Special contribution

MTBE: recent carcinogenicity studies

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MTBE, a gasoline oxygenate, has contaminated drinking water sources for many years. Carcinogenicity studies conducted in animals in the 1990s raised concerns of potential human health risks. Recent industry-sponsored studies have confirmed the carcinogenic effects of this agent and have identified additional sites of tumor induction (i.e., brain). However, the petroleum industry has attempted to portray these recent findings as demonstrating either no effect or no concern for humans. Our paper briefly summarizes the new findings and puts into perspective the totality of carcinogenic effects and health risks on this environmental chemical.

Keywords: Gasoline oxygenate, MTBE, Carcinogenicity, Brain tumors, Water contaminant

Chronic (18-month or 2-year) studies on the gasoline oxygenate methyl tertiary butyl ether (MTBE) that were reported in the 1990s showed exposure-related increases in the incidences of kidney tumors and testicular tumors in male F344 rats and liver tumors in male and female CD-1 mice after inhalation exposure,[1] and testicular tumors in male Sprague–Dawley rats and lymphomas-leukemias in female Sprague–Dawley rats after oral (gavage) exposure.[2] These studies were used to develop cancer-based public health guidelines in California, New York, other states, and internationally.

Two recently released chronic study reports of MTBE in rats sponsored by the petroleum industry[3,4] also found carcinogenic effects associated with exposure to this chemical. This special contribution provides a brief synopsis of the more recent studies. We are continuing to review the thousands of pages of documentation received to date on these studies; thus, the information highlighted below must be considered as preliminary and incomplete. However, due to the seriousness of effects observed in these studies, and public health concerns arising from the increasing detections of MTBE in drinking water supplies in the USA,[5] it is prudent to provide an initial summary of cancers documented to date.

A 2-year drinking water study of MTBE in male and female Wistar rats was carried out at the Hamner Institute (HI),[3] with microscopic diagnoses performed by pathologists from Experimental Pathology Laboratories. This work was carried out at the request of the legal departments of ExxonMobil, Chevron, and Shell Oil. The study evaluated responses in three dose groups plus a control group for each sex. Each group was comprised of 50 animals. The planned drinking water concentrations of MTBE were 0, 0.5, 3.0, and 7.5 mg/ml for males and 0, 0.5, 3.0, and 15.0 mg/ml for females.

The HI study housed all animals of each gender in one room, with MTBE, a volatile chemical, provided *ad libitum* in water bottles. This resulted in airborne exposures of all animals, including the intended 'controls', as documented in airborne measurements taken at 6-month intervals. Due to their exposure, we regarded 'control' animals as a 'very low dose' exposure group. The unintended exposures increased the likelihood that cancer would be observed in 'control' animals, and therefore influences analyses of pairwise comparisons between exposed groups and 'controls'. Although it may have been difficult to eliminate these unintended exposures, the data analyses should have been based on the exposures in the control groups.

An additional problem with exposure quantification resulted from the poor palatability of MTBE in drinking water. This resulted in water consumption levels that decreased with increasing concentrations of MTBE. Water consumption was significantly reduced in all of the intended exposure groups compared to the 'control' groups. As a result of spillage, volatile loss of MTBE from the drinking water bottles during the study, and the unintentional inhalation exposure of all animals, dose levels in this study cannot be reliably calculated.

The most notable effect reported in the drinking water carcinogenicity study of MTBE was a statistically significant trend in male rats for astrocytomas, a

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rare brain cancer.[3] Although comparisons between the concurrent control group and the exposure groups are most valid for identifying chemical-induced effects, comparisons with historical control data are also helpful in interpreting treatment-related effects; this is particularly true for rare tumors.[6–8] Use of historical control data is essential for this study because it lacked a 'true unexposed control' group. To address the comparison between the incidence of astrocytomas in the high-dose group (8%) and in historical controls, Dodd *et al.*[3] relied on a 1994 report on spontaneous tumors in control Wistar rats, which was based on 10 2-year studies conducted in the 1980s.[9] The early data listed a range of 0–8% for astrocytomas in male rats. However, Dodd *et al.*[3] neglected to note that a follow-up report by the same authors reported a range of astrocytomas in control male Wistar rats of 0–2% (based on five 2-year studies conducted between 1990 and 1995).[10] More importantly, the authors opted to ignore larger databases reporting the incidence of astrocytomas in control male Wistar rats of 0.35% among 3696 male rats in 2-year studies at Harlan Laboratories.[11] The difference in incidence of astrocytomas in the high-dose group of male rats (4/50, 8%) in the HI study compared to the historical control incidence of this rare cancer in male Wistar rats (13/3696, 0.35%) was highly significant ($P<0.0001$ by a Fisher's exact test). Dismissing an important tumor result based on an outdated outlier control rate of 8% is misleading and lacks scientific credibility. We are still reviewing other cancers that might have occurred at elevated rates in this study.

The HI study had problems in addition to the repeated exposure of controls and the misuse of historical control data. Widespread infection and inflammatory disease of the feet, referred to as pododermatitis, occurred early in the study and necessitated treatment and in some cases euthanization of animals. This finding was unexpected because animals were housed in polycarbonate cages, eliminating the possibility of irritation from wire cage floors. In addition, this disease occurred in young animals that were not overweight. Ninety out of the 200 of the male rats (all exposure groups) were affected.

Treatments for this condition included twice daily application of triple antibiotics and disinfectants, such as chlorhexidine. The potential impact of pododermatitis, stress responses, and pharmaceutical treatments on the physiological, pharmacokinetic, or toxicological responses in animals exposed to MTBE are not known.

Histopathological evaluations of the low-dose and mid-dose groups were limited to gross lesions identified at necropsy and to two specified target organs: brain and kidney. Severely limiting histopathology evaluations can result in an under diagnosis of lesions that are not detected by gross visual inspection at necropsy. Although mention was made of differences in diagnosis between the original and quality assessment pathologists, a description of how those differences were resolved was not included in the final study report. The study also lacked a pathology working group evaluation of the microscopic findings, which is recommended for all carcinogenicity studies performed by the National Toxicology Program.[12] These shortcomings add to the uncertainty regarding the thoroughness of the pathological evaluations.

The second recent chronic (2-year) study sponsored by the American Petroleum Institute (required under TSCA), involved inhalation of evaporative emissions from unleaded gasoline or gasoline containing MTBE.[4] Groups of 50 male and 50 female F344 rats were exposed to total vapor concentrations of 0, 2, 10, or 20 g/m^3 for both gasoline vapor condensates. In the gasoline plus MTBE groups, the MTBE concentrations were 0.4, 2, or 4 g/m^3 (110, 555, or 1110 ppm).

All tumor incidence data were analyzed using a two-sided test with a significance level set at $P=0.05$, rather than a one-sided test which is conventionally used in carcinogenicity studies that are designed to determine if an agent causes an increase in tumor incidence compared to controls. Incidences of testicular tumors and mononuclear cell leukemia were significantly increased in male rats exposed to the evaporative emissions containing MTBE, but not with exposures lacking MTBE. The authors of this study[4] discounted the leukemia findings by suggesting that they were due to sampling bias in the low- and mid-dose groups (spleens from 11 low-dose male rats and 12 mid-dose male rats were not examined microscopically). Since spleens from all male rats should have been examined as a potential target organ, the improper dismissal of this positive finding reflects poor laboratory practice.

Incidences of kidney tumors in male rats were significantly increased with exposure to gasoline alone and gasoline plus MTBE. However, in the high-dose groups, incidence of kidney tumors was elevated only in the gasoline plus MTBE group. The exposure concentration of MTBE in this study's high-dose group was less than 40% of the concentration of MTBE that induced a significant increase in kidney neoplasms in the previous study of MTBE by Bird *et al.*[1]

Some significant effects observed in gasoline plus MTBE exposure groups that were not highlighted by the authors[4] include increased incidences of mammary gland fibroadenomas in female rats and

olfactory epithelial degeneration that was significantly increased in male rats and elevated but not significantly in female rats.

Alpha2u-Globulin nephropathy has been hypothesized to be a male rat-specific, non-genotoxic mode-of-action for the induction of kidney tumors by MTBE.[1,4,13,14] However, experimental data demonstrate that MTBE fails to meet several criteria established by International Agency for Research on Cancer[15] and the US Environmental Protection Agency[16] for judging whether an agent might cause kidney tumors by an alpha2u-globulin associated response: (1) the MTBE metabolite, formaldehyde, is a definite genotoxic chemical; (2) severe nephropathy is induced by MTBE in female rats;[1] (3) most of the characteristic sequence of histopathological changes associated with alpha2u-globulin nephropathy have not been observed with MTBE; (4) much of the accumulating protein in renal tubule cells of treated rats is likely not alpha2u-globulin;[17,18] (5) binding of MTBE to kidney proteins is extremely weak; (6) similarities in dose–response relationships for tumor outcome and histopathological endpoints have not been observed.

In summary, the recent studies on MTBE noted above confirm and extend the carcinogenicity database on this chemical. The totality of data shows that MTBE induces neoplasms in multiple studies, at multiple sites, by multiple routes of exposure (inhalation, oral gavage, and drinking water), in multiple species and strains of laboratory animals, and in both genders. In addition, exposure to MTBE is associated with the induction of rare and uncommon tumors. MTBE is rapidly absorbed after inhalation or oral exposures, and distributed in the blood to all major tissues.[19] With substantial evidence of genotoxicity now available, it is entirely plausible that MTBE would cause cancer in multiple organs. Metabolism of MTBE produces equimolar amounts of t-butanol and formaldehyde,[20] a genotoxic chemical and known human carcinogen (causing nasopharyngeal tumors and leukemia).[21,22] Thus, the overall scientific evidence on MTBE is consistent with criteria established by national and international health agencies for classifying an agent in the absence of human data as a 'probable human carcinogen' (International Agency for Research on Cancer),[23] 'reasonably anticipated to be a human carcinogen' (National Toxicology Program),[24] or 'likely to be carcinogenic to humans' (US Environmental Protection Agency).[25] Hence, the elimination of human exposures to MTBE continues to be an important public health need.

Declaration of Interest

The authors have worked on MTBE for governmental agencies since the 1990s and currently provide assistance and consultative services on MTBE to law offices and non-profit agencies.

References

1 Bird MG, Burleigh-Flayer HD, Chun JS, Douglas JF, Kneiss JJ, Andrews LS. Oncogenicity studies of inhaled methyl tertiary-butyl ether (MTBE) in CD-1 mice and F-344 rats. J Appl Toxicol 1997;17:S45–55.

2 Belpoggi F, Soffritti M, Maltoni C. Pathological characterization of testicular tumors and lymphomas–leukaemias, and of their precursors observed in Sprague–Dawley rats exposed to methyl-tertiary-butyl-ether (MTBE). Eur J Oncol 1998;3:201–6.

3 Dodd DE, Layko DK, Bermudez E. Methyl tertiary-butyl ether (MTBE): two-year combined chronic toxicity/carcinogenicity drinking water study in Wistar rats. Protocol Number 07001. Research Triangle Park, NC: Hamner Institute for Health Sciences; 2010. Available from the following: http://hero.epa.gov/index.cfm?action=search.view&reference_id=835572 http://hero.epa.gov/index.cfm?action=search.view&reference_id=835686 http://hero.epa.gov/index.cfm?action=search.view&reference_id=835697 http://hero.epa.gov/index.cfm?action=search.view&reference_id=835726 http://hero.epa.gov/index.cfm?action=search.view&reference_id=835761

4 Benson JM, Gigliotti AP, March TH, Barr EB, Tibbetts BM, Skipper BJ, *et al*. Chronic carcinogenicity study of gasoline vapor condensate (GVC) and GVC containing methyl tertiary-butyl ether in F344 rats. J Toxicol Environ Health A2011;74:638–57

5 US Environmental Protection Agency. Methyl tertiary butyl ether (MTBE) overview; 2008. [cited 2011] Available from: http://www.epa.gov/mtbe/faq.htm

6 Haseman JK, Huff J, Boorman GA. Use of historical control data in carcinogenicity studies in rodents. Toxicol Pathol 1984;12:126–35.

7 Haseman JK, Boorman GA, Huff J. Value of historical control data and other issues related to the evaluation of long-term rodent carcinogenicity studies. Toxicol Pathol 1997;25:524–7.

8 Keenan C, Elmore S, Francke-Carroll S, Kemp R, Kerlin R, Peddada S, *et al*. Best practices for use of historical control data of proliferative rodent lesions. Toxicol Pathol 2009;37:679–3

9 Walsh KM, Poteracki J. Spontaneous neoplasms in control Wistar rats. Fundam Appl Toxicol 1994;22:65–72.

10 Poteracki J, Walsh KM. Spontaneous neoplasms in control Wistar rats: a comparison of reviews. Toxicol Sci 1998;45:1–8.

11 Weber K, Garman RH, Germann PG, Hardisty JF, Krinke G, Millar P, *et al*. Classification of neural tumors in laboratory rodents, emphasizing the rat. Toxicol Pathol 2011;39:129–51.

12 Boorman GA, Montgomery CA, Jr, Eustis SL, Wolfe MJ, McConnell EE, Hardisty JF. Quality assurance in pathology for rodent carcinogenicity studies. In: Milman HA, Weisburger EK, editors. Handbook of carcinogen testing. Park Ridge, NJ: Noyes Publications; 1985. p. 345–67.

13 Prescott-Mathews JS, Poet TS, Borghoff SJ. Evaluation of the in vivo interaction of methyl tert-butyl ether with alpha2u-globulin nephropathy in male F-344 rats. Toxicol Appl Pharmacol 1999;157:60–7.

14 Prescott-Mathews JS, Wolf DC, Wong BA, Borghoff SJ. Methyl tert-butyl ether causes alpha2u-globulin nephropathy and enhanced renal cell proliferation in male Fischer-344 rats. Toxicol Appl Pharmacol 1997;143:301–14.

15 Capen CC, Dybing E, Rice JM, Wilbourn JD, editors. Species differences in thyroid, kidney and urinary bladder carcinogenesis. IARC Scientific Publication No. 147. Lyon: International Agency for Research on Cancer; 1999.

16 US Environmental Protection Agency (US EPA). Alpha 2u-globulin association with chemically induced renal toxicity and neoplasia in the male rat. Risk Assessment Forum. EPA/625/3-91/019F. Washington, DC: US Environmental Protection Agency; 1991.

17 Swenberg JA, Dietrich DR. Immunohistochemical localization of α2u-globulin in kidneys of treated and control rats of a 13-week vapor inhalation study undertaken with methyl tertiary butyl ether (MTBE). Report to the MTBE Health Effects Testing Task Force. 1991.

18 Melnick RL, Kohn MC. Possible mechanisms of induction of renal tubular cell neoplasms in rats associated with α2u-globulin: role of protein accumulation versus ligand delivery to the kidney. In: Capen CC, Dybing E, Rice JM, Wilbourn JD,

editors. Species differences in thyroid, kidney and urinary bladder carcinogenesis. IARC Scientific Publication No. 147. Lyon: International Agency for Research on Cancer; 1999. p. 119–37.
19 Melnick RL, White MC, Davis JM, Hartle RW, Ghanayem B, Ashley DL, *et al*. Potential health effects of oxygenated gasoline. In: Interagency assessment of oxygenated fuels. Washington, DC: National Science and Technology Council; 1997. p. 4.1–4.38
20 Brady JF, Xiao F, Ning SM, Yang CS. Metabolism of methyl tertiary-butyl ether by rat hepatic microsomