-insufficient phenotype such that a second (p53 LOH) event is not required (French et al. 1990; Venkatachalam et al. 1998).

**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. Therefore, short-term (26 week) exposure to test and positive control agents during the period between 7 and 33 weeks of age makes it possible to distinguish between treatment-induced and spontaneous tumors.

**Table 1. Comparison of results from 14 known human carcinogens tested in rodent NCI/NTP cancer bioassays, Salmonella (Sal) and/or in vivo micronuclei (Mn) genotoxicity assays, and/or three transgenic mouse cancer bioassays.**

| Agent             | CAS no. | IARC group | NTP ROC | NCI/NTP bioassays | Genotoxicity (Sal; Mn) | p53 +/- | Tg AC | RasH2 |
|-------------------|---------|------------|---------|-------------------|------------------------|---------|-------|-------|
| Benzene           | 71-43-2 | 1          | Known   | +; +; +; + (NT 1986b) | +; + (Storer et al. 2001) | +d; + (Eastin et al. 1998) | +d; + (Blanchard et al. 1998; Spalding et al. 1999) | +g; + (Usui et al. 2001) |
| Cyclophosphamide  | 6055-19-2 | 1         | Known   | +; +; +; +; + (Weisburger 1977) | + (Storer et al. 2001) | +d; + (Eastin et al. 2001) | +d; + (Usui et al. 2001) | +g; + (Usui et al. 2001) |
| Melphalan         | 148-82-3 | 1         | Known   | +; +; +; +; +; + (Weisburger 1977) | + (Eastin et al. 1998; Storer et al. 2001) | +d; + (Eastin et al. 1998; Storer et al. 2001) | +d; + (Usui et al. 2001) | +g; + (Usui et al. 2001) |
| Cyclosporin A     | 79217-60-0 | 1       | Known   | NT | +; +; +; + (Storer et al. 2001) | -; - (Eastin et al. 1998; Storer et al. 2001) | +d; + (Eastin et al. 1998; Storer et al. 2001) | +d; + (Usui et al. 2001) |
| Diethy stilbestrol | 56-53-1 | 1          | Known   | NT | -; NT | -; - (Eastin et al. 1998; Storer et al. 2001) | +d; - (Eastin et al. 1998; Storer et al. 2001) | +d; - (Usui et al. 2001) |
| 17b-Estradiol     | 50-28-2 | 1          | Reasonable | NT | +; +; +; + (Storer et al. 2001) | -; - (Eastin et al. 1998; Storer et al. 2001) | +d; + (Eastin et al. 1998; Storer et al. 2001) | +d; + (Usui et al. 2001) |
| TCDD²             | 1746-0-1 | 1          | Known   | +; +; +; + (NCI/NTP 1982a) | -; - (Eastin et al. 1998; Storer et al. 2001) | +d; + (Eastin et al. 1998; Storer et al. 2001) | +d; + (Usui et al. 2001) | NT |
| UVR (312–450 nM)  | NA      | N          | Known   | NT | +; +; +; + (Storer et al. 1986b) | +d (Liang et al. 1999) | +d (Trempeus et al. 1998) | NT |
| Asbestos fibrils   | 1332-21-4 | 1         | Known   | +; +; +; + (NCI/NTP 1982a) | -; - (NT 1986a) | +d (Maronpot et al. 2000; Usui et al. 2001) | +d; + (Usui et al. 2001) | NT |
| Beryllium         | 7440-41-7 | 1          | Known   | NT | -; - (NT 1986a) | +d (Fitch et al. 1998) | NT | NT |
| Plutonium-239     | NA      | N          | Known   | NT | +; +; +; + (NT 1986a) | +d (Fitch et al. 1998) | NT | NT |
| Cobalt-60 (LET)   | NA      | N          | Known   | NT | +; +; +; + (NT 1986a) | +d (Fitch et al. 1998) | NT | NT |
| Sodium arsenate   | 7784-46-5 | 1         | Known   | NT | -; -; -; - (NT 1986a) | +d (Liang et al. 1999) | NT | NT |
| Thio-TEPA         | 52-24-4 | 1          | Known   | NT | +; +; +; + (NT 1986a) | +d (Maronpot et al. 2000; Usui et al. 2001) | +d; + (Usui et al. 2001) | NT |

Abbreviations: –, negative; +, positive; ±, equivocal; d, dermal; f, food; g, gavage; i, inhalation; ip, intraperitoneal; LET, linear energy transfer; NT, not tested or no published record; sc, subcutaneous; wb, whole body. Individual results were found in the cited references or in the IARC (2002) or the NTP databases (NTP 2002). NCI/NTP peer-reviewed conclusions are reported for male rat, female rat, male mouse, and 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.

