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Toxicity and Carcinogenicity of the Water Disinfection Byproduct, Dibromoacetic Acid, in Rats and Mice

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Abstract

Dibromoacetic acid (DBA) is a water disinfection byproduct formed by the reaction of chlorine oxidizing compounds with natural organic matter in water containing bromide. Male and female F344/N rats and B6C3F₁ mice were exposed to DBA in drinking water for 2 weeks (N=5), 3 months (N=10), or 2 years (N=50). Concentrations of DBA in drinking water were 0, 125, 250, 500, 1,000, and 2,000 mg/L in the 2-week and 3-month studies, and 0, 50, 500, and 1,000 mg/L in the 2-year studies. Toxic effects of DBA in the prechronic studies were detected in the liver (hepatocellular cytoplasmic vacuolization in rats and mice) and testes (delayed spermiation and atypical residual bodies in male rats and mice, and atrophy of the germinal epithelium in rats). In the 2-year studies, neoplasms were induced at multiple sites in rats and mice exposed to DBA; these included mononuclear cell leukemia and abdominal cavity mesotheliomas in rats, and neoplasms of the liver (hepatocellular adenoma or carcinoma and hepatoblastoma) and lung (alveolar adenoma or carcinoma) in mice. The increase in incidence of hepatocellular neoplasms in male mice was significant even at the lowest exposure concentration of 50 mg/L, which is equivalent to an average daily dose of approximately 5 mg/kg. These studies provide critical information for future re-evaluations of health-based drinking water standards for haloacetic acids.

Keywords

dibromoacetic acid; water disinfection byproduct; delayed spermiation; mesothelioma; hepatoblastoma; leukemia; hepatocellular adenoma/carcinoma

Introduction

Haloacetic acids are formed when drinking water supplies containing natural organic matter are disinfected with chlorine oxidizing compounds. If bromide is also present in the source

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water, it may be oxidized to hypobromous acid-hypobromite ion, which can react with organic matter to form brominated organic compounds (Richardson et al., 2003).

Haloacetic acids are second to trihalomethanes as the most commonly detected disinfection byproducts in surface drinking water supplies in the United States. The relative amounts of these two families of chemicals as well as other disinfection byproducts produced in drinking water supplies are affected by several factors, including the nature and concentration of the organic precursor materials, water temperature, pH, the type of disinfectant, the disinfectant dose and contact time (Liang and Singer, 2003;Huang et al., 2004). Levels of haloacetic acids in drinking water are regulated by the US Environmental Protection Agency. Under the disinfection byproduct rule (US EPA, 1998) the sum of five haloacetic acids {monochloroacetic acid, dichloroacetic acid (DCA), trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid (DBA)} are limited to 60 µg/L (60 ppb). In a nationwide study of disinfection byproduct occurrence in diverse geographic regions of the United States, concentrations of DBA in the finished water ranged up to 18 µg/L and estimates of DBA in distribution systems ranged up to 22 µg/L (Weinberg et al., 2002).

Dihaloacetates are rapidly absorbed from the gastrointestinal tract after oral exposure. The maximum blood concentration of dibromoacetate in F344 rats was reached in one hr after administration by gavage (Schultz et al., 1999). The major metabolites identified in the urine and blood of F344 rats or B6C3F₁ mice administered DCA are glyoxylate, glycolate, and oxalate (Lin et al., 1993;Narayanan et al., 1999). In addition to these metabolites, approximately 30% of radioactivity from orally administered ¹⁴C-DCA was exhaled as carbon dioxide (Lin et al., 1993;Xu et al., 1995). Biotransformation of dihaloacetates to glyoxylate occurs primarily in liver cytosol of rats and humans by a glutathione dependent process (James et al, 1997) catalyzed by glutathione-S-transferase zeta (GSTζ) (Tong et al., 1998). Glyoxylate can undergo transamination to glycine, decarboxylation to form carbon dioxide, and oxidation to oxalate. Pretreatment of rats or mice with dihaloacetates reduces the rate of elimination of subsequent doses of these agents (Gonzalez-Leon et al., 1997;Austin and Bull, 1997); a similar effect has been observed in children and adults given therapeutic doses of DCA (Curry et al., 1991;Stacpoole et al., 1998). The reduced elimination of dihaloacetates in pretreated animals is due to irreversible inactivation of GSTζ (Anderson et al., 1999;Saghir and Schultz, 2002).

