

Responses of Transgenic Mouse Lines p53^{+/-} and Tg·AC to Agents Tested in Conventional Carcinogenicity Bioassays

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The haplo-insufficient p53 knockout (p53^{+/-}) and zetaglobin v-Ha-ras (Tg·AC) transgenic mouse models were compared to the conventional two rodent species carcinogen bioassay by prospectively testing nine chemicals. Seven of the chemicals classified as carcinogens in the conventional bioassay induced tumors in the liver or kidneys of B6C3F₁ mice, and one (pentachlorophenol) also induced tumors in other tissues. Only three chemicals, furfuryl alcohol, pyridine, and pentachlorophenol, induced tumors in rats. The tumorigenic effect of pyridine was seen in F344 rats but not in Wistar strain rats. None of the chemicals induced tumors in the p53^{+/-} transgenic mice, which is consistent with the absence of genotoxicity of these chemicals. Only two of the seven nongenotoxic carcinogens were positive in the Tg·AC model (lauric acid diethanolamine and pentachlorophenol). These results show that these transgenic models do not respond to many chemicals that show strain- or species-specific responses in conventional bioassays.

Key Words: Tg·AC, p53^{+/-}; transgenic; mouse bioassays; cancer.

Environmental or chemical-induced carcinogenesis is a multicomponent process involving the chemical, physical, and biological properties of the inducing agent as well as the genetic complexity of the exposed organism. Through the genetic and molecular analyses of tumors, the development of neoplasia has become recognized as a multigene, multistep process that can involve alterations in specific genes via mutation or functional dysregulation. The complexity of these events is the principal basis for the difficulties associated with the interspecies extrapolation of rodent carcinogenicity results to human risk. The studies reported here were based on the hypothesis that transgenic mouse lines that possess an inducible v-Ha-ras gene (Tg·AC) (Cannon *et al.*, 1997; Leder *et al.*, 1990) or that carry an inactivated allele of the p53 tumor suppressor gene (p53^{+/-}) (Harvey *et al.*, 1993) would exhibit enhanced sensitivity to agents with carcinogenic activity and

reduce the complexity of identifying potential carcinogens (Tennant *et al.*, 1999). An important feature of these transgenic mouse lines is that the 26-week chemical exposure studies are completed before any significant strain-related sporadic tumor incidence occurs that might confound interpretation of chemically induced effects.

The process of validating assays such as transgenic mouse models as alternatives to the conventional rodent bioassay is difficult. The conventional rodent bioassay, which has been in use for over three decades, has been accorded credibility through the experience of conducting hundreds of tests. Although a great deal of uncertainty is encountered in efforts to extrapolate the outcome of such tests to human risk, the bioassay is generally considered the best available method. The primary basis for confidence in extrapolating animal results to human risk is that most of the substances known to be human carcinogens have tested positive in the conventional bioassay. The primary uncertainty is whether all substances inducing a positive response in the rodent represent a human risk.

Previous studies have indicated the p53^{+/-} line can discriminate between genotoxic carcinogens and noncarcinogens (French *et al.*, 1999; Tennant, 1995; Tennant, 1999) and that the Tg·AC model can discriminate between both genotoxic and nongenotoxic carcinogens and noncarcinogens (Spalding *et al.*, 1999; Tennant, 1995). In a recent study, Eastin *et al.* (1998) tested four human carcinogens in the Tg·AC and p53^{+/-} lines. One other human carcinogen, benzene, had been tested previously (French *et al.*, 1999; Spalding *et al.*, 1999). The two mutagenic substances, benzene and melphalan, induced a positive response in the p53^{+/-} assay. These chemicals, as well as the nonmutagens, cyclosporin A, diethylstilbestrol, and 2,3,7,8-tetrachlorodibenzodioxin, were all positive in the Tg·AC line. These results suggest that in parallel to the conventional rodent bioassay, the transgenic models are sensitive to known human carcinogens. Although these results are informative, several issues remain unresolved. For example, a major concern has been that genetically altered rodents would prove to be more sensitive than conventional rodents and thus

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provide additional false positive responses for agents that would be of little risk to humans (Ashby, 1997).

In an effort to make the evaluation process as objective as possible, a group of nine agents being tested for carcinogenicity in the conventional bioassay were used to prospectively evaluate the specificity of the two transgenic mouse lines. These studies complement other Tg·AC and p53^{+/-} mouse studies that have evaluated carcinogens and noncarcinogens in a retrospective fashion (Eastin, *et al.*, 1998; Spalding *et al.*, 1999, Tennant *et al.*, 1995). The selection of these agents for carcinogenicity testing was made by the National Toxicology Program (NTP), and their evaluation in the transgenic lines therefore eliminates investigator bias. Lauric acid diethanolamine (LADA), coconut oil acid diethanolamine (COD), oleic acid diethanolamine (OAD), 1-chloro-2-propanol (CP), pyridine (PY), and pentachlorophenol (PCP), were tested in both Tg·AC and p53^{+/-} transgenic lines. Three additional agents, diethanolamine (DEA), triethanolamine (TEA), and furfuryl alcohol (FA), were tested in Tg·AC mice. Fortuitously, several of the agents were tested by the NTP via dermal application, which allows for the results of the Tg·AC studies to be compared directly with those obtained in the NTP bioassay. However, for other agents such as furfuryl alcohol, which was tested by inhalation in the conventional bioassay, it was not possible to duplicate the route of exposure in either the Tg·AC or the p53^{+/-} transgenic lines. In general, the same doses used in the conventional bioassay were used in the transgenic bioassays; however, in some studies, higher doses were used. Under such circumstances, it was judged that a negative response in the transgenic lines at the higher doses would have greater credibility, as the duration of exposure was much less than that in the conventional bioassay.

In this report, we compare the activity of these agents in the transgenic mice with their activity in the conventional bioassay and discuss the implications of these observations.

MATERIALS AND METHODS

Test and control chemicals. Acetone (ACS grade, $\geq 99.5\%$ purity), and 95% ethanol (vendor purity, 95%) were obtained from Midwest Research Institute (MRI), Kansas City, MO. Tap water from the Durham County water supply served as the vehicle for 1-chloro-2-propanol and pyridine and as the vehicle control. Analytical water sample results did not reveal any contaminants that would have affected the study. 12-O-Tetradecanoyl-phorbol-13-acetate (TPA, approximately 99% pure), obtained from Sigma Chemical Co., St. Louis, MO, served as a positive control.

1-Chloro-2-propanol (CP) (CAS No. 127-00-4), furfuryl alcohol (FA) (CAS No. 98-00-0), and oleic acid diethanolamine (OAD) (CAS No. 93-83-4) were obtained from MRI, the NTP chemistry contractor. Coconut oil diethanolamine (COD) (CAS No. 68603-42-9) and pyridine (PY) (CAS No. 110-86-1) were obtained from Battelle Memorial Institute (BMI), Columbus, OH. Diethanolamine (DEA) (CAS No. 111-42-2), lauric acid diethanolamine (LADA) (CAS No. 120-40-1), triethanolamine (TEA) (CAS No. 102-71-6), and pentachlorophenol (PCP) (CAS No. 87-86-5) were obtained from Research Triangle Institute (RTI), Research Triangle Park, NC. DEA, TEA, LADA, CP, and FA were from the same chemical batch (lot number) as that used in the conventional 2-year bioassay conducted by the National Toxicology Program.

Animals. Hemizygous or homozygous female Tg·AC mice (zetaglobin promoted v-Ha-ras on a FVB background) and heterozygous male and female p53^{+/-} mice (C57BL/6-Trp53(+/-)tm1Dol; N5) were obtained from the NTP breeding colony at Taconic Laboratory Animals and Services, (Germantown, NY) at 4 to 5 weeks and 5 to 8 weeks of age (Tg·AC and p53^{+/-}, respectively). Homozygous female Tg·AC mice were used in two earlier studies with DEA and TEA. Hemizygous female Tg·AC mice were used in the studies with the remaining seven chemicals in order to agree with the choice made by the NTP to use Tg·AC hemizygous animals (Eastin *et al.*, 1998).

The female Tg·AC mice were group housed (either 4 or 5/cage), while the p53^{+/-} mice were either single (male) or group (female, 5/cage) housed in polycarbonate shoebox style cages with absorbent hardwood bedding (Beta Chips, Northeastern Products Corp., Warrensburg, NY) in an AALAC-accredited facility. Female mice were group housed as a cost-saving measure. For future studies, single housing is recommended. The cages, with stainless steel tops, were changed at least once a week (single housed) or twice a week (group housed). Mice were maintained on a 12-h (fluorescent) light:dark cycle. Relative humidity and temperature ranges across all studies were 7–94% and 59–82°F, respectively. At the start of the study, the Tg·AC mice were 14 weeks of age and the p53^{+/-} mice were 8–11 weeks of age. The Tg·AC mice were held longer than usual before the study start, because animal availability and delivery occurred prior to the completion of the chemical characterization of the chemicals to be administered.

Each mouse was uniquely identified by tail tattoo prior to being placed on study. At study start, the mice were weighed to the nearest 0.1 g and were allocated to treatment or control groups using a stratified (by cage mean body weight for Tg·AC mice) randomization procedure. Tg·AC mice that exhibited evidence of skin abrasions or bite wounds at the site of chemical application were eliminated from the study. All mice had free access to tap water. The p53^{+/-} mice were fed NIH-07 open block formula *ad libitum* (Ziegler Bros., Gardener, PA). Tg·AC mice were maintained *ad libitum* on Purina Picolab No. 5058 chow (Granville Milling, Creedmoor, NC). We have routinely used the Picolab No. 5058 diet because, in our experience, Tg·AC mice have a more favorable weight gain and are less aggressive. Picolab diet No. 5058 has a 9% fat content in contrast to the NIH-07 formula, which has a 5% fat content.

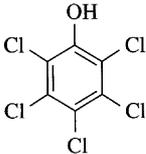
Treatment

Dose selection. Dose selection was based on the MTD (maximum tolerated dose) used in the 2-year conventional bioassay (Table 1). In most instances, doses higher than the MTD were used if those doses had not caused overt toxicity in the conventional 13-week subchronic assay.

Skin paint studies. All nine chemicals were administered topically to Tg·AC mice. DEA and TEA were administered to homozygous female mice, whereas hemizygous female Tg·AC mice were used for the other seven agents. The doses originally selected for the PCP study in Tg·AC mice were 3.0, 6.0, or 12.0 mg/mouse. However, overt toxicity was observed at the two higher doses after the first application. Thereafter, for the remainder of the study, the dose group receiving 12.0 mg was placed on 0.75 mg/day, and the dose group receiving 6.0 mg was placed on 1.5 mg/day. Three of the agents, COD, LADA, and OAD, were administered topically to p53^{+/-} mice.

The application site (from the dorsal interscapular region to a point ~1 cm from the base of the tail) was closely shaved prior to the first treatment; care was taken to avoid abrasions or cuts. The site of application (SOA) was shaved weekly or as needed prior to subsequent treatments. The topically applied test agents were solubilized in acetone or 95% ethanol and administered in 200- μ l volumes 5 \times /week for 20 weeks (Tg·AC) or 26 weeks (p53^{+/-}). At the time these studies were conducted, a 20-week dermal exposure period was the standard protocol used for studies in Tg·AC mice. We now recommend a 26-week exposure period by all routes of administration for both transgenic mouse models. Concurrent negative control groups were treated with 200 μ l acetone or 95% ethanol. The positive control agent for the Tg·AC studies was TPA 1.25 μ g 3 \times /week or 1.5 μ g, 2 \times /week for 20 weeks. A positive control

TABLE 1—Continued

Chemical report no.	Genotoxicity route	Chronic bioassay results tumor sites			
		♂ Rat	♀ Rat	♂ Mouse	♀ Mouse
Pyridine 	SA-; MN-				
	Dose	0, 7, 14, or 33 (0, 8, 17, or 36) ^b	0, 7, 14, or 33	0, 35, 65, or 110	0, 15, 35, or 70
	Call	SE (EE) ^b	EE	CE	CE
	Site and incidence	K 2/50, 4/48, 6/50, 10/49		L 38/50, 47/50, 46/49, 47/50	L 41/49, 42/50, 45/50, 44/50
TR-470	Water				
Pentachlorophenol 	SA-; MN-				
	Dose	0, 10, 20, or 30 stop exposure 60 mg/kg for 52 wks	0, 10, 20, or 30; stop exposure 60 mg/kg for 52 wks	0, 100, 200, or 600 ppm	0, 100, 200, or 600 ppm
	Call	NE (stop exposure = SE)	NE (stop exposure = NE)	CE	CE
	Site and incidence	MT 1/50, 0/50, 2/50, 0/50, 9/50 N 1/50, 3/50, 1/50, 0/50, 5/50		L 6/35, 19/48, 21/48, 34/49 AG 1/34, 4/48, 21/48, 45/49	L 1/34, 4/50, 6/49, 31/48 AG 0/35, 2/49, 2/46, 38/49 SP 0/34, 0/50, 2/50, 2/47
TR-349/483 ^c	Feed				

Note. NE, no evidence; EE, equivocal evidence; SE, some evidence; CE, clear evidence of carcinogenicity; L, liver; K, kidney; N, nose; AG, adrenal gland; SP, spleen; MT, mesothelial tissue.

^a Doses are in mg/kg unless noted otherwise and represent control, low, medium, high levels of administration.

^b Pyridine was also tested in male Wistar rats.

^c F344/N rats were tested using purified pentachlorophenol (~99% pure) and the results were reported in TR-483; B6C3F₁ mice were tested using Dowicide EC-7 (~91% pure) and the results were reported in TR-349.

group was not included in the p53^{+/-} mouse studies. Dose groups in the Tg·AC studies comprised 15–20 female mice. Dose groups in the p53^{+/-} studies comprised 10 female and 10 male mice.

Oral studies. CP and PY were delivered in water *ad libitum* 7 days/week to p53^{+/-} mice for 26 weeks. PCP was prepared as dietary admixtures in NIH-07 open formula at doses of 100, 200, or 400 ppm and fed *ad libitum* 7 days/week for 26 weeks.

Dose preparation. Dose preparations were prepared approximately every 3 weeks and were stored at 4°C. The stability of these preparations under these conditions had been demonstrated previously by NTP. The preparations for each substance were analytically analyzed for accuracy three or four times during each study. With few exceptions, all dose formulation samples were within 10% of the targeted concentrations. Those several formulations that varied more than 10% were not considered to have a negative impact on the studies.

Clinical observations. All mice were observed twice a day for morbidity and mortality (once a day on weekends and holidays). Detailed records of body weights and clinical observations were recorded once per week during the treatment and post-treatment periods. Observations included evidence of systemic or local toxicity, the number of skin tumors (skin painting studies) recorded after a thorough examination of the application site and all orifices, and the presence of odontomas or malocclusions (Tg·AC mice).

Papillomas at the site of application had to reach at least 1 mm in size and

persist for at least 3 weeks to be considered a “true” response. The latency time from the onset of treatment to the appearance of the first tumor and the maximum tumor burden was determined for each mouse. In comparing the frequency of papillomas among the different treatment groups, the maximum papilloma burden that occurred for each animal during the study period was used in the computation. Because an accurate accounting of tumor burdens exceeding 30 papillomas per mouse is problematic, an upper limit of 30 tumors was arbitrarily assigned when that limit was reached. Animals that did not survive the first 10 weeks of the study were not included in either data summaries or data calculations.

Necropsy and histopathology. Gross necropsies were conducted on all natural deaths and moribund sacrifice animals. Six weeks after the conclusion of treatment (Tg·AC mice) or at the completion of treatment (p53^{+/-} mice) (i.e., 26 weeks total), all surviving mice were euthanized and subjected to a complete necropsy, including collection of a cross section of the skin at the SOA for the dermal studies. Tissues from control and high-dose group animals only were fixed in 10% neutral buffered formalin (NBF), then trimmed, embedded in paraffin, cut at 5–6 microns, stained with hematoxylin and eosin (H&E), and examined microscopically. The following tissues were routinely examined during necropsy: kidney, liver, lungs, thymus, spleen, mandibular lymph node, mandibular salivary gland, stomach, adrenal glands, pituitary gland, thyroid gland, and mammary gland. In addition, any gross lesions were collected and fixed in 10% NBF. Tissues were submitted to NTP contractors

TABLE 2
Doses and Routes of Administration Used in the Transgenic Mouse Studies

Chemical	Genotoxicity	Tg·AC			p53 [±]		
		Route	Dose	Call	Route	Doses	Call
Diethanolamine	SA-; MN-	Skin paint	0, 5.0, 10.0, or 20.0 mg ^a	-		Not tested	
Triethanolamine	SA-; MN-	Skin paint	0, 3.0, 10.0, or 30.0 mg ^a			Not tested	
Coconut oil acid diethanolamine	SA-; MN+	Skin paint	0, 2.5, 5.0, or 7.5 mg ^a	-	Skin paint	0, 2.5, 5.0, or 7.5 mg ^a	-
Lauric acid diethanolamine	SA-; MN-	Skin paint	0, 5.0, 10.0, or 20.0 mg ^a	+	Skin paint	0, 5.0, 10.0, or 20.0 mg ^a	-
Oleic acid diethanolamine	SA-; MN nt	Skin paint	0, 0.4, 0.8, or 1.2 mg ^a	-	Skin paint	0, 0.4, 0.8, or 1.2 mg ^a	-
1-Chloro-2-propanol	SA+; MN-	Skin paint	0, 1.2, 6.0, or 12.0 mg ^a	-	Water	0, 250, 500, or 1000 ppm	-
Furfuryl alcohol	SA-; MN-	Skin paint	0, 0.25, 0.75, or 1.5 mg ^a	-		Not tested	
Pyridine	SA-; MN-	Skin paint	0, 1.5, 3.0, or 6.0 mg ^a	-	Water	0, 250, 500, or 1000 ppm (male) 0, 125, 250, or 500 ppm (female)	-
Pentachlorophenol	SA-; MN-	Skin paint	0, 0.75, 1.5, or 3.0 mg ^{a,b}	+	Feed	0, 100, 200, or 400 ppm	-

^a Doses are "dose/mouse".

^b Dose level was reduced from 12.0 mg to 0.75 and 6.0 mg to 1.5 mg/animal in 200 μ L acetone after one treatment due to mortality.

(Pathco, Research Triangle Park, NC, and Pathology Associates International, Durham, NC) for histologic processing and evaluation, respectively.

RESULTS

Nine chemicals (diethanolamine, triethanolamine, coconut oil acid diethanolamine, lauric acid diethanolamine, oleic acid diethanolamine, 1-chloro-2-propanol, furfuryl alcohol, pyridine, pentachlorophenol) that were undergoing evaluation for carcinogenicity in the conventional 2-year bioassay were tested prospectively for tumorigenic activity using the Tg·AC and p53^{+/-} transgenic mouse models. The chemical structure, evidence of genotoxicity based upon the *Salmonella* mutagenicity or *in vivo* erythrocyte micronucleus assays, the doses tested, and results of the conventional 2-year bioassay (i.e., tumor organ sites and specific tumor incidence data) are provided for each chemical in Table 1. Of the nine chemical agents tested, only two, OAD (NTP 1999d) and CP (NTP 1998), were judged to show no evidence (NE) of carcinogenicity in the conventional bioassay.

There was no evidence of carcinogenic activity for DEA (NTP 1999c), COD (NTP 1999f), LADA (NTP 1999e), or TEA (NTP 1999h) in F344/N rats, but all four chemicals were judged to show either some evidence (SE) or clear evidence (CE) of activity in male and/or female B6C3F₁ mice (Table 1). The mouse liver was the major target organ site for this group of agents. FA (NTP 1999a) induced adenomas in the nose of male rats (SE) and kidney tumors in male mice (SE). PY (NTP 1999g) induced kidney tumors in male rats (SE) and multiple hepatocellular carcinomas in male and female mice (CE). Wistar rats treated concurrently with PY at similar doses did not develop the kidney tumors induced in F344/N rats or tumors at any other organ site. PCP (NTP 1989, 1999b) was a transspecies carcinogen and induced nose tumors and mesothelial tumors in male rats (SE), and liver, adrenal gland, and

spleen tumors in male and female mice (CE). None of the seven carcinogenic chemicals induced tumors in female F344/N rats.

Fortuitously for the evaluation of the Tg·AC model, DEA, TEA, and the three diethanolamine condensates (COD, LADA, OAD) were all topically administered in the conventional bioassay (Table 1). Thus, they make possible a direct route-to-route comparison and eliminate route of exposure as an important variable in the comparative evaluation. All nine chemicals were topically administered for the Tg·AC studies. The three diethanolamine condensates were administered topically to p53^{+/-} mice. The routes of administration used for CP, PY, and PCP in p53^{+/-} mice were the same as those used in the 2-year bioassay (Table 2). One chemical, FA, was conducted in the conventional bioassay by inhalation, and because inhalation facilities were not available for the transgenic studies, it was evaluated only in the Tg·AC model via topical application. Dose selection for the transgenic mouse studies was based on the MTDs used in the conventional bioassay (Table 2). In most cases, the MTDs used in the transgenic studies were higher than the MTDs used in the conventional bioassay if the latter doses had not caused morbidity or reduced survival in the 90-day subchronic studies (Fig. 1). Figure 1 shows the relationship between the doses used in the conventional and transgenic bioassays for triethanolamine, diethanolamine, and the diethanolamine condensates.

Tg·AC Mice

Using a 20-week exposure protocol, homozygous Tg·AC mice were topically treated with DEA and TEA; the remaining seven chemicals were tested using hemizygous female Tg·AC mice. The treated mice were monitored closely and there was no evidence of chronic irritation or ulceration at the SOA during the exposure period at the doses tested.

