

Evaluation of Dichloroacetic Acid for Carcinogenicity in Genetically Modified Tg.AC Hemizygous and p53 Haploinsufficient Mice

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There has been considerable interest in the use of genetically modified mice for detecting potential environmental carcinogens. For this reason, the National Toxicology Program has been evaluating Tg.AC hemizygous and p53 haploinsufficient mice as models to detect potential carcinogens. It was reasoned that these mouse models might also prove more effective than standard rodent models in evaluating the numerous disinfection byproducts that are found in low concentrations in drinking water. Dichloroacetic acid (DCA) is one of the most frequently found disinfection byproducts and DCA has been consistently shown to cause hepatocellular tumors in rats and mice in standard rodent studies. Tg.AC hemizygous and p53 haploinsufficient mice were exposed in the drinking water to DCA for up to 41 weeks. In a second study Tg.AC mice were subjected to dermal DCA exposure for up to 39 weeks. Increased incidences and severity of cytoplasmic vacuolization of hepatocytes were seen in the p53 mice, but there was no evidence of carcinogenic activity at exposures of up to 2000 mg/l in the drinking water. Increased incidences and severity of cytoplasmic vacuolization of hepatocytes were seen in the drinking water study with Tg.AC mice and a modest non-dose-related increase in pulmonary adenomas was observed in males exposed to 1000 mg/l in the drinking water. Dermal exposure up to 500 mg/kg for 39 weeks resulted in increased dermal papillomas at the site of application in Tg.AC mice. No significant increase in papillomas under the same study conditions was seen in the 26-week study. For DCA under these study conditions, the p53 and Tg.AC mice appear less sensitive to hepatocarcinogenesis than standard rodent models. These results suggest caution for the use of Tg.AC and p53 mice to screen unknown chemicals in drinking water for potential carcinogenicity.

Key Words: bioassays; Tg.AC; p53; mice; dichloroacetic acid; drinking water.

The obvious advantage of having a very sensitive and rapid screening test of drinking water for potential carcinogens led

the U.S. Environmental Protection Agency (U.S. EPA) and the National Toxicology Program (NTP) to consider the use of genetically modified mice in the assessment of disinfection byproducts (Boorman *et al.*, 1999). In the past few years the NTP has been evaluating a variety of genetically modified mice as potential models for detecting carcinogenic chemicals. The combination of two genetically modified mice, such as the Tg.AC hemizygous and p53 haploinsufficient mouse, has been suggested as an effective and rapid means of identifying chemical carcinogens (Pritchard *et al.*, 2003; Tennant *et al.*, 1995). One advantage of the Tg.AC mouse is that it responds rapidly to carcinogens with dermal tumors that are visible, allowing both time to tumor and tumor multiplicity to be assessed without the costs associated with histopathology (Tennant *et al.*, 1995). An advantage of the p53 haploinsufficient mouse is that it is expected to have a short latency period for tumors induced by genotoxic chemicals because it is heterozygous for the Trp53 tumor-suppressor gene (Pritchard *et al.*, 2003).

Therefore, we decided to test several chemicals commonly found in low concentrations in the drinking water and also known to cause cancer in standard rodent models at carcinogenic doses in these genetically modified mouse models to determine their relative sensitivity. We felt that this was necessary before using these models to evaluate unknown chemicals in water that often occur at very low concentrations. Dichloroacetic acid (DCA) was the first chemical selected because it is a common disinfectant that gives a very consistent carcinogenic response in rodents. DCA is often found in water at concentrations between 5 and 125 µg/l (IARC, 1995). Increased incidences of hepatocellular tumors were found in DCA-exposed male and/or female B6C3F₁ mice in eight drinking water studies (IARC, 2004). DCA is also considered genotoxic *in vivo* and *in vitro* (IARC, 2004). In these previous studies, DCA exposures associated with increased liver tumors in traditional mouse models generally exceeded one year and were often at drinking water concentrations of 1000–2000 mg/l (IARC, 2004). In this study, we evaluated exposures to genetically modified mice at 500, 1000, and 2000 mg/l in drinking water, for 26–41 weeks.