Spermatotoxicity in male rats has been identified as one of the most sensitive toxic endpoints of exposure to DCA and DBA; the primary effects appear to be delayed spermiation (retention of mature sperm), formation of atypical residual bodies, abnormal sperm morphology, and decreased sperm motility (Linder et al., 1994a,b;1997). The formation of atypical residual bodies was suggested to be a result of impairment of degradative processes of Sertoli cells. At doses of DBA that caused changes in sperm quality, the germinal epithelium appeared intact and there were no obvious changes in sperm production. At higher doses (250 mg/kg), seminiferous tubule atrophy was observed (Linder et al., 1997). Exposures to DBA induced liver changes (e.g., glycogen accumulation) in B6C3F₁ mice (Kato-Weinstein et al., 2001) and neuromuscular toxicity in F344 rats (Moser et al., 1999).

No studies have been reported on the carcinogenicity of DBA in animals; however, several studies have shown that DCA administered in drinking water is carcinogenic to the liver of mice and rats (Herren-Freund et al., 1987;DeAngelo et al., 1991;Daniel et al., 1992;DeAngelo et al., 1996).

No studies have been reported on the carcinogenicity or reproductive and developmental effects of DBA in humans; however, several studies have indicated an association between exposure to chlorination byproducts and alterations in reproductive function, fetal development

(Nieuwenhuijsen et al., 2000), and human cancer risk (Morris et al., 1992;McGeehin et al., 1993;Cantor et al., 1999).

Because there is widespread human exposure to DBA and because a related chemical, DCA, was found to be carcinogenic to the liver of rats and mice, the present studies were conducted to characterize the toxicity and carcinogenicity of this brominated water disinfection byproduct in rats and mice. Drinking water was selected as the route of exposure to mimic human exposure to this chemical.

Materials and Methods

Test chemical and dose formulation

DBA was obtained as a clumped white powder or moist crystalline solid from Fluka (Buchs, Switzerland) in one lot for use in the 2-week, 3-month and 2-year studies. The identity of the material was confirmed by infrared, UV/visible, and NMR spectroscopy. Periodic HPLC analyses indicated that the purity of the test material was >99% during these studies; a single impurity with a relative peak area of 0.34% had the same retention time as monobromoacetic acid. Karl Fisher titration indicated 0.27% water.

Dose formulations were prepared by mixing DBA with tap water, adjusted to pH 5 with 0.1N sodium hydroxide, and stored in sealed opaque glass or Nalgene® containers at 5° C for up to 6 weeks. Measured levels of DBA in tap water samples were approximately 30 µg/L during the 2-week studies, 20 µg/L during the 3-month studies, and 4 µg/L during the 2-year studies. Formulations were analyzed by ion chromatography and those used in these studies differed by 10% or less from the target concentrations.

Animal maintenance and exposure

Four-week-old male and female F344/N rats and B6C3F₁ mice were obtained from Taconic Farms, Inc. (Germantown, NY). Animals were quarantined for 2-weeks and were approximately 6 weeks old at the beginning of each study. Animals were housed in polycarbonate cages containing heat-treated irradiated hardwood chips (five per cage for male and female rats and female mice, except in the 2-year study in which male rats were housed up to three per cage). Male mice were housed individually. Feed (NTP-2000) and water were available *ad libitum*. The animal room environment was maintained at 72 ± 3° F, 50 ± 15% relative humidity, with 10 room air changes per hour; fluorescent lighting was provided 12 hr per day.

Drinking water containing DBA at concentrations of 0, 125, 250, 500, 1,000, and 2,000 mg/L were provided to groups of 5 male and female rats and mice for 2-weeks and to groups of 10 animals per sex and species for 3 months. In the 2-year studies, groups of 50 male and female rats and mice were exposed to 0, 50, 500, or 1000 mg/L of DBA in the drinking water. All animals were observed twice daily. Water consumption was recorded weekly in the 2-week and 3-month studies; in the 2-year studies water consumption was recorded weekly for the first 13 weeks, then for 7 days every 4 weeks. Body weights were recorded weekly in the 2-week and 3-month studies; in the 2-year studies body weights were recorded weekly during the first 13 weeks, at 4-week intervals thereafter, and at terminal sacrifice.

Histopathology

All animals that died during each study or that were killed at the end of each exposure period received a complete necropsy. Organ weights measured at terminal necropsies in the 2-week and 3-month studies included the heart, right kidney, liver, lung, right testis, and thymus. In the 2-week study, microscopic examinations were limited to gross lesions, as well as kidney,

liver, lung, testis, and thymus in the 0 and 2,000 mg/L groups and to no-effect levels. Complete histopathologic examinations were performed on animals exposed to 0 or 2,000 mg/L and in lower dose groups to no-effect levels in the 3-month study and on all animals in all dose groups in the 2-year studies. All major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 μ m, and stained with hematoxylin and eosin. For complete histopathologic evaluations, the following tissues were examined microscopically: gross lesions and tissue masses, adrenal gland, bone marrow, brain, clitoral gland, esophagus, eye, gallbladder (mice), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, trachea, urinary bladder, and uterus. An independent quality assessment pathologist evaluated all pathology data and materials; final diagnoses were based on a consensus of an NTP pathology working group (Boorman et al., 1985).

Historical control data provided in Tables 4 and 5 were obtained from NTP drinking water studies conducted (within 5 years of the DBA study) in F344 rats or B6C3F₁ mice that were given NTP-2000 diet.

Statistical analyses

Differences in survival were analyzed by survival analysis methods (Cox, 1972; Tarone, 1975). In the 3-month studies, incidences of lesions (the ratio of the number of animals bearing such lesions at a specific anatomical site to the number of animals in which that site was examined) were analyzed with the Cochran-Armitage trend test and Fisher's exact test (Gart et al., 1979). Lesion incidences in the 2-year studies were analyzed by the survival-adjusted Poly-3 quantal response test (Bailer and Portier, 1988; Portier and Bailer, 1989). Tests of significance included pairwise comparisons of each exposed group with controls and a test for an overall exposure-related trend.

Results

2-Week Studies

Exposure to DBA in drinking water for two weeks at concentrations up to 2000 mg/L had no adverse effects on water consumption, survival, body weight gain, or clinical signs in rats or mice. Based on water consumption and body weight during the 2-week exposures, average daily doses of DBA ranged from 17 to 270 mg/kg in rats and from 22 to 370 mg/kg in mice (Table 1).

Liver weights of all exposed male and female rat groups were 12–44% greater than controls (results not shown) and the incidences of diffuse cytoplasmic alteration of hepatocytes were increased in males exposed to 250 mg/L or greater and in the 2000 mg/L females (Table 2). Affected hepatocytes were minimally enlarged, granular or vacuolated (0% to 25% greater than normal). The cytoplasm of affected cells appeared pale and stained lightly eosinophilic.

Relative testis weights of male rats exposed to 500 mg/L or greater concentrations were decreased by 8–15% compared to controls. Testicular lesions in these dose groups were characterized by delayed spermiation. Step 19 spermatids were retained and found in the outermost part of the seminiferous epithelium adjacent to the lumen in stages IX and X of the spermatogenic cycle. These changes were occasionally accompanied by large atypical residual bodies. Atypical residual bodies were also observed in male mice exposed to 500 mg/L or

greater concentrations (Table 2). These round to oval amphophilic to eosinophilic bodies were present free in the lumen and also near the basement membrane of the seminiferous tubules.

Incidences of thymic atrophy were increased in male mice exposed to 1000 or 2000 mg/L and in female mice exposed to 2000 mg/L (Table 2). This lesion consisted of a minimal to mild decrease in the thickness of the thymic cortex.

3-Month Studies

Rats and mice were exposed to the same drinking water concentrations of DBA as in the 2-week study. Final mean body weights or body weight gains in the 2000 mg/L groups were approximately 10% less than those of controls for male and female rats and mice. There were no effects on water consumption or survival, and there were no clinical findings related to the DBA exposures in rats or mice. Average daily doses of DBA ranged from 10 to 181 mg/kg in rats and from 16 to 260 mg/kg in mice (Table 1).