²Both dermal and gavage studies in the Tg.AC mice employed ethinyl estradiol (CAS no. 57-63-6), a synthetic form of 17β-estradiol.
induce transformation in NIH3T3 cells. Approximately three 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- to 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 comparisons.
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 nongenetically altered co-isogenic reference controls. 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 NTP carcinogenicity testing is prediction of human carcinogenicity of chemicals, the merit of each transgenic model was evaluated by determining the ability of the model to identify human carcinogens. Classification of human carcinogens was based on evaluations by the NTP Report on Carcinogens (ROC) (NTP 2002) and the International Agency for Research on Cancer (IARC 2002) chemical evaluations/classifications. Both the ROC 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 IARC (group 1) or the ROC requires definitive data from human epidemiologic 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 in the 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 ROC has no equivalent to IARC group 3 and does not list such chemicals. Rodent carcinogenicity was not used as the primary targeted response in our analysis. Nevertheless, for completeness we considered the correlation of each transgenic model with the outcomes of National Cancer Institute (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 nongenotoxic chemicals, as defined by either a positive result in the Salmonella (Ames) test and/or in vivo rodent micronucleus assay.

| Agent                      | CAS no. | IARC group | ROC | NCI/NTP bioassays | Genotoxicity (Sal, Mn) | p53+/− | Tg.AC | RasH2                |
|----------------------------|---------|------------|-----|-------------------|------------------------|--------|-------|----------------------|
| p-Anisidine                | 90-04-0 | NE         | Not listed | +, − | −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 | +; NT | −g (Tennant et al. 1999) | −d (Tennant et al. 1999) | NT |
| 2,6-Diaminotoluene         | 620-40-5| NE         | Not listed | −, −, −, −f | +; NT | −g (Tennant et al. 1999) | −d (Tennant et al. 1999) | NT |
| 8-Hydroxyquinoline         | 148-24-3| 3          | Not listed | −, − | +; −f (Eastin et al. 1998) | −d (Eastin et al. 1998) | NT |
| Coconut oil diethanolamine | 69603-42-9| NE         | Not listed | −, −, −, +d | +; NT | −d (Spalding et al. 2000) | −d (Spalding et al. 2000) | NT |
| Diethanolamine             | 111-42-2| 3          | Not listed | −, −, − | −d (Spalding et al. 2000) | −d (Spalding et al. 2000) | NT |
| Ethyl acrylate             | 140-88-5| 2B         | Delisted | +, +, +, +g | −, − | −d (Nylander-French and French 1998, Tise et al. 1997) | +g (Yamamoto et al. 1998b) |
| Furfuryl alcohol           | 98-00-0 | NE         | Not listed | +, +, +, i | −, − | −d (Spalding et al. 2000) | −d (Spalding et al. 2000) | NT |
| Lauric acid diethanolamine | 120-40-1| NE         | Not listed | −, −, +d | −f (Spalding et al. 2000) | +d (Spalding et al. 2000) | NT |
| Methylisocyanide           | 924-42-5| 3          | Not listed | −, −, +, + | −, − | −g (Tennant et al. 1995) | −d, −g (Eastin et al. 1998) | NT |
| Methylphenidate            | 298-59-9| NE         | Not listed | −, −, −, −f, + | −, − | −f (Tennant et al. 1995) | −d (Tennant et al. 1999) | NT |
| Pyridine                   | 110-86-1| 3          | Not listed | +, +, +, +w | −, − | −g (Spalding et al. 2000) | −d (Spalding et al. 2000) | NT |
| Reserpine                  | 50-55-5 | 3          | Reasonable | −, − | −f (Tennant et al. 1995) | −d, −g (Tennant et al. 1995) | −f (Yamamoto et al. 1998b) |
| Rotenone                   | 83-79-4 | NE         | Not listed | −, −, −, −f | −, − | −f (Eastin et al. 1998) | +d, −g (Eastin et al. 1998) | −g (Yamamoto et al. 1998b) |
| Resorcinol                 | 108-46-3| 3          | Not listed | −, −, − | −d (Eastin et al. 1998) | +d (Eastin et al. 1998) | −g (Maronpot et al. 2000) |
| Oleic acid diethanolamine  | 93-83-4 | NE         | Not listed | −, −, −, −d | −, − | −d (Spalding et al. 2000) | −d (Spalding et al. 2000) | NT |
| Clofibrate                 | 637-07-0| 3          | Not listed | −, −, − | −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 (Storer et al. 2001) | −f (Storer et al. 2001) | −f (Usui et al. 2001) |
| Methylyliden HCl           | 135-23-9| NE         | Not listed | +, +, NT, NT | −, −, − | −g, −f (Storer et al. 2001) | −d (Eastin et al. 2001) | −g (Yamamoto et al. 1996) |
| Haloperidol                | 52-88-8 | NE         | Not listed | −, − | −f (Storer et al. 2001) | NT | −g (Usui et al. 2001) | −g (Usui et al. 2001) |
| Chlorpromazine HCl         | 69-09-0 | NE         | Not listed | −, − | −f (Storer et al. 2001) | NT | −g (Usui et al. 2001) | −g (Usui et al. 2001) |
| Metaproterenol             | 52962-23-4| NE         | Not listed | −, − | −f (Storer et al. 2001) | NT | −f (Yamamoto et al. 1998b) |
| WY-14643                   | 117-81-7| 3          | Reasonable | +, +, +f | −, − | −f (Storer et al. 2001) | −f (Storer et al. 2001) | −f (Storer et al. 2001) |
| Sulfinpyrazone             | 723-46-6| 3          | Not listed | −, −, − | −f (Storer et al. 2001) | −f (Storer et al. 2001) | −f (Usui et al. 2001) |
| Sulfapyrazone              | 127-69-5| 3          | Not listed | −, −, − | −f (Storer et al. 2001) | −f (Storer et al. 2001) | −f (Usui et al. 2001) |
| Ampicillin                 | 7171-48-2| 3          | Not listed | −, −, − | −f (Storer et al. 2001) | −f (Storer et al. 2001) | −f (Usui et al. 2001) |