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Our results indicate that for DCA evaluated in the drinking water for up to 9 months, the Tg.AC hemizygous and p53 haploinsufficient mice appear less sensitive for detecting a carcinogenic response than did the traditional mouse models. A carcinogenic response was not found in either sex of both genetically modified mouse models at 6 months. Even with 9 months exposure, no liver tumors were found and a modest increase in pulmonary adenomas was seen in only the male Tg.AC mice. In the Tg.AC mice, a positive papilloma response at the site of application was seen following 9 months of dermal exposure but not at 6 months. This suggests that caution is warranted in using these mouse models for evaluating chemicals in the drinking water for which carcinogenic potential is unknown.

MATERIALS AND METHODS

Mice. Male and female FVB/N-TgN(v-Ha-ras)^{Led} (Tg.AC) hemizygous and B6.129-Trp53^{tm1Brd} (N5) haploinsufficient mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY) for use in the 26-, 39-, and 41-week studies. Tg.AC hemizygous mice were quarantined for 11 (drinking water) or 14 (dermal) days, and p53 haploinsufficient mice were quarantined for 12 days before the beginning of the studies. Five male and five female mice per strain were randomly selected for parasite evaluation and gross observation of disease prior to study start. Tg.AC hemizygous mice were approximately 6 (dermal) or 7–8 (drinking water) weeks old and p53 haploinsufficient mice were 6–7 weeks old at the beginning of the studies. Blood samples were collected from five male and five female sentinel mice from each study at 4 and 26 weeks, from five male and five female mice from the group with the best survival in each study at 39 or 41 weeks. The sera were analyzed and found negative for antibody titers to rodent pathogens. Animals were housed individually with feed and water available *ad libitum*. Water consumption for each mouse was measured weekly during the drinking water studies. Cages and racks were rotated every two weeks. The protocol and studies were reviewed and conducted under the supervision of Battelle's animal care and use committee following the standards outlined in the guide for the care and use of animals (NRC, 1996).

Chemicals. DCA was obtained from Aldrich Chemical Company (Milwaukee, WI) in two lots and found to be approximately 99% pure. The stability of the bulk chemical was checked monthly during the study using high-performance liquid chromatography (HPLC) and degradation was not observed. The positive TPA (12-*O*-tetradecanoylphorbol-13-acetate) control for the Tg.AC dermal studies was also purchased from Sigma-Aldrich Chemical Company (St Louis, MO) and found to be 99% pure by HPLC and stable during the course of the study. USP-grade acetone (vehicle for dermal studies) was obtained from Spectrum Chemicals and Laboratory Products (Gardena, CA) in two lots and the overall purity of both lots was determined to be greater than 99.9%. More details on the chemical analysis and spectra are available in the NTP Technical Report (NTP, 2006).

Dermal studies. Groups of 15 male and 15 female Tg.AC hemizygous mice were administered 0, 31.25, 125, or 500 mg DCA/kg body weight in 3.3 ml water:acetone/kg body weight, neutralized to a pH of 6 to 8, 5 days per week for 26 weeks. Groups of 10 male and 10 female Tg.AC hemizygous mice were administered the same doses for 39 weeks. Vehicle control mice were administered water:acetone only. Doses were applied to the clipped dorsal skin from the mid-back to the interscapular area.

Drinking water studies. Groups of 15 male and 15 female Tg.AC hemizygous and p53 haploinsufficient mice were exposed to 0, 500, 1000, or

2000 mg DCA/l drinking water for 26 weeks. Groups of 10 male and 10 female Tg.AC hemizygous and p53 haploinsufficient mice were exposed to the same concentrations for 41 weeks.