Liver weights were increased by up to 40% in exposed male and female rats, and exposure-related increases in centrilobular to midzonal glycogen-like hepatocellular cytoplasmic vacuolization were observed (Table 3). This lesion was also present in most control and exposed mice; however, severity grades were increased in the 1000 and 2000 mg/L groups of males and females. The changes in severity of this lesion corresponded with increased liver weights.

Testis weights were decreased by 50% in male rats exposed to 2000 mg/L; this change was associated with testicular atrophy in this group (Table 3). The latter lesion was characterized by marked degeneration of the germinal epithelium, syncytial cell formation, vacuolization of Sertoli cells, mild nonsuppurative inflammation around some venules, and more prominent interstitial cells (Figure 1). Hypospermia was significantly increased in the epididymis, and sperm motility and sperm concentrations were significantly reduced in male rats exposed to 2000 mg/L. In the 500 and 1000 mg/L exposure groups, testicular changes in male rats were similar to those seen in the 14-day study, i.e., retained spermatids and large atypical residual bodies (Figure 2). In mice, testicular lesions were characterized by the presence of large atypical eosinophilic bodies in seminiferous tubules (and in the epididymis), occasional spermatid retention, and vacuolization of Sertoli cells (Table 3, Figures 3).

2-Year Studies

In the 2-year studies, groups of 50 male and female rats and mice were exposed to drinking water solutions containing 0, 50, 500, or 1000 mg DBA/L. The highest concentration (1000 mg/L) was selected based on body weight and organ weight effects and the severity of microscopic lesions observed at 2000 mg/L in the 3-month studies. Based on the dose-response data for liver tumors induced by dichloroacetic acid in mice (DeAngelo et al., 1991), the lower exposure concentration of DBA was selected to extend the dose range of any exposure related effects observed with this chemical.

Treatment with DBA had no effect on survival of rats or mice. Mean body weights of exposed mice were similar to those of controls, while mean body weights of the 500 and 1000 mg/L groups of male or female rats were at most 5 to 15% less than those of controls. These body weight differences were in part due to decreased water consumption. Average daily doses of DBA in rats and mice during the 2-year studies are shown in Table 1. At comparable exposure levels, average daily doses of DBA expressed as mg/kg body weight were lower with increasing durations of exposure (2 weeks, 3 months, and 2 years). Lower average daily doses occur because water consumption does not change proportionally with increasing body weight during animal growth phases.

The major neoplastic effects of DBA exposure in rats were induction of malignant mesotheliomas in males and increased incidences of mononuclear cell leukemia (MCL) in males and females (Table 4). The incidence of mesothelioma in the 1000 mg/L group of male rats was significantly increased compared to controls. This incidence also exceeded the historical incidence in controls from drinking water studies. Because neoplastic cells were found throughout the peritoneum, on the abdominal wall and serosal surface of several abdominal organs (Figure 4), all mesotheliomas in this study were classified as malignant.

A positive trend was observed for MCL in female rats and the incidence in the 1000 mg/L group was significantly increased compared to the incidences in controls. This incidence exceeded the historical range for drinking water controls. In males, a significant increase in MCL also occurred at 50 mg/L, and the incidences in the 50 and 500 mg/L groups exceeded the historical control rates for drinking water studies. The incidence of MCL in the 1000 mg/L group was similar to that of concurrent controls and historical controls.

The incidences of several nonneoplastic lesions were also significantly increased in rats exposed to DBA; these included minimal to mild cystic degeneration in the liver of males (6% control, 18% low dose group, 22% mid dose group, and 30% in the high dose group), alveolar epithelial hyperplasia in females (6%, 14%, 26%, and 28%), and nephropathy in females (36%, 64%, 74%, and 80%).

The major neoplastic effects of DBA exposure in mice were observed in the liver (Table 5). The incidences of hepatocellular adenoma or carcinoma were increased at all exposure levels and exceeded historical control rates for drinking water studies. In addition, the incidences of hepatoblastoma were increased in all exposure groups of male mice and exceeded historical control rates for drinking water studies. Hepatoblastomas are often observed within or adjacent to an existing hepatocellular adenoma or carcinoma and are considered to be an undifferentiated variant of hepatocellular neoplasms (Harada et al., 1999). The malignant potential of hepatoblastoma is similar to that of hepatocellular carcinoma.

In addition to liver neoplasms, the incidence of alveolar/bronchiolar adenoma or carcinoma was increased significantly in the 500 mg/L group of male mice. The incidences of these neoplasms in mid-dose and high-dose males and in high-dose females exceeded their historical rates in drinking water controls. A marginal increase in the incidence of alveolar epithelial hyperplasia was also noted in exposed male mice (4% control, 12 % low dose group, 12% mid dose group, and 14% in the high dose group).

Discussion

The liver and testes were the primary target organs of DBA-induced toxicity in rats and mice. In the liver, exposure to DBA produced increased incidences or severity of hepatocellular cytoplasmic vacuolization. Testicular lesions induced in rats in the 2-week and 3-month studies were similar to those described by Linder et al. (1994b;1997) and were characterized by a delay in spermiation with retention of step 19 spermatids in the seminiferous epithelium adjacent to the lumen of stage IX and stage X tubules of the spermatogenic cycle. These changes were accompanied by the presence of atypical residual bodies. The formation of atypical residual bodies in rats exposed to DBA was suggested to be a result of impaired degradative function in Sertoli cells (Linder et al., 1997). The lowest concentration of DBA showing testicular effects in the 3-month study was 500 mg/L, equivalent to a mean daily dose of 40 mg/kg. Decreased testicular weight and testicular atrophy were observed in the 2000 mg/L group (166 mg/kg). Linder et al. (1997) observed altered spermiation at 10 mg/kg or higher doses in Sprague-Dawley rats exposed to DBA by gavage for 31 or 79 days and seminiferous tubule atrophy at 250 mg/kg/day. The difference in dose-response between the present study and that of Linder

et al. (1997) might be due differences in rat strains used in the two studies or different methods of exposure (drinking water versus gavage). Sperm morphology evaluations in the present study showed reductions in epididymal sperm counts, sperm motility, and spermatid heads at 2000 mg/L (166 mg/kg) but not at 1000 mg/L (90 mg/kg). Hypospermia was also diagnosed in the epididymis of rats in the present study. Linder et al. (1994b) observed significant reductions in sperm motility and altered sperm morphology after 14 daily gavage doses of 270 mg/kg.

Testicular lesions induced in mice exposed to 1000 or 2000 mg/L were similar to those observed in rats. These lesions, which had not been reported previously in mice, were characterized as spermatid retention and large atypical residual bodies in seminiferous tubules. The atypical residual bodies in mice were generally larger than those observed in rats; however, this effect was detected at a lower average daily dose in rats (40 mg/kg) than in mice (115 mg/kg). As noted above, the formation of these bodies was probably due to impaired degradative functions carried out by Sertoli cells. Evaluations of sperm from exposed mice did not demonstrate any effects on morphology, motility, or epididymal counts. Neither testicular atrophy nor decreases in testicular weight were observed in mice exposed to 2000 mg/L, the concentration of DBA that caused these effects in rats.

In the 2-year studies, neoplastic effects were induced at multiple sites in rats and mice exposed to DBA. The incidence of malignant mesotheliomas in the 1000 mg/L group of male rats (20%) was significantly increased and exceeded the NTP's historical control rate for mesotheliomas in 2-year drinking water studies (mean historical incidence in male rats: $6.0\% \pm 4.2\%$). This malignant lesion was observed at multiple sites throughout the abdominal cavity. Chemically induced mesotheliomas have been observed predominantly in male rats compared to female rats or mice of either sex. Of over 500 carcinogenicity studies reported by the NTP, 16 agents produced positive evidence of chemically induced neoplasms in the mesothelium; of those agents, 15 were active in male rats, two in female rats and mice, and one in male mice. Both mutagenic and non-mutagenic chemicals induced these neoplasms. Phenoxybenzamine was the only chemical that induced neoplasms in the abdominal cavity mesothelium of both sexes of rats and mice (NCI, 1978). Potassium bromate also induced mesotheliomas of the peritoneum in male F344 rats (Kurokawa et al., 1983). Based on the above studies, there is no apparent relationship between chemical structure and this neoplastic response.