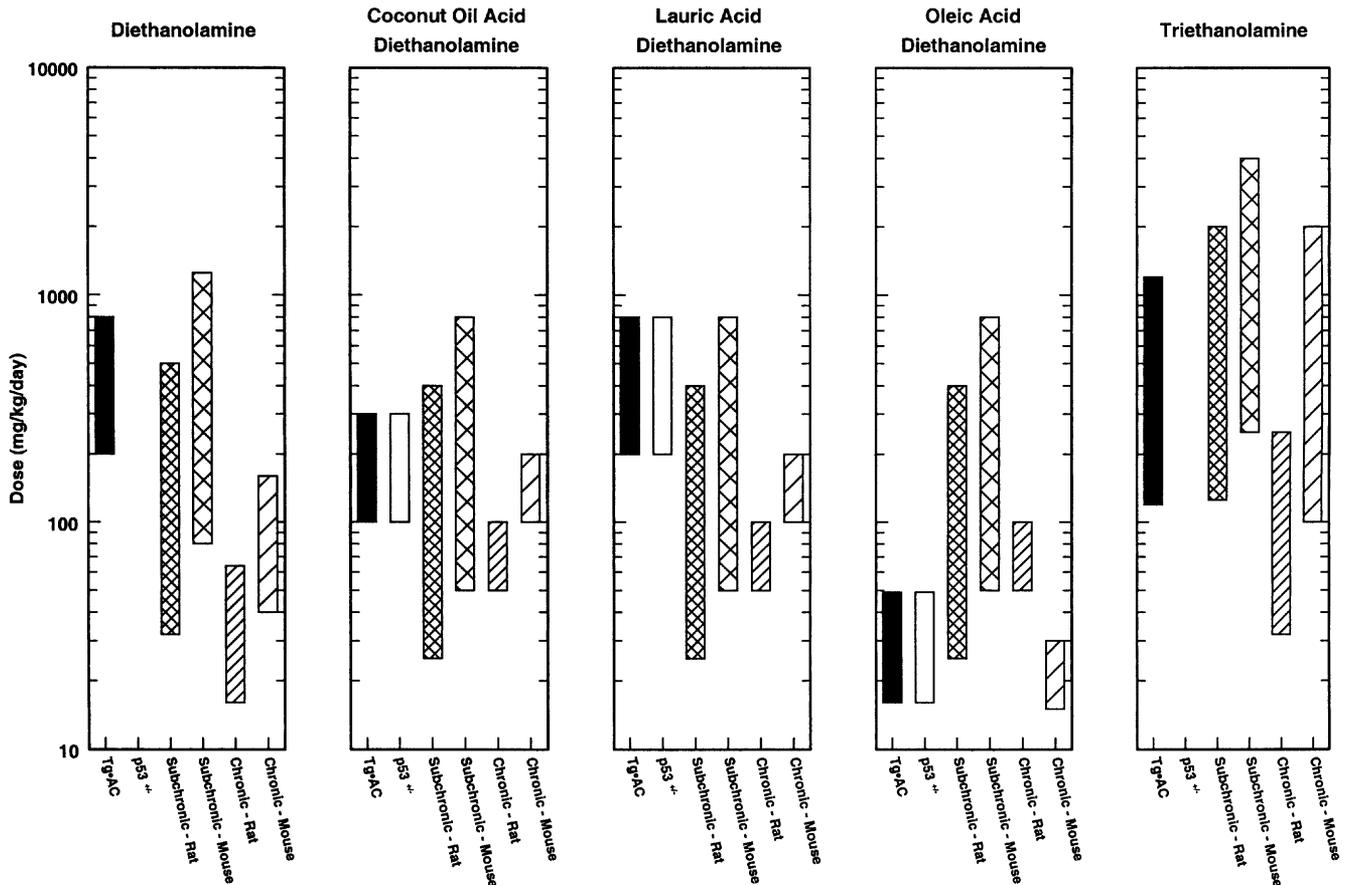


FIG. 1. The dose ranges, expressed as mg/kg/day, selected for topical application of diethanolamine, the three diethanolamine condensates, and triethanolamine in the Tg·AC and p53^{def} transgenic mouse studies are compared to the dose ranges used in the conventional subchronic and chronic bioassay.

Two agents, OAD and CP, that were inactive in the conventional bioassay were also inactive in Tg·AC mice (Table 3). The higher incidence (26%) of mice with papillomas among animals treated with OAD at 1.2 mg is likely due to a "cage effect," as four of the five mice with papillomas were cage mates. When female Tg·AC mice are group housed, a dominant female can inflict bite wounds on the other cage mates, and papillomas can occur at the wound sites. Only one other mouse in the other three cages receiving the same OAD dose developed a papilloma (one only) at the SOA. The tumor incidence among the acetone- and 95% ethanol- treated negative control groups was very low, with usually no more than a single mouse exhibiting a single papilloma at the SOA (Table 3).

Of the seven agents active in the conventional bioassay, only LADA and PCP were active in Tg·AC mice. LADA induced papillomas at the SOA in a dose-related manner, and at the highest dose, 20 mg/mouse/day (800 mg/kg/day), tumors were visible after only 7 weeks of treatment (Table 3). The dose levels of 10 or 20 mg/mouse/day (400 and 800 mg/kg/day, respectively) were 2- and 4-fold higher than the highest dose (200 mg/kg/day) that caused liver tumors in female B6C3F₁

mice (SE). Only the highest dose of PCP, 3.0 mg/mouse/day (120 mg/kg/day), was clearly active in Tg·AC mice. The incidence was 100% and the mean maximum tumor burden was 11 papillomas per mouse. The high dose of 120 mg/kg was twice that of the highest estimated MTD dose (66 mg/kg/day) received by the B6C3F₁ mice in the conventional bioassay. Originally, the doses of PCP selected for testing in Tg·AC mice were 3.0, 6.0, or 12.0 mg/mouse/day, but the two higher doses proved to be excessively toxic after just one treatment. In response to this overt toxicity, the remaining mice in each of these dose groups were subsequently exposed to lower doses of PCP (Table 3).

The other five bioassay carcinogens (DEA, TEA, COD, FA, and PY) were inactive in Tg·AC mice (Table 3). The average incidence of mice bearing papillomas was uniformly high among all groups of the TEA-treated mice, as well as in the acetone control group. However, the average tumor multiplicity was less than 1.0 in every instance. There was no indication that this low multiplicity was related to treatment, as the average multiplicity of papillomas for Tg·AC mice was in the range observed for negative (vehicle) control groups (Spalding *et al.*, 1999). The incidence of mice bearing papillomas was

TABLE 3
Prospective Evaluation of Chemical Activity in the Tg·AC Transgenic Mouse Model