Positive control mice. For each route of administration, groups of 15 male and 15 female Tg.AC hemizygous mice were dermally administered 1.25 µg TPA in 100 µg acetone three times per week for 26 weeks. Positive control mice were removed from study after the appearance of 20 or more skin papillomas. All TPA groups showed a positive response.

Clinical examination and pathology. All animals were observed twice daily. Clinical findings and body weights were recorded at the beginning of the study, weekly and at the end of the study. Any wart-like dermal masses were recorded as papillomas after 3 consecutive weeks. Necropsies were performed on all mice except the positive control groups. At necropsy all organs and tissues were examined for grossly visible lesions and major tissues were fixed and preserved in 10% neutral buffered formalin, processed, and embedded in paraffin. A study pathologist examined and recorded all lesions in a toxicology data management system. A second pathologist reviewed all tumor diagnoses and potential target tissues. A pathology working group addressed potential inconsistencies (Boorman and Eustis, 1986). The pathology materials such as slides, paraffin blocks, and wet tissues were sent to the NTP archives for an independent audit. More details of recording of gross lesions, pathology examination, reviews and audits are included in the NTP Technical report (NTP, 2006).

Statistical analysis. Incidences of neoplasms or nonneoplastic lesions in each dosed group were compared with those in the control group using Fisher's exact test (Gart *et al.*, 1979).

RESULTS

Dermal Exposure

The positive control groups responded to three times weekly dermal TPA exposure with more than 90% of Tg.AC males and females developing more than 20 dermal papillomas each before week twenty. Skin papillomas were not found in the vehicle control or low-dose DCA male and female Tg.AC by 26 weeks. One mid-dose (125 mg/kg) male, two high-dose (500 mg/kg) males and two high-dose females developed papillomas at the site of application by 26 weeks (Table 1). The mid-dose male, one high-dose male and both high-dose females had a single papilloma. In the 39-week exposure study, 8 of 10 male mice and 6 of 10 female mice exposed to 500 mg/kg had dermal papillomas at site of application; the majority of these mice had multiple papillomas. In the lower doses and vehicle controls, dermal papillomas at the site of application were seen in only two male mice exposed to 125 mg/kg for 39 weeks (Table 1). A significant increase in the incidence of hyperkeratosis at the site of application was seen in all DCA dermally exposed males and the high-dose females in the 39-week study. A significant increase in hyperplasia at the site of application was also seen in the high-dose and mid-dose males and in the high-dose females in the 39-week study (Table 2).

Drinking Water Exposure

In male and female Tg.AC and p53 mice, body weight decreased significantly with increasing DCA drinking water

TABLE 1
Skin Papilloma Formation at the Site of application in Tg.AC Hemizygous Mice in the Dermal Studies of DCA

	26-week study (<i>n</i> = 15 per group)					39-week study (<i>n</i> = 10 per group)			
	Positive control ^a	Vehicle control	31.25 mg/kg	125 mg/kg	500 mg/kg	Vehicle control	31.25 mg/kg	125 mg/kg	500 mg/kg
Males									
Number with papilloma	14** (93%)	0 (0%)	0 (0%)	1 (7%)	2 (13%)	0 (0%)	0 (0%)	2 (20%)	8** (80%)
Females									
Number with papilloma	15** (100%)	0 (0%)	0 (0%)	0 (0%)	2 (13%)	0 (0%)	0 (0%)	0 (0%)	6** (60%)

**Differs from controls at *p* < 0.01.

^aTPA.

exposure in both the 26- and 41-week studies. Growth curves for the 41-week studies are in Figure 1.

Because increased liver tumors were found in 10 studies of DCA in B6C3F₁ mice (Table 6 and Fig. 2), we reviewed our pathology findings for incidental liver tumors in DCA-exposed genetically modified mice to see if we were missing a subtle effect. Exposure of 180 male and female Tg.AC and p53 mice to 500, 1000, or 2000 mg/l DCA in the drinking water for 26 weeks resulted in hepatocellular tumors in none of the mice. A review of the 120 male and female Tg.AC and p53 mice exposed to the same DCA levels in the drinking water for 41 weeks revealed an hepatocellular adenoma in one male Tg.AC mouse in the high-dose group and two male p53 haploinsufficient mice in the low-dose group for a total of three liver tumors among all 300 DCA-exposed animals.

Cytoplasmic vacuolization of hepatocytes was found in both Tg.AC and P53 haploinsufficient mice and was characterized by poorly demarcated cytoplasmic clear spaces that lacked distinct borders and that were partially separated by irregular strands of eosinophilic cytoplasm. This change was considered consistent with cytoplasmic glycogen accumulation. The vacuole area sometimes displayed light basophilic stippling, a discoloration

that was only occasionally observed in controls but was more frequent in the treated animals. The pattern of accumulation tended to be centrilobular, but as severity increased, it involved more of the hepatocytes within the lobules. Cytoplasmic vacuolization of the hepatocytes in the p53 haploinsufficient mice was generally more pronounced than in the Tg.AC mice.

In Tg.AC mice exposed to DCA in the drinking water for 26 weeks, increased incidences and severities of cytoplasmic vacuolization of hepatocytes were seen in males exposed to 500 mg/l DCA and in males and females exposed to 1000 and 2000 mg/l DCA (Table 3). In the 41-week study, nearly all Tg.AC mice had cytoplasmic vacuolization of hepatocytes, including the control animals, and the severity increased with DCA concentration.

In the 26-week and 41-week drinking water studies of p53 haploinsufficient mice, cytoplasmic vacuolization of hepatocytes was found in nearly all males and females, so an increased incidence was generally not observed (Table 4). However, the severity of cytoplasmic vacuolization of hepatocytes increased with DCA concentration in both males and females in the 26-week study and in females in the 41-week study (Table 4).

TABLE 2
Incidences of Nonneoplastic Lesions of the Skin at the Site of Application in Tg.AC Hemizygous Mice in the Dermal Studies of DCA

	26-week study (<i>n</i> = 15 per group)				39-week study (<i>n</i> = 10 per group)			
	Vehicle control	31.25 mg/kg	125 mg/kg	500 mg/kg	Vehicle control	31.25 mg/kg	125 mg/kg	500 mg/kg
Males								
Epidermis, hyperkeratosis	2 (13%)	7 (47%)	15** (100%)	14** (93%)	2 (20%)	8* (80%)	9** (90%)	10** (100%)
Epidermis, hyperplasia	0 (0%)	2 (13%)	11** (73%)	13** (87%)	0 (0%)	0 (0%)	8** (80%)	9** (90%)
Females								
Epidermis, hyperkeratosis	8 (53%)	9 (60%)	14* (93%)	14* (93%)	5 (50%)	8 (80%)	9 (90%)	10* (100%)
Epidermis, hyperplasia	0 (0%)	1 (7%)	10** (67%)	13** (87%)	0 (0%)	0 (0%)	3 (30%)	6** (60%)

*Differs from controls at *p* < 0.05.