In a subsequent 2-year study of potassium bromate administered to male F344 rats in drinking water at concentrations ranging from 0.02 to 0.4 g/L, mesothelioma was detected on the tunica vaginalis testis in one animal euthanized after 52 weeks of treatment (0.2 g/L); whereas, after 78 weeks of treatment, mesotheliomas were present on multiple abdominal organs (Wolf et al., 1998). Based on these findings, Wolf et al. (1998) suggested that the tunica vaginalis might be the site of origin of bromate-induced mesotheliomas. However, the time-dependent incidence of preneoplastic lesions and mesotheliomas in male rats exposed to potassium bromate was consistent with either the tunica vaginalis or the spleen as the site of origin of these neoplasms (Crosby et al., 2000b). In the present study of DBA, the earliest death of a male rat with mesothelioma occurred after 73 weeks of exposure. Neoplastic lesions were observed on multiple organs throughout the abdominal cavity in early death animals in the DBA study. Thus, it is not possible at this time to draw a definitive conclusion on the site of origin of the abdominal mesotheliomas.

MCL is a commonly occurring hematopoietic neoplasm in F344 rats. It is a rapidly progressive, lethal neoplastic disease that first develops in the spleen, with infiltrates of neoplastic cells occurring in the liver, lung, lymph nodes, bone marrow, and other organs (Elwell et al., 1996). A significantly increased incidence and positive trend for MCL was observed in female rats. The incidence in the 1000 mg/L group (44%) far exceeded the historical control rate for

2-year drinking water studies in which female rats were given NTP-2000 diet (mean incidence of $23.5\% \pm 4.4\%$). In male rats, the incidence of MCL was increased significantly in the 50 mg/L group (62% versus 34% in controls), and a marginal increase was observed in the 500 mg/L group (48%). Both of these incidences exceeded the historical control rate for 2-year drinking water studies in male rats fed NTP-2000 diet (mean incidence of $31.6 \pm 3.3\%$). There was no difference in the incidence of MCL between controls and high-dose males (1,000 mg/L) (26%). The MCL response in female rats was considered to be related to DBA exposure based on the positive trend, the significantly increased incidence that far exceeded historical rates, and the equivocal evidence of DBA-related MCLs in male rats.

A nonmonotonic dose response does not always occur with chemically induced MCLs in male rats. In most instances in which such a response was observed, survival was reduced in the higher dose groups (e.g., NTP 1988;1993;1996). However, in the DBA study, survival of male rats was not significantly reduced in any exposure group. Because there is no satisfactory explanation for the significantly increased incidence of MCL in only the low-dose group, the MCL response in male rats was considered to represent equivocal evidence of carcinogenic activity. This conclusion is consistent with that of other 2-year studies in which MCL was increased in the low-dose or mid-dose groups (e.g., NTP 1997;2001).

Exposure of mice to DBA produced significant dose-related increases in hepatocellular adenomas or carcinomas in males and females and hepatoblastomas in male mice. Of particular note is that the increase in hepatocellular neoplasms in male mice was significant even at the lowest exposure concentration tested (50 mg/L), which is equivalent to an average daily dose of approximately 5 mg/kg. Administration of DCA in drinking water for two years also induced hepatocellular neoplasms in male B6C3F1 mice (DeAngelo et al., 1991); however, DCA did not induce hepatocellular neoplasms at doses as low as that of DBA in the current study. The potent liver tumor response observed in this study provides clear evidence of carcinogenic activity for DBA in mice. The increases in hepatocellular neoplasms were not associated with hepatocellular necrosis or regenerative hyperplasia.

DBA also induced a significant increase in alveolar/bronchiolar neoplasms in male mice and a nonsignificant dose-related increase in lung neoplasms in female mice. Also observed in male mice was a dose-related increase in alveolar epithelial hyperplasia, a lesion that is considered to be part of the continuum of proliferative changes in lung neoplasia. Thus, the lung neoplastic response in male mice is likely related to DBA exposures, while the weaker response observed in female mice may have been related to DBA exposure.