Treatment	Number of animals	Incidence (%)	Mean weeks to first tumor	Multiplicity (tumors/total animals per group)	Mean weeks to maximum tumor burden	Survival at 20 weeks (%)
Diethanolamine^a						
5.0 mg	15	1/15 (6.7)	18.0	1/15 (0.07)	18.0	14/15 (93.3)
10.0 mg	15	0/15 (0.0)	—	—	—	14/15 (93.3)
20.0 mg	15	1/15 (6.7)	18.0	1/15 (0.07)	18.0	12/15 (80.0)
95% Ethanol 200 μ l	19	1/19 (5.3)	19.0	1/19 (0.05)	19.0	17/19 (89.5)
TPA 1.25 μ g (2 \times /week)	20	18/20 (90.0)	12.7	179/20 (8.95)	19.0	18/20 (90.0)
Triethanolamine^a						
3.0 mg	14	4/14 (28.6)	11.8	5/14 (0.36)	13.8	11/14 (78.6)
10.0 mg	13	5/13 (38.5)	13.4	10/13 (0.77)	14.8	12/13 (92.3)
30.0 mg	19	4/19 (21.1)	8.8	10/19 (0.53)	13.5	15/19 (78.9)
Acetone 200 μ l	14	4/14 (28.6)	13.3	4/14 (0.29)	13.3	14/14 (100)
TPA 1.25 μ g (2 \times /week)	20	19/20 (95.0)	7.6	390/20 (19.5)	12.4	9/20 (45.0)
Coconut oil acid diethanolamine^{b,c}						
2.5 mg	15	1/15 (6.7)	9.0	1/15 (0.07)	9.0	13/15 (86.7)
5.0 mg	15	0/15 (0.0)	—	—	—	13/15 (86.7)
15.0 mg	19	3/19 (15.8)	6.0	47/19 (2.47)	13.0	15/19 (78.9)
95% Ethanol 200 μ l	19	1/19 (5.3)	11.0	1/19 (0.05)	11.0	17/19 (89.5)
TPA 1.5 μ g (2 \times /week)	10	10/10 (100.0)	8.6	203/10 (20.3)	16.0	10/10 (100)
Lauric acid diethanolamine^b						
5.0 mg	15	3/15 (20.0)	10.3	9/15 (0.60)	16.6	12/15 (80)
10.0 mg	15	11/15 (73.3)	11.3	25/15 (1.67)	13.4	13/15 (86.7)
20.0 mg	15	13/15 (86.7)	6.4	75/15 (5.0)	16.2	13/15 (86.7)
Acetone 200 μ l	18	1/18 (5.6)	18.0	1/18 (0.06)	18.0	18/18 (100)
TPA 1.5 μ g (2 \times /week)	10	8/10 (80.0)	9.8	238/10 (23.8)	18.4	10/10 (100)
Oleic acid diethanolamine^{b,c}						
0.4 mg	14	1/14 (7.1)	19.0	1/14 (0.07)	19	14/14 (100)
0.8 mg	14	1/14 (7.1)	15.0	1/14 (0.07)	15	14/14 (100)
1.2 mg	19	5/19 (26.3)	12.8	17/19 (0.90)	14.8	16/19 (84.2)
95% Ethanol 200 μ l	19	1/19 (5.3)	11.0	1/19 (0.05)	11.0	17/19 (89.5)
TPA 1.5 μ g (2 \times /week)	10	10/10 (100.0)	8.6	203/10 (20.3)	16.0	10/10 (100)
1-Chloro-2-propanol^{b,d}						
1.2 mg	15	2/15 (13.3)	12.5	2/15 (0.13)	12.5	14/15 (93.3)
6.0 mg	15	1/15 (6.7)	15.0	1/15 (0.07)	15.0	15/15 (100)
12.0 mg	20	0/20 (0.0)	—	—	—	19/20 (95.0)
Acetone 200 μ l	14	0/14 (0.0)	—	—	—	14/14 (100)
TPA 1.25 μ g (3 \times /week)	14	14/14 (100)	5.8	310/14 (22.1)	15.6	12/14 (85.7)
Furfuryl alcohol^{b,d}						
0.25 mg	15	0/15 (0.0)	—	—	—	15/15 (100)
0.75 mg	14	1/14 (7.1)	19.0	1/14 (0.07)	19.0	14/14 (100)
1.50 mg	20	0/20 (0.0)	—	—	—	18/20 (90.0)
Acetone 200 μ l	14	0/14 (0.0)	—	—	—	14/14 (100)
TPA 1.25 μ g (3 \times /week)	14	14/14 (100)	5.8	310/14 (22.1)	15.6	12/14 (85.7)
Pyridine^{b,e}						
1.5 mg	15	2/15 (13.3)	10.0	2/15 (0.13)	10.0	15/15 (100)
3.0 mg	15	0/14 (0.0)	—	—	—	13/14 (92.9)
6.0 mg	20	1/20 (5.0)	20.0	1/20 (0.05)	20	17/20 (85.0)
Acetone 200 μ l	15	1/15 (6.7)	20.0	1/15 (0.07)	20.0	13/15 (86.7)
TPA 1.25 μ g (3 \times /week)	15	15/15 (100)	6.9	405/15 (27.0)	16.5	13/15 (86.7)
Pentachlorophenol^{b,e}						
0.75 mg	13	1/13 (7.7)	5.0	1/13 (0.08)	5.0	8/13 (61.5)
1.50 mg	13	8/13 (61.5)	10.9	11/13 (0.85)	12.9	10/13 (76.9)
3.0 mg	14	14/14 (100)	7.1	162/14 (11.6)	16.4	12/14 (85.7)
Acetone 200 μ l	15	1/15 (6.7)	20.0	1/15 (0.07)	20.0	13/15 (86.7)
TPA 1.25 μ g (3 \times /week)	15	15/15 (100)	6.9	405/15 (27.0)	16.5	13/15 (86.7)

^a Chemicals tested in homozygous Tg·AC mice.

^b Chemicals tested in hemizygous Tg·AC mice.

^c Coconut oil acid diethanolamine and oleic acid diethanolamine shared vehicle and positive controls.

^d 1-Chloro-2-propanol and furfuryl alcohol shared vehicle and positive controls.

^e Pyridine and pentachlorophenol shared vehicle and positive controls.

TABLE 4
Prospective Comparison of Results between the NTP 2-Year Bioassay and the 26-Week Studies
in C57BL/6 Mice Heterozygous p53 (\pm) Deficient Mice (N5)

Chemical	TR	Mut ^a	Route	F344/N		B6C3F ₁		p53 ^{+/-} def	
				Sex	Target	Sex	Target	Sex	Result
COD	479	-; +	SP	M, F	-; -	M, F	L, K; L	M, F	-; -
LADA	480	-; -	SP	M, F	-; -	M, F	-; L	M, F	-; -
OAD	481	-; -	SP	M, F	-; -	M, F	-; -	M, F	-; -
CP	477	+; -	DW	M, F	-; -	M, F	-; -	M, F	-; -
PY	470	-; -	DW	M, F	K; -	M, F	L; L	M, F	-; -
PCP	483/349	-; -	F	M, F	MT; -	M, F	-; -	M, F	-; -

Note. K, kidney; MT, mesothelial tissue; L, liver. 26-week studies replicated NCI/NTP 2 year bioassay using the same route of administration and dose range.

^a *In vitro* Salmonella mutagenesis assay; *in vivo* micronucleus assay.

16% in the highest dose group of COD-treated mice and was twice that seen in the concurrent negative control group. Three of the five mice in one cage had a total of 47 papillomas among them, and the average time to appearance of the first tumor was 6 weeks. None of the other mice among three cages treated with the same dose had papillomas at the SOA. This result strongly suggests that the response was a cage effect potentially due to bite wounds inflicted by a dominant female. We now conduct all of our chemical evaluation studies among singly housed animals in order to eliminate the cage effect responses.

Four different concurrent positive control groups among the hemizygous mice were treated with TPA at doses of 1.25 μ g 3 \times /week or 1.5 μ g 2 \times /week. The combined incidence of tumor response was 96% (47/49) (Table 3). Thirty-six of the 47 responding mice had a tumor burden of 20 or more papillomas each. Fifty-five percent (26/47) had enlarged spleens, a response characteristic of TPA-treated Tg \cdot AC mice that carry high (20 or more) papilloma burdens. There was no significant difference among the four positive control groups with respect to latency times or the mean maximum tumor burden per mouse.

The hemizygous Tg \cdot AC mice used in these studies were received in the spring of 1996. The high incidence (96%) and uniformity of the tumor response to treatment with TPA, the positive control, indicates that the nonresponder phenotype that appeared later among Tg \cdot AC mice (Weaver *et al.*, 1998; Thompson *et al.*, 1998) was not in evidence at this time. The two mice that did not respond to TPA (see the positive control group for LADA, Table 3) were not genotyped, so it is not known whether they were of the nonresponder genotype.

p53^{+/-} Mice

The routes of administration used in the p53^{+/-} mouse studies were the same as those selected for the conventional bioassays (Table 2). The doses used for the topical studies were similar to those used in Tg \cdot AC mouse studies and were equivalent to or exceeded the MTDs selected for mice in the

bioassays (Fig. 1). The highest doses selected for the other agents (CP, PY, PCP) were comparable to the bioassay MTDs.

The two bioassay noncarcinogens, CP, a mutagenic noncarcinogen, and OAD, a nonmutagenic noncarcinogen, were also inactive in the p53^{+/-} transgenic mice (Tables 2 and 4). The other four agents (COD, LADA, PY, PCP), all conventional bioassay carcinogens, were also inactive. There was no evidence of overt chemical toxicity by any of the agents during the 26 weeks of exposure. The lack of carcinogenic activity of LADA, PCP, and PY was predictable because nongenotoxic carcinogens have not induced tumors in the p53^{+/-} model. Coconut oil acid diethanolamine, a genotoxic condensate of diethanolamine, was also inactive. Survival was high among all dose groups (95% for male and 97% for female mice).

Histopathology. Animals sacrificed because of moribundity or at termination of exposure were examined carefully for gross lesions. Tissues from the negative control and top dose groups were examined for microscopic lesions. With the exception of squamous cell papillomas and two dermal sarcomas (NOS) at the SOA, there were no other treatment-associated neoplastic lesions observed in Tg \cdot AC mice. One of the dermal sarcomas was found associated with a papilloma. Squamous cell papillomas of the forestomach, mandibular salivary gland adenomas, ovarian cysts, and small papillomas at sites away from the SOA were noted, but these were not treatment related; the incidence of these lesions was similar to that reported by Mahler *et al.*, (1998). There was a non-treatment-related incidence of odontomas (19.7%; 88/446) among the hemizygous Tg \cdot AC mice. However, these lesions did not have a negative impact on the studies, because most of the mice (90.1%) survived to the end of the 20-week exposure period when they were 34 weeks old.

There were no treatment-related gross or microscopic neoplastic lesions observed among any of the exposed p53^{+/-} mice in these prospective studies. The incidence of non-treatment-related spontaneous lesions associated with the p53^{+/-} genotype was very low and did not impact survival.

TABLE 3
Prospective Evaluation of Chemical Activity in the Tg·AC Transgenic Mouse Model

Treatment	Number of animals	Incidence (%)	Mean weeks to first tumor	Multiplicity (tumors/total animals per group)	Mean weeks to maximum tumor burden	Survival at 20 weeks (%)
Diethanolamine^a						
5.0 mg	15	1/15 (6.7)	18.0	1/15 (0.07)	18.0	14/15 (93.3)
10.0 mg	15	0/15 (0.0)	—	—	—	14/15 (93.3)
20.0 mg	15	1/15 (6.7)	18.0	1/15 (0.07)	18.0	12/15 (80.0)
95% Ethanol 200 μ l	19	1/19 (5.3)	19.0	1/19 (0.05)	19.0	17/19 (89.5)
TPA 1.25 μ g (2 \times /week)	20	18/20 (90.0)	12.7	179/20 (8.95)	19.0	18/20 (90.0)
Triethanolamine^a						
3.0 mg	14	4/14 (28.6)	11.8	5/14 (0.36)	13.8	11/14 (78.6)
10.0 mg	13	5/13 (38.5)	13.4	10/13 (0.77)	14.8	12/13 (92.3)
30.0 mg	19	4/19 (21.1)	8.8	10/19 (0.53)	13.5	15/19 (78.9)
Acetone 200 μ l	14	4/14 (28.6)	13.3	4/14 (0.29)	13.3	14/14 (100)
TPA 1.25 μ g (2 \times /week)	20	19/20 (95.0)	7.6	390/20 (19.5)	12.4	9/20 (45.0)
Coconut oil acid diethanolamine^{b,c}						
2.5 mg	15	1/15 (6.7)	9.0	1/15 (0.07)	9.0	13/15 (86.7)
5.0 mg	15	0/15 (0.0)	—	—	—	13/15 (86.7)
15.0 mg	19	3/19 (15.8)	6.0	47/19 (2.47)	13.0	15/19 (78.9)
95% Ethanol 200 μ l	19	1/19 (5.3)	11.0	1/19 (0.05)	11.0	17/19 (89.5)
TPA 1.5 μ g (2 \times /week)	10	10/10 (100.0)	8.6	203/10 (20.3)	16.0	10/10 (100)
Lauric acid diethanolamine^b						
5.0 mg	15	3/15 (20.0)	10.3	9/15 (0.60)	16.6	12/15 (80)
10.0 mg	15	11/15 (73.3)	11.3	25/15 (1.67)	13.4	13/15 (86.7)
20.0 mg	15	13/15 (86.7)	6.4	75/15 (5.0)	16.2	13/15 (86.7)
Acetone 200 μ l	18	1/18 (5.6)	18.0	1/18 (0.06)	18.0	18/18 (100)
TPA 1.5 μ g (2 \times /week)	10	8/10 (80.0)	9.8	238/10 (23.8)	18.4	10/10 (100)
Oleic acid diethanolamine^{b,c}						
0.4 mg	14	1/14 (7.1)	19.0	1/14 (0.07)	19	14/14 (100)
0.8 mg	14	1/14 (7.1)	15.0	1/14 (0.07)	15	14/14 (100)
1.2 mg	19	5/19 (26.3)	12.8	17/19 (0.90)	14.8	16/19 (84.2)
95% Ethanol 200 μ l	19	1/19 (5.3)	11.0	1/19 (0.05)	11.0	17/19 (89.5)
TPA 1.5 μ g (2 \times /week)	10	10/10 (100.0)	8.6	203/10 (20.3)	16.0	10/10 (100)
1-Chloro-2-propanol^{b,d}						
1.2 mg	15	2/15 (13.3)	12.5	2/15 (0.13)	12.5	14/15 (93.3)
6.0 mg	15	1/15 (6.7)	15.0	1/15 (0.07)	15.0	15/15 (100)
12.0 mg	20	0/20 (0.0)	—	—	—	19/20 (95.0)
Acetone 200 μ l	14	0/14 (0.0)	—	—	—	14/14 (100)
TPA 1.25 μ g (3 \times /week)	14	14/14 (100)	5.8	310/14 (22.1)	15.6	12/14 (85.7)
Furfuryl alcohol^{b,d}						
0.25 mg	15	0/15 (0.0)	—	—	—	15/15 (100)
0.75 mg	14	1/14 (7.1)	19.0	1/14 (0.07)	19.0	14/14 (100)
1.50 mg	20	0/20 (0.0)	—	—	—	18/20 (90.0)
Acetone 200 μ l	14	0/14 (0.0)	—	—	—	14/14 (100)
TPA 1.25 μ g (3 \times /week)	14	14/14 (100)	5.8	310/14 (22.1)	15.6	12/14 (85.7)
Pyridine^{b,e}						
1.5 mg	15	2/15 (13.3)	10.0	2/15 (0.13)	10.0	15/15 (100)
3.0 mg	15	0/14 (0.0)	—	—	—	13/14 (92.9)
6.0 mg	20	1/20 (5.0)	20.0	1/20 (0.05)	20	17/20 (85.0)
Acetone 200 μ l	15	1/15 (6.7)	20.0	1/15 (0.07)	20.0	13/15 (86.7)
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Pentachlorophenol^{b,e}						
0.75 mg	13	1/13 (7.7)	5.0	1/13 (0.08)	5.0	8/13 (61.5)
1.50 mg	13	8/13 (61.5)	10.9	11/13 (0.85)	12.9	10/13 (76.9)
3.0 mg	14	14/14 (100)	7.1	162/14 (11.6)	16.4	12/14 (85.7)
Acetone 200 μ l	15	1/15 (6.7)	20.0	1/15 (0.07)	20.0	13/15 (86.7)
TPA 1.25 μ g (3 \times /week)	15	15/15 (100)	6.9	405/15 (27.0)	16.5	13/15 (86.7)

^a Chemicals tested in homozygous Tg·AC mice.

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^c Coconut oil acid diethanolamine and oleic acid diethanolamine shared vehicle and positive controls.

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Chemical	TR	Mut ^a	Route	F344/N		B6C3F ₁		p53 ^{+/-} def	
				Sex	Target	Sex	Target	Sex	Result
COD	479	-; +	SP	M, F	-; -	M, F	L, K; L	M, F	-; -
LADA	480	-; -	SP	M, F	-; -	M, F	-; L	M, F	-; -
OAD	481	-; -	SP	M, F	-; -	M, F	-; -	M, F	-; -
CP	477	+; -	DW	M, F	-; -	M, F	-; -	M, F	-; -
PY	470	-; -	DW	M, F	K; -	M, F	L; L	M, F	-; -
PCP	483/349	-; -	F	M, F	MT; -	M, F	-; -	M, F	-; -

Note. K, kidney; MT, mesothelial tissue; L, liver. 26-week studies replicated NCI/NTP 2 year bioassay using the same route of administration and dose range.

^a *In vitro* Salmonella mutagenesis assay; *in vivo* micronucleus assay.

16% in the highest dose group of COD-treated mice and was twice that seen in the concurrent negative control group. Three of the five mice in one cage had a total of 47 papillomas among them, and the average time to appearance of the first tumor was 6 weeks. None of the other mice among three cages treated with the same dose had papillomas at the SOA. This result strongly suggests that the response was a cage effect potentially due to bite wounds inflicted by a dominant female. We now conduct all of our chemical evaluation studies among singly housed animals in order to eliminate the cage effect responses.

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The hemizygous Tg \cdot AC mice used in these studies were received in the spring of 1996. The high incidence (96%) and uniformity of the tumor response to treatment with TPA, the positive control, indicates that the nonresponder phenotype that appeared later among Tg \cdot AC mice (Weaver *et al.*, 1998; Thompson *et al.*, 1998) was not in evidence at this time. The two mice that did not respond to TPA (see the positive control group for LADA, Table 3) were not genotyped, so it is not known whether they were of the nonresponder genotype.

p53^{+/-} Mice

The routes of administration used in the p53^{+/-} mouse studies were the same as those selected for the conventional bioassays (Table 2). The doses used for the topical studies were similar to those used in Tg \cdot AC mouse studies and were equivalent to or exceeded the MTDs selected for mice in the

bioassays (Fig. 1). The highest doses selected for the other agents (CP, PY, PCP) were comparable to the bioassay MTDs.

The two bioassay noncarcinogens, CP, a mutagenic noncarcinogen, and OAD, a nonmutagenic noncarcinogen, were also inactive in the p53^{+/-} transgenic mice (Tables 2 and 4). The other four agents (COD, LADA, PY, PCP), all conventional bioassay carcinogens, were also inactive. There was no evidence of overt chemical toxicity by any of the agents during the 26 weeks of exposure. The lack of carcinogenic activity of LADA, PCP, and PY was predictable because nongenotoxic carcinogens have not induced tumors in the p53^{+/-} model. Coconut oil acid diethanolamine, a genotoxic condensate of diethanolamine, was also inactive. Survival was high among all dose groups (95% for male and 97% for female mice).

Histopathology. Animals sacrificed because of moribundity or at termination of exposure were examined carefully for gross lesions. Tissues from the negative control and top dose groups were examined for microscopic lesions. With the exception of squamous cell papillomas and two dermal sarcomas (NOS) at the SOA, there were no other treatment-associated neoplastic lesions observed in Tg \cdot AC mice. One of the dermal sarcomas was found associated with a papilloma. Squamous cell papillomas of the forestomach, mandibular salivary gland adenomas, ovarian cysts, and small papillomas at sites away from the SOA were noted, but these were not treatment related; the incidence of these lesions was similar to that reported by Mahler *et al.*, (1998). There was a non-treatment-related incidence of odontomas (19.7%; 88/446) among the hemizygous Tg \cdot AC mice. However, these lesions did not have a negative impact on the studies, because most of the mice (90.1%) survived to the end of the 20-week exposure period when they were 34 weeks old.

There were no treatment-related gross or microscopic neoplastic lesions observed among any of the exposed p53^{+/-} mice in these prospective studies. The incidence of non-treatment-related spontaneous lesions associated with the p53^{+/-} genotype was very low and did not impact survival.

Pentachlorophenol induced a moderate to marked centrilobular hypertrophy in the livers of all 400-ppm male and female mice, indicating that a toxic threshold was reached in these studies. These lesions were accompanied by mononuclear and/or lymphocytic infiltration in all male and female mice of the 400-ppm treatment group as compared to 5 out of 10 male and female mice each in the untreated control group. No other findings were considered significant or related to treatment.

Pyridine induced a mild increase in the incidence and severity of centrilobular hyperplasia in the liver of the 1000-ppm male treatment group, which was minimal in the 500-ppm high-dose female treatment group. The only neoplastic lesion observed was an alveolar-bronchiolar adenoma in a male mouse in the drinking water control group. Survival of male mice exposed to pyridine in the drinking water was lowest in the 500-ppm group (7/10 males) and may have been related to treatment. In the control group, 9/10 males and 8/10 females survived to study termination. Only 1 male out of 10 died in the 1000-ppm treatment group. Weight gain among the treated male mice was reduced (relative to control) in a dose-related manner. This was not observed among dosed female mice. The MTD may have been exceeded in the pyridine studies but it did not affect survival or induction of neoplasia.

DISCUSSION

The primary purpose of this study was to provide an objective prospective evaluation of the utility of two transgenic models in identifying carcinogens. The study was equally important in providing data that could be compared directly to results from the conventional bioassays. Fortuitously, in the conventional 2-year bioassay, five of the nine tested substances were administered by dermal application, the principal route of exposure used with the Tg·AC model based on the skin papilloma reporter phenotype. The results for four of these chemicals (DEA, TEA, COD, LADA) in the conventional bioassay were single-species effects (i.e., they occurred only in mice and not in rats). Skin tumors were not induced, even though chronic exposure at the SOA was associated with nonneoplastic skin toxicity such as inflammation and hyperplasia. Further, the pattern of tumors observed (i.e., increases in hepatocellular and renal tumors) appears to be consistent with the response to DEA. In the two studies with the diethanolamine condensates (COD, LADA) in which tumors were seen, the response was judged to be related to the concentration of free DEA present in the preparation. OAD, the condensate that showed no evidence of carcinogenicity, caused significant non-neoplastic toxicity in the skin but had the lowest concentration of DEA. It is also quite notable that the effects in the liver were judged solely on a statistical basis, as the incidence of liver tumors in the concurrent (nonexposed) control groups was at the high end of the historical control range of the spontaneous liver tumor incidence observed among B6C3F₁ mice (Haseman *et al.*,

1998). The renal tumors likewise represent an increased incidence of tumors that occur spontaneously in the concurrent controls.

Based upon the absence of *in vitro* genotoxicity of DEA and its condensates, the absence of LADA activity in the p53^{+/-} model was predictable; that is, no induction of neoplasia was observed within the 6-month exposure period. Though coconut oil acid diethanolamine is genotoxic by the criteria of the *in vivo* mouse bone marrow micronucleus assay, its tumor activity in the conventional bioassay follows the pattern induced by diethanolamine in B6C3F₁ mice and was attributed to the presence of the free DEA in the preparation of COD condensate. Therefore, under these circumstances, COD would not be expected to have activity in p53^{+/-} mice.

The tumor response in the conventional assay to the diethanolamine condensates was judged to be due to the free diethanolamine in those preparations. In the case of the Tg·AC model, DEA was clearly nontumorigenic, and with the exception of LADA, the condensates, even at doses higher than the MTDs used in the conventional bioassay, failed to be tumorigenic. These results indicate that DEA lacks the capacity to induce the expression of the transgene in the Tg·AC model. The effects observed with LADA are quite interesting and would appear to be the consequence of some property of the lauric acid component rather than that of DEA.

These results therefore present a unique opportunity to assess how information derived from conventional rodent bioassays and transgenic bioassays can be related to potential human health consequences from exposure to substances containing DEA. In the traditional interpretation of conventional bioassay results, any induction of tumors at any site in either of the test species has been taken as indicative of a potential consequence for human exposure. However, it can be argued that the effects of the diethanolamines represent the manifestation of a species-specific response to DEA, as they are observed only in the B6C3F₁ mice and not in the comparably exposed Fischer F344/N rats. The responses may relate only to the amplification of tumors that occur spontaneously as a consequence of the genotype of the B6C3F₁ animals. Such results can then arguably be interpreted to mean that there is relatively little human health risk from exposure to DEA and related compounds. The absence of an effect in the transgenic animals, therefore, is not necessarily representative of a false-negative result, but rather an indication that the conventional bioassay has given a false positive result. Thus, the response in transgenic animals may provide a more accurate assessment of potential human risk. It has been proposed that the genes associated with neoplastic pathways represented in the two transgenic models render the animals less susceptible to the induction of strain- or species-specific effects that result from chronic exposure in conventional bioassays (Tennant *et al.*, 1999). The Tg·AC line produced results that were concordant with the conventional bioassay for OAD, which was negative in both assays, and for LADA, which was positive. However, it is unlikely that the

response to LADA observed in the Tg·AC animals is related to the same pathway by which the liver tumors were increased in the conventional assay. The results, therefore, are probably related to the specific capacity of LADA to induce transgene expression in the Tg·AC model and further studies will be required to develop a plausible hypothesis about the mode of action of this substance.

Among the other agents evaluated in this study, concordant results were obtained between the transgenic models and conventional bioassay with CP, which failed to induce tumors in either bioassay. FA was administered in the 2-year bioassay via inhalation, and resources were not available to evaluate the substance via inhalation in the two transgenic models. Because FA was not mutagenic, it would be predictably negative in the p53 model. Skin exposure of Tg·AC mice to FA did not induce papillomas. PY, which was also not genotoxic, induced tumors in both B6C3F₁ mice and Fischer F344/N rats. However, it can be argued that the effects observed in both species represent strain-specific responses; the effects in the liver of B6C3F₁ mice were related to a high spontaneous background tumor incidence and the effects observed in the Fischer rats were not reproduced in comparably exposed Wistar rats.

The pentachlorophenol studies conducted in the conventional 2-year bioassay are not directly comparable between mice and rats. Whereas the F344 rats were exposed to pure (99%) PCP (NTP, 1999b), B6C3F₁ mice were exposed in an earlier study to technical grade (90%) PCP and Dowicide EC-7 which was composed of 91% PCP (NTP, 1989). However, the technical grade PCP contained 100- to 1000-fold greater amounts of such impurities as polychlorinated dibenzodioxins and dibenzofurans than did Dowicide EC-7. PCP was a trans-species carcinogen, causing malignant mesotheliomas and nasal tumors in female rats and tumors of the liver, adrenal gland, and spleen in exposed mice. Papillomas were induced in dermally exposed Tg·AC mice at the highest dose (120 mg/kg) of pure (99%) PCP, which was comparable to the exposure of B6C3F₁ mice to the highest estimated dose (116 mg/kg) of Dowicide EC-7. Tg·AC mice were initially exposed to higher doses (240 and 480 mg/kg) of PCP based on the doses used in the 6-month exposure studies conducted with pure (99%) PCP in B6C3F₁ mice (NTP, 1989). However, these doses caused acute toxicity, indicating immediate systemic effects via the dermal route, so that the doses were adjusted to lower levels.

In the context of current toxicologic assessments, one proposed strategy for using transgenic mouse models is to include them in subchronic toxicity testing. With this strategy the exposure period could be extended to 6 months (180 days) rather than the conventional 90-day period used by the NTP and others. The longer exposure period would allow for an assessment of carcinogenic potential in the transgenic models. It would also provide improved toxicologic data for setting doses for the 2-year conventional bioassay should it be judged necessary to conduct them. However, it is quite possible that the transgenic assays alone could provide sufficient evidence of

carcinogenic potential with appropriate dosimetric data. The transgenic results could also provide insight to the possible mechanism of carcinogenicity. If tumors are seen in the p53^{+/-} model, the tumor tissue can be analyzed for the status (loss of heterozygosity or mutations) of the wild-type allele (Dunnick *et al.*, 1997). A positive response in the Tg·AC model alone would be indicative of a possible nongenotoxic carcinogen and reflect the capacity of the chemical to induce specific gene expression.

The studies reported here serve as another step in the process of evaluating the use of transgenic mouse models for assessing human risk from chemical exposure. They will be important when judged in the context of results that will come from the large international evaluation of alternative carcinogenicity methods currently being conducted under the coordination of the International Life Sciences Institute, Health Effects Institute (Robinson, 1998).

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