**Differs from controls at *p* < 0.01.

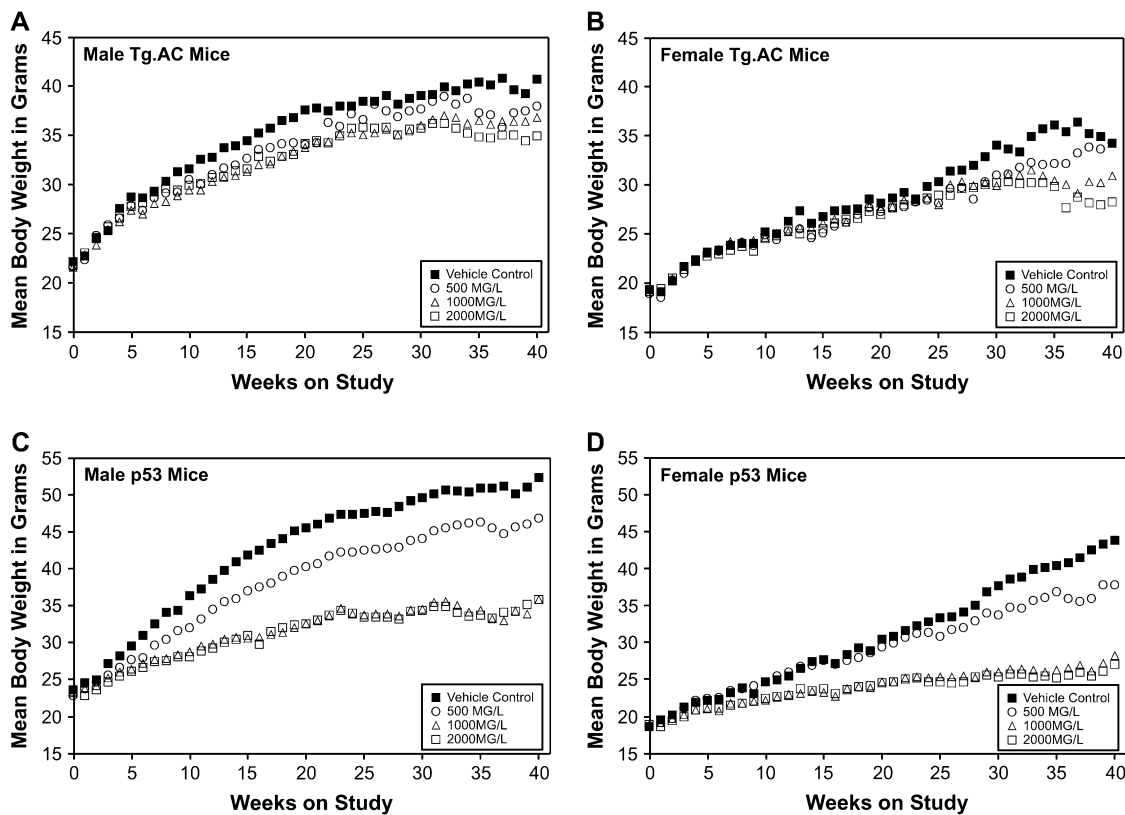


FIG. 1. Mean body weights (g) of male and female Tg.AC hemizygous and p53 haploinsufficient mice exposed to DCA in drinking water for 41 weeks. Mean body weights of 500, 1000, and 2000 mg/l Tg.AC males were less than those of controls after weeks 35, 8, and 11, respectively (A); mean body weights of 500, 1000, and 2000 mg/l Tg.AC females were less than those of controls after weeks 27, 28, and 26, respectively (B). Mean body weights of 500, 1000, 2000 mg/l p53 hemizygous male mice were less than those of controls after 4, 3, and 1 weeks, respectively (C); mean body weights of 500, 1000, 2000 mg/l p53 hemizygous female mice were less than those of controls after 27, 9, and 9 weeks, respectively (D).

At 26 weeks, three alveolar/bronchiolar carcinomas were seen in DCA-exposed Tg.AC mice including a mid-dose male and a low-dose and high-dose female (Table 5). In the 41-week study, alveolar/bronchiolar carcinomas were not seen but

a significant increase in alveolar/bronchiolar adenomas was found in male Tg.AC mice exposed to 1000 mg/l; at 2000 mg/l only three males and two females were found with these pulmonary adenomas (Table 5).