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A total of 99 chemicals have been studied in one or more of these three transgenic models. For this analysis, we divided these chemicals into three groups: a) known human carcinogens (IARC group 1 and/or ROC known; 14 chemicals, Table 1); b) probable/possible human carcinogens (IARC groups 2A and 2B or ROC reasonably anticipated; 32 chemicals, Table 2); and c) chemicals with inadequate evidence of carcinogenicity (IARC group 3, NTP bioassay negative, and/or not listed in the ROC or by IARC; 53 chemicals, Table 3).

Tables 1–3 identify each chemical by Chemical Abstracts Service (CAS) number and give the IARC and/or the 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, 10 possible strategies were considered for using transgenic models to identify chemicals as known or suspected human carcinogens or as noncarcinogens. For comparison, the standard 2-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, 12 strategies in all were considered:

- **Strategy 1:** NTP rat bioassay
- **Strategy 2:** NTP rat bioassay plus the Trp53+/– model for genotoxic chemicals
- **Strategy 3:** Tg.AC model
- **Strategy 4:** RasH2 model
- **Strategy 5:** NTP rat bioassay plus the Trp53+/– model for genotoxic chemicals
- **Strategy 6:** Tg.AC model for nongenotoxic chemicals
- **Strategy 7:** RasH2 model for nongenotoxic chemicals
- **Strategy 8:** NTP rat bioassay plus the Trp53+/– model for genotoxic chemicals
- **Strategy 9:** Tg.AC model for all chemicals
- **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

| Agent | CAS No. | IARC | NTP ROC | NCI/NTP bioassays | Genotoxicity (Sal, Mn) | p53+/– | Tg.AC | RasH2 |
|-------|---------|------|---------|------------------|-----------------------|-------|-------|-------|
| o-Mannitol | 69-65-8 | NE | Not listed | NCI/NTP 1982(d) | −; −; −; −f | −; − | −f (Storer et al. 2001) | NT |
| 1,1,2-Trichloroethane | 79-00-5 | 3 | Not listed | NCI/NTP 1979g | −; −; +; + | NT | −g (Yamamoto et al. 1998b) |
| Xylenes (mixed) | 1330-20-7 | 3 | Not listed | NCI/NTP 1979g | −; −; −; −g | −; NT | NT | −g (Yamamoto et al. 1998b) |
| Furfural | 98-01-1 | 3 | Not listed | NCI/NTP 1990c | +; −; −; +; + | NT | NT | +g (Yamamoto et al. 1998b) |
| 5-Nitro-o-toluidine | 99-55-8 | 3 | Not listed | NCI/NTP 1990c | −; −; −; −f | +; NT | NT | −f (Yamamoto et al. 1998b) |
| Benzenethonium chloride | 121-54-0 | NE | Not listed | NCI/NTP 1995b | −; −; −; −f | −; NT | NT | −f (Spalding et al. 1999) |
| o-Benzyl-p-chlorophenol | 120-32-1 | NE | Not listed | NCI/NTP 1994 | −; −; −; −f | −; NT | NT | −d (Spalding et al. 1999) |
| 2-Chloroethanol | 107-07-3 | NE | Not listed | NCI/NTP 1985a | −; −; −; −f | −; −NT | NT | −d (Spalding et al. 1999) |
| Phenol | 108-95-2 | 3 | Not listed | NCI/NTP 1990g | +; −; −; −; − | −; + | + (Spalding et al. 1999) |
| Triethanolamine | 102-71-6 | 3 | Not listed | NCI/NTP 1999g | +; −; −; −; −f | −; −; − | −d (Spalding et al. 1999) |
| Acetic anhydride | 108-24-7 | NE | Not listed | NCI/NTP 1999g | −; −; −; − | −; −NT | NT | −d (Spalding et al. 1999) |
| 2,4-Dinitro-1-fluorobenzene | 70-34-8 | NE | Not listed | NCI/NTP 1995b | −; −; −; −f | −; −; − | −d (Spalding et al. 1999) |
| Bisopropylcarboximide | 690-13-0 | NE | Not listed | NCI/NTP 1999g | −; −; −; −f | −; −; NT | NT | NT |
| Dicyclohexylcarbodiimide | 538-75-0 | NE | Not listed | NCI/NTP 1995b | −; −; −; −f | −; −; − | −d (Spalding et al. 1999) |
| Fluoroacetone acetone | 67-73-2 | NE | Not listed | NCI/NTP 1995b | −; −; −; −f | −; −; − | −d (Spalding et al. 1999) |
| Tripropylene glycol diacylate | 42098-66-5 | NE | Not listed | NCI/NTP 1995b | −; −; −; −f | −; −; − | −d (Spalding et al. 1999) |
| p-Limonene | 5989-27-5 | 3 | Not listed | NCI/NTP 1995b | −; −; −; −f | −; −; − | −d (Spalding et al. 1999) |
| Foreign body (transponder) | NA | NE | Not listed | NCI/NTP 1995b | −; −; −; −f | −; −; − | −d (Spalding et al. 1999) |
| Acetone | 67-64-1 | NE | Not listed | NCI/NTP 1995b | −; −; −; −f | −; −; − | −d (Spalding et al. 1999) |
| Benzoyl peroxide | 94-36-0 | 3 | Not listed | NCI/NTP 1995b | −; −; −; −f | −; −; − | −d (Spalding et al. 1999) |
| Ethanol | 64-17-5 | NE | Not listed | NCI/NTP 1995b | −; −; −; −f | −; −; − | −d (Spalding et al. 1999) |
| Methyl ethyl ketone peroxide | 133823-4 | NE | Not listed | NCI/NTP 1995b | −; −; −; −f | −; −; − | −d (Spalding et al. 1999) |
| 4-Nitro-o-phenylenediamine | 99-56-9 | 3 | Not listed | NCI/NTP 1995b | −; −; −; −f | −; −; − | −d (Spalding et al. 1999) |
| 6-Nitrobenzimidazole | 94-52-0 | NE | Not listed | NCI/NTP 1995b | −; −; −; −f | −; −; − | −d (Spalding et al. 1999) |
| Cholestryamine | 10141-12-6 | NE | Not listed | NCI/NTP 1995b | −; −; −; −f | −; −; − | −d (Spalding et al. 1999) |
| Magnetic fields (60 mHz) | NA | NE | Not listed | NCI/NTP 1995b | −; −; −; −f | −; −; − | −d (Spalding et al. 1999) |

Abbreviations: −, negative; +, positive; a, equivocal; d, dermal; f, food; g, gavage; l, inhalation; i-o, initiation-promotion; NE, not evaluated; NT, not tested; sc, subcutaneous; w, drinking water; wb, whole body. Individual results are found in the cited references or in the IARC (2002) or the NTP databases (NTP 2002). NCI/NTP peer-reviewed conclusions are reported for male F344 rat, female F344 rat, male B6C3F1 mouse, and female B6C3F1 mouse, respectively. Results from transgenic models are presented as the summary conclusion for each route of exposure using one or both sexes.
• 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: a) a chemical was considered genotoxic if either the Salmonella or in vivo micronuclei assays were positive; b) a chemical was considered nongenotoxic only if both assays were negative; and c) 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/ROC 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. Although 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 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 ensure 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, 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 reassignment 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 on the authoritative judgments of IARC and the ROC. Likewise, we accepted the authors’ interpretations of the data. However, there was uniformity of study design and interpretation for a sizable number of the studies involved in the International Life Sciences Institute (ILSI) Alternatives to Carcinogenicity Testing consortium. It was beyond the scope of our analysis to reevaluate and reinterpret each individual study.

Fourth, we 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 multisite, multisex, carcinogenic effects. Although future refinements in statistical evaluation may permit subclassification 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+/−, 77% 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

| Table 4. Summary performance of each strategy versus likely human cancer. |
|---|
| Strategy | Positive for carcinogens | Negative for noncarcinogens | Positive for noncarcinogens | Negative for carcinogens | Overall accuracy |
| Trp53+/− | 21 | 12 | 0 | 10 | 77% (33/43) |
| Trp53+/− (genotoxic) | 16 | 5 | 0 | 4 | 84% (21/25) |
| Tg.AC | 17 | 10 | 2 | 6 | 77% (27/35) |
| RasH2 | 21 | 9 | 0 | 7 | 81% (30/37) |
| Trp53+/− (genotoxic), RasH2 (nongenotoxic) | 17 | 10 | 0 | 6 | 82% (27/33) |
| Trp53+/− (genotoxic), RasH2 (all) | 30 | 7 | 0 | 4 | 90% (34/31) |
| Trp53+/− (genotoxic), Tg.AC (nongenotoxic) | 21 | 8 | 0 | 6 | 83% (25/30) |
| Trp53+/− (genotoxic), Tg.AC for all | 25 | 7 | 2 | 4 | 84% (32/38) |

All chemicals in Tables 1 and 2 are included as human carcinogens, but only those chemicals in Table 3 with negative results in the NTP bioassay results are regarded as true human noncarcinogens. 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% (9/9) | 57% (4/7) | 79% (22/28) |
| Group 2A | 62% (5/8) | 50% (2/4) | 100% (9/9) | 76% (16/21) |
| Group 2B | 55% (6/11) | 64% (7/11) | 89% (9/13) | 63% (22/35) |
| Group 3 | 0% (0/13) | 21% (3/14) | 29% (4/14) | 17% (7/41) |
| Not evaluated | 7% (1/15) | 29% (7/24) | 0% (0/8) | 17% (8/47) |

*Two of the three that were not positive were equivocal. Includes 7,12-dimethylbenzanthracene, 4-nitroquinoline N-oxide, and 4-hydroxyaminquinoline-1-oxide.
that use more than one transgenic model (strategies 5–8; as defined above) were somewhat more predictive, ranging from 82 to 90%. The best strategy (Trp53+/- for genotoxic chemicals and RasH2 for all chemicals; strategy 6) correctly predicted the human outcome for 90% of the agents (Table 4). Strategy 8 (Trp53+/- for genotoxic chemicals and Tg.AC for all chemicals) was only slightly less predictive (84%).

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 shown in Table 5. Note that a) the transgenic models (considered collectively) are more apt to be positive for the more certain human carcinogens (IARC groups 1 and 2A) than for the less certain human carcinogens (group 2B); b) there is a striking difference in the proportion of positive transgenic responses between the 1/2A/2B chemicals and the group 3 chemicals or those not evaluated; and c) the IARC group 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 weaknesses. 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.

Table 6. Summary of performance of each strategy versus likely human cancer when all chemicals in Table 3 are regarded as true human noncarcinogens.