The 2-years studies presented here demonstrate that DBA is a multiple organ carcinogen in laboratory animals; the primary sites of tumor induction identified are the abdominal cavity mesothelium of male rats, hematopoietic system in female rats, and the liver and lung of mice. The mechanism(s) of neoplasm induction by DBA or the related compound, DCA, are not known. For DCA, Carter et al. (2003) suggested that the induction of liver tumors in mice is due to selective growth of a phenotypic cell-type that does not respond to mitoinhibitory homeostatic control mechanisms. Neither hepatocellular necrosis nor regenerative hyperplasia was associated with the development of preneoplastic lesions or liver tumors in DCA-treated or DBA-treated mice. An early increase in hepatocyte proliferation is not likely involved in the mode of action for DBA, because no increases in the hepatocyte DNA labeling index were observed in mice exposed for 26 days (results not shown), and a slight increase that occurred in male F344 rats was not accompanied by an increase in liver tumor response. DNA hypomethylation and increased expression of c-myc and IGF-II genes were suggested as possible early events in the hepatocarcinogenicity of dihaloacetic acids in mice (Pereira et al., 2001; Tao et al., 2004). DNA damage due to oxidative stress in the livers of mice exposed to halogenated acetic acids, including DBA (Austin et al., 1996), may also contribute to the

hepatocarcinogenicity of these chemicals. A study of gene expression in immortalized rat peritoneal mesothelial cells incubated with potassium bromate detected increases in oxidative stress responsive genes, as well as changes in genes that regulate DNA repair and cell cycling (Crosby et al., 2000a). The carcinogenicity of DBA may involve a genotoxic mechanism since this chemical induces DNA damage in *E. coli* (Giller et al., 1997), mutations in *Salmonella typhimurium* strain TA98 and TA100 with and without metabolic activation (Kargalioglu et al., 2002), and DNA strand breaks in Chinese hamster ovary cells (Plewa et al., 2002). In addition, glyoxylate, a metabolite of dihaloacetate biotransformation, is mutagenic in *Salmonella* TA100, TA102, and TA104 (Sayato et al., 1987). Thus, it is possible that oxidative stress, DNA damage, and selective growth of preneoplastic cells are involved in the carcinogenesis of DBA.

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Figure 1. Marked (grade 4) seminiferous atrophy (end stage lesion) in the testis of a rat treated with 2000 mg/L of DBA for 13 weeks. All tubules (arrows) in the field are atrophic.

Figure 2.
Presence of numerous abnormal residual bodies (arrows) in the testis of a rat treated with 1000 mg/L of DBA for 13 weeks.

Figure 3. Presence of numerous abnormal residual bodies (arrows) in the testis of a mouse treated with 2000 mg/L of DBA for 13 weeks.

Figure 4. Malignant mesothelioma (arrow) growing on the serosal surface of the testis in a male rat treated with 1000 mg/L of DBA for 2 years. A spontaneous interstitial cell tumor is also present.

Table 1

Average daily doses of DBA (mg/kg/day) in rats and mice exposed via drinking water for 2 weeks, 3 months, or 2 years

Concentration in water, mg/L	Male rats			Female rats			Male mice			Female mice		
	2wk	3mo	2 yr	2wk	3mo	2 yr	2wk	3mo	2 yr	2wk	3mo	2 yr
50	-	-	2	-	-	0 ^a	-	-	4	-	-	4
125	17	10	-	17	12	-	24	16	-	22	17	-
250	31	20	-	33	23	-	47	30	-	53	34	-
500	67	40	20	67	48	25	95	56	45	88	67	35
1000	134	90	40	135	93	45	178	115	87	166	132	65
2000	270	166	-	257	181	-	370	230	-	309	260	-

^aThe levels of DBA in the tap water used in these studies resulted in average daily doses of <0.01 mg/kg DBA in control rats and mice.

Table 2Incidences^a of lesions induced in rats and mice exposed to DBA via drinking water for 2 weeks

Sex, species	Liver: hepatocyte cytoplasmic alteration						Organ: lesion						Thymus: atrophy	
	0	125	250	500	1000	2000	0	125	250	500	1000	2000	0	125
	mg/L						mg/L							
Male rats	0	0	2	5	5	5	0	0	0	4	5	5		
Female rats	0	0	0	0	0	5								
Male mice							0	0	0	1	5	5	0	0
Female mice													0	0

^aNumber of animals with lesion in groups of 5 animals per sex per species per dose.

Table 3
Liver and testicular weights and lesions in rats and mice exposed to DBA via drinking water for 3 months

		Concentration in water, mg/L					
		0	125	250	500	1000	2000
Male rats							
Liver							
Weight (grams)		10.7	12.3**	13.3**	13.3**	14.2**	15.0**
Hepatocellular vacuolization ^a		3	5	7	9*	9*	10*
Testis							
Weight (grams)		1.42	1.44	1.41	1.44	1.41	0.68**
Germinal epithelial atrophy ^a		0	0	0	0	0	10**
Delayed spermiation, atypical residual bodies ^a		0	0	0	8**	8**	0
Female rats							
Liver							
Weight (grams)		6.10	6.54*	7.37**	7.48**	7.92**	8.19**
Hepatocellular vacuolization ^a		0	0	1	0	3	10**
Male mice							
Liver							
Weight (grams)		1.63	1.76	1.80	1.84*	1.87**	2.10**
Hepatocellular vacuolization ^b		1.6	1.6	1.5	1.5	2.1	2.9
Testis							
Delayed spermiation, atypical residual bodies ^a		0	0	0	0	9**	10**
Female mice							
Liver							
Weight (grams)		1.19	1.24	1.35	1.39*	1.55**	1.67**
Hepatocellular vacuolization ^b		1.5	1.5	1.5	1.7	2.3	2.7

^a Data represent the number of animals with this lesion in groups of 10

^b Because essentially all control and exposed mice had this lesion, the data for mice are group mean severity grades (1=minimal, 2=mild, 3=moderate, 4=marked)

* Significantly different ($P \leq 0.05$) from the control group by Fisher's exact test;

** $P \leq 0.01$

Table 4

Incidences^a of malignant mesothelioma and mononuclear cell leukemia (MCL) in rats in the 2-year drinking water study of DBA

	Concentration in water, mg/L			
	0	50	500	1000
Males				
Mesothelioma ^b	3/50 7**	1/50 2	0/50 0	10/50 23*
MCL ^c	17/50 37	31/50 66**	24/50 56	13/50 30
Females				
MCL ^d	11/50 24**	13/50 27	16/50 35	22/50 47*

^aThe first value for each group is the overall incidence of this lesion; the second value is the Poly-3 mortality-adjusted incidence (%).

^bHistorical incidence: mean 6.0% ± 4.2%, range 0%–12%.

^cHistorical incidence: mean 31.6% ± 3.3%, range 26%–34%.

^dHistorical incidence: mean 23.5% ± 4.4%, range 20%–30%.

An asterisk by a control incidence indicates significance with the trend test of the mortality-adjusted rates. An asterisk by an exposure group incidence indicates significance by pairwise comparisons between the controls and that exposure group.

* Significantly different ($P \leq 0.05$) from the control group by the Poly-3 test;

** $P \leq 0.01$

Table 5
Incidences^a of liver and lung neoplasms in male and female mice in the 2-year drinking water study of DBA

	Concentration in water, mg/L			
	0	50	500	1000
Males				
Hepatocellular adenoma or carcinoma ^b	28/49	41/50	42/50	47/50
	61 **	86 **	88 **	96 **
Hepatoblastoma ^c	0/49	4/50	6/50	18/50
	0 **	9	13 *	39 **
Alveolar/bronchiolar adenoma or carcinoma ^d	12/49	12/50	22/50	17/50
	28	26	49 *	37
Females				
Hepatocellular adenoma or carcinoma ^e	22/49	28/50	37/50	37/49
	48 **	61	80 **	80 **
Alveolar/bronchiolar adenoma or carcinoma ^f	2/50	5/50	5/50	7/50
	4	11	11	15

^aThe first value for each group is the overall incidence of this lesion; the second value is the Poly-3 mortality-adjusted incidence (%).

^bHistorical incidence: mean 49.7% ± 31.1%, range 48%–85%.

^cHistorical incidence: mean 4.5% ± 6.2%, range 0%–13%.

^dHistorical incidence: mean 16.5% ± 10.7%, range 12%–26%.

^eHistorical incidence: mean 44.4% ± 18.1%, range 20%–63%.

^fHistorical incidence: mean 6.4% ± 3.9%, range 2%–12%.

An asterisk by a control incidence indicates significance with the trend test of the mortality-adjusted rates. An asterisk by an exposure group incidence indicates significance by pairwise comparisons between the controls and that exposure group.

* Significantly different ($P \leq 0.05$) from the control group by the Poly-3 test;

** $P \leq 0.01$