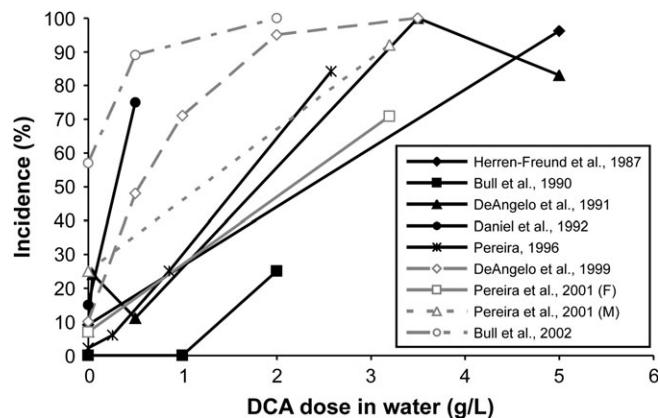


FIG. 2. Liver tumor incidences (%) from studies of DCA in B6C3F₁ mice. The length of exposure ranged from 31 weeks (Pereira *et al.*, 2001) to 104 weeks (Daniel *et al.*, 1992), with most studies lasting at least 52 weeks. In all studies, the incidence of liver tumors increased with increasing concentration of DCA. Also see Table 6.

DISCUSSION

Long-term rodent studies are one of the major tools for determining potential carcinogenicity of environmental chemicals. Rodent studies are costly and time consuming however, their strengths and weaknesses are fairly well understood (Eastin *et al.*, 1998; Haseman, 2000). Cost, time and sensitivity are major limitations in evaluations of potential carcinogenicity of chemicals but this is especially true for the hundreds of disinfection byproducts (DBPs) that occur at low concentrations in the drinking water. This prompted the NTP and U.S. EPA to suggest that genetically modified mice might be more useful in providing toxicity and carcinogenicity data on drinking water chemicals and DBPs for setting research priorities and guiding regulatory policies (Boorman *et al.*, 1999).

The Tg.AC hemizygous and the p53 haploinsufficient mice were reported to be more rapid, less expensive and more

TABLE 3
Incidences and Severities of Cytoplasmic Vacuolization of the Hepatocytes in Tg.AC Hemizygous Mice in the Drinking Water Studies of DCA

	26-week study (<i>n</i> = 15 per group)				41-week study (<i>n</i> = 10 per group)			
	0 mg/l	500 mg/l	1000 mg/l	2000 mg/l	0 mg/l	500 mg/l	1000 mg/l	2000 mg/l
Males	7 (47%), 1.0 ^a	13* (87%), 1.8	15** (100%), 2.7	15** (100%), 3.7	9 (90%), 2.0	10 (100%), 2.3	9 (90%), 3.2	10 (100%), 3.8
Females	6 (40%), 1.3	10 (67%), 2.7	14** (93%), 3.1	14** (93%), 3.7	7 (70%), 2.0	9 (90%), 2.6	9 (90%), 2.9	10 (100%), 3.0

*Differs from controls at $p < 0.05$.

**Differs from controls at $p < 0.01$.

^aAverage severity, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

sensitive in detecting potential carcinogens than standard rodent models (Tennant *et al.*, 1995). Because Tg.AC transcript expression is found in both the skin and forestomach (Cannon *et al.*, 1997), it was possible that a good correlation might be found in papilloma response at the two sites between drinking water and dermal exposures. Should this correlation prove accurate, it would be possible to screen and rank numerous DBPs for relative carcinogenic potency using dermal exposures at a fraction of the cost of 2-year studies (Boorman *et al.*, 1999).

The NTP selected DCA, bromodichloromethane, and bromate as chemicals that are frequently found in the drinking water and for which considerable rodent and mechanistic data was available. DCA is especially well studied with eight mouse studies showing increased liver tumor incidence with drinking water exposure (IARC, 2004) (also see Table 6 and Fig. 2).

In addition to the 39- to 41-week exposures, we included a 26-week exposure to determine whether the shorter exposure would be sufficient to detect a carcinogenic effect in genetically modified mice. DCA exposure by the dermal route in Tg.AC hemizygous mice and by the drinking water route in both Tg.AC and p53 haploinsufficient mice failed to cause a significant increase in tumors in any of the groups of mice at 26 weeks. Exposures longer than we conducted have diminishing utility because in both Tg.AC and P53 mice

increasing background tumor incidences and increasing mortality begin to decrease sensitivity to detect an effect.

The top doses in these studies were at or near the maximum feasible concentrations. The highest dermal dose (500 mg/kg) is approximately 150 g DCA/l of dosing solution and the higher drinking water concentrations (1 or 2 g/l) resulted in both decreased water consumption and body weights in the Tg.AC and p53 mice. The decreases were more severe in the p53 mice where mean body weights in the top two dose groups were at least 15% lower than controls in the females and more than 20% lower in the males.

In the 39-week dermal study in Tg.AC mice, there was a clear papilloma response at the site of application in the 500 mg/kg dose group. However, at the next lowest dose (125 mg/kg), dermal papillomas were not significantly increased, suggesting that dermal exposure of water mixtures is unlikely to be a sensitive indicator of potential carcinogenicity for the family of haloacetic acids that might be found in the drinking water.

The p53 haploinsufficient mice failed to demonstrate a tumor response to exposures up to 2000 mg/l for 26 or 41 weeks. This may not be surprising because the p53 model is considered more sensitive to mutagenic carcinogens (Tennant *et al.*, 1995). A recent workshop concluded that the p53 haploinsufficient mouse is a useful model for risk assessment of genotoxic chemicals (MacDonald *et al.*, 2004). DCA is considered to be genotoxic

TABLE 4
Incidences and Severities of Cytoplasmic Vacuolization of the Hepatocytes in p53 Haploinsufficient Mice in the Drinking Water Studies of DCA

	26-week study (<i>n</i> = 15 per group)				41-week study (<i>n</i> = 10 per group)			
	0 mg/l	500 mg/l	1000 mg/l	2000 mg/l	0 mg/l	500 mg/l	1000 mg/l	2000 mg/l
Males	15 (100%), 2.7 ^a	15 (100%), 3.4	15 (100%), 3.4	15 (100%), 4.0	9 (90%), 3.6	10 (100%), 3.0	10 (100%), 3.7	10 (100%), 3.8
Females	3 (20%), 1.0	15** (100%), 2.2	15** (100%), 3.1	15** (100%), 3.5	10 (100%), 1.9	10 (100%), 2.7	10 (100%), 3.7	10 (100%), 3.6

**Differs from controls at $p < 0.01$.

^aAverage severity, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

TABLE 5
Incidences of Alveolar/Bronchiolar Neoplasms in Tg.AC Hemizygous Mice in the Drinking Water Studies of DCA

	26-week study (n = 15 per group)				41-week study (n = 10 per group)			
	0 mg/l	500 mg/l	1000 mg/l	2000 mg/l	0 mg/l	500 mg/l	1000 mg/l	2000 mg/l
Males	0 (0%)	0 (0%)	1 ^a (7%)	0 (0%)	1 ^b (10%)	2 ^b (20%)	7 ^{**b} (70%)	3 ^b (30%)
Females	0 (0%)	1 ^a (7%)	0 (0%)	1 ^a (7%)	0 (0%)	0 (0%)	0 (0%)	2 ^b (20%)

**Differs from controls at $p < 0.01$.

^aAlveolar/bronchiolar carcinoma.

^bAlveolar/bronchiolar adenoma.

in vivo and *in vitro*, but the results for mutagenesis in bacteria and mouse lymphoma cell lines are inconsistent (IARC, 2004).

The consistent positive liver tumor response to DCA in drinking water in ten B6C3F₁ mouse studies (IARC, 2004; Table 6) and in one rat study (DeAngelo *et al.*, 1996) suggested that we might find a liver tumor response in these genetically modified mouse models. A review of all of the pathology data in the current studies revealed only three benign (adenoma) hepatocellular tumors in the 300 mice of both strains and both sexes that were exposed to 500–2000 mg/l DCA in the drinking water. The negative results in the p53 haploinsuffi-

cient mouse is perhaps not surprising because it has previously been shown that this model generally does not respond to other chemicals that induce predominantly mouse liver tumors (Spalding *et al.*, 2000). What received less attention was that the Tg.AC mouse also failed to detect five of the seven nonmutagenic rodent carcinogens (Spalding *et al.*, 2000).

The drinking water exposure in the Tg.AC hemizygous mouse appeared to cause an increase in pulmonary tumors. In the 41-week study there was a significant increase in pulmonary adenomas (7/10) in the male mice exposed to 1000 mg/l DCA. Pulmonary adenomas were found in only three males and two

TABLE 6
DCA Studies of Liver Tumor Incidence in Nongenetically Modified Mice^a

Study	Authors	Citation	Sex	Route of exposure	DCA doses (g/l)	Maximum duration of exposure	Liver tumor response
1	Herren-Freund, Pereira, Khoury, Olson	<i>Toxicol. Appl. Pharmacol.</i> 90 , 183–189, 1987	M	Water, with 0, 2.5, 10 µg/g bwt ip ENU	2, 5	61 weeks	+
2	Bull, Sanchez, Nelson, Larson, Lansing	<i>Toxicology</i> 63 , 341–359, 1990	M, F	Water	1, 2 (M); 2 (F)	52 weeks	+(M); –(F)
3	DeAngelo, Daniel, Stober, Olson	<i>Fundam. Appl. Toxicol.</i> 16 , 337–347, 1991	M	Water	0.05, 0.5, 3.5, 5	75 weeks	+
4	Daniel, DeAngelo, Stober, Olson, Page	<i>Fundam. Appl. Toxicol.</i> 19 , 159–168, 1992.	M	Water	0.5	104 weeks	+
5	Pereira	<i>Fundam. Appl. Toxicol.</i> 31 , 192–199, 1996	F	Water	2.0, 6.67, 20.0 mmol/l	83 weeks	+
6	DeAngelo, George, House	<i>J. Toxicol. Environ. Health</i> 58 , 485–507, 1999	M	Water	0.05, 0.5, 1, 2, 3.5	100 weeks	+
7	Pereira, Kramer, Conran, Tao	<i>Carcinogenesis</i> 22 , 1511–1519, 2001	M, F	Water, with 30 mg/g MNU ip and chloroform in water	3.2	31 weeks	+(M); +(F)
8	Bull, Orner, Cheng, Stillwell, Stauber, Sasser, Lingohr, Thrall	<i>Toxicol. Appl. Pharmacol.</i> 182 , 55–65, 2002	M	(1) Water; (2) Water	(1) 0.5, 2; (2) 0.1, 0.5, 2	87 weeks	+
9	Carter, Carter, Deddens, Hurst, George, DeAngelo	<i>Environ. Health Perspect.</i> 111 , 53–64, 2003	M	Water	0.05, 0.5, 1, 2, 3.5	100 weeks	+
10	Bull, Sasser, Lei	<i>Toxicology</i> 199 , 169–183, 2004	M	Water, with TCA and CT	0.1, 0.5, 2	36 weeks	+

Note. bwt = body weight; ENU = ethylnitrosourea; MNU = N-methyl-N-nitrosourea; TCA = trichloroacetate; CT = carbon tetrachloride. Also see Figure 2.

^aAll were B6C3F₁ mice.

females exposed to 2000 mg/l. In the 26-week DCA drinking water exposure study one dosed male and two dosed females were diagnosed with pulmonary alveolar/bronchiolar carcinomas. Although these carcinomas did not appear to be dose-related, they may have been related to DCA exposure.

This study, suggests that routine use of the Tg.AC hemizygous and p53 haploinsufficient mouse may underestimate the number of chemicals that would demonstrate carcinogenic activity if evaluated in standard rodent studies. Genetically modified mouse models continue to be an excellent research model to address mechanistic questions for a variety of disease processes including carcinogenicity. Their use in routine screening of unknown chemicals should be done with caution.

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