| Strategy | Positive for carcinogens | Negative for noncarcinogens | Positive for noncarcinogens | Negative for carcinogens | Overall accuracy |
|----------|--------------------------|-----------------------------|-----------------------------|--------------------------|------------------|
| Trp53+/- | 21                       | 27                          | 1                           | 10                       | 81% (48/59)      |
| Trp53+/- (genotoxic) | 16                      | 6                           | 0                           | 4                        | 85% (25/26)      |
| Tg.AC    | 21                       | 18                          | 10                          | 6                        | 74% (44/62)      |
| RasH2    | 21                       | 18                          | 5                           | 7                        | 76% (35/45)      |
| Trp53+/- (genotoxic); RasH2 (nongenotoxic) | 17                      | 18                          | 3                           | 6                        | 80% (35/44)      |
| Trp53+/- (genotoxic); RasH2 (all) | 20                      | 15                          | 5                           | 4                        | 83% (43/53)      |
| Trp53+/- (genotoxic); Tg.AC (nongenotoxic) | 21                       | 23                          | 3                           | 6                        | 83% (44/53)      |
| Trp53+/- (genotoxic); Tg.AC for all | 25                      | 22                          | 10                          | 4                        | 77% (47/61)      |
| NTP rodent bioassay | 27                      | 17                          | 18                          | 0                        | 69% (40/58)      |
| NTP rat bioassay; Tg.AC (nongenotoxic) | 35                      | 13                          | 9                           | 0                        | 84% (48/57)      |
| NTP rat bioassay; RasH2 (nongenotoxic) | 33                      | 12                          | 8                           | 0                        | 85% (45/53)      |
| NTP rat bioassay; genotoxicity | 36                      | 7                           | 23                          | 0                        | 65% (43/66)      |

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 NTP rodent noncarcinogens; negative for carcinogens, negative assay results for NTP rodent carcinogens.

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 |
|----------|--------------------------|-----------------------------|-----------------------------|--------------------------|------------------|
| Trp53+/- | 7                        | 12                          | 0                           | 16                       | 54% (19/35)      |
| Trp53+/- (genotoxic) | 7                      | 5                           | 0                           | 4                        | 75% (12/16)      |
| Tg.AC    | 14                       | 10                          | 2                           | 14                       | 60% (24/40)      |
| RasH2    | 15                       | 9                           | 0                           | 8                        | 75% (24/32)      |
| Trp53+/- (genotoxic); RasH2 (nongenotoxic) | 10                      | 10                          | 0                           | 0                        | 0% (0/10)        |
| Trp53+/- (genotoxic); RasH2 (all) | 17                      | 7                           | 0                           | 10                       | 89% (24/27)      |
| Trp53+/- (genotoxic); Tg.AC (nongenotoxic) | 10                      | 8                           | 0                           | 14                       | 56% (18/32)      |
| Trp53+/- (genotoxic); Tg.AC for all | 16                      | 7                           | 2                           | 13                       | 61% (23/38)      |

Definitions: positive for carcinogens, positive assay results for NTP rodent carcinogens; negative for noncarcinogens, negative assay results for NTP rodent noncarcinogens; positive for noncarcinogens, positive assay results for NTP rodent noncarcinogens; negative for carcinogens, negative assay results for NTP rodent carcinogens.

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/ROC known carcinogens among the total of 53 chemicals tested in both (Table 6).

In contrast, the NTP two-species bioassay identified all IARC/NTK 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 determinations 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 76%, and Tg.AC 74%; Table 6). Of course, this difference is also reflected in the modest success (54–75%) 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 become 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., diethylhexyl phthalate) we used the most recent determination. Moreover, these 18 chemicals collectively were positive in only 23% (7/30) 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.
Because 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 nongenotoxic chemicals) provided an improvement in performance. Overall concordance increased to 84% versus the 69% of the bioassay itself. More important, 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 similar results, with an overall concordance of 85% (45/53), or just 15% (8/53) 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 nongenotoxic 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-methoxyacrylamide, 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 adenomas (males only). N-Methoxyacrylamide 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 the Trp53+/– model. Thus, to provide a more complete assessment of possible testing strategies, we compared an additional strategy (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 65% (43 of 66), but it has 23 positive results for noncarcinogens out of 66 chemicals (35%). 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 use the standard rat bioassay in conjunction with Trp53+/– for genotoxic chemicals and Tg.AC or RasH2 for nongenotoxic 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 ensure 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 used (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 transgens 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 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 8 of the 53 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 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 suspected 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 a 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 to evaluate new models and/or modify 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 itself to the evaluation and validation of any new model, transgenic or otherwise.

Development of models. In the current analysis we 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 (p16ink4a and p19Arf 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 mutatation 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 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 develop an arsenal of models. Likewise, site-specific or mechanism-specific models could be developed and used 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.

Correction
After the manuscript was published online in EHP-In-Press, the authors discovered errors in several values, most of them in Tables 4–7. These errors have been corrected. None of the errors affected the interpretation of the data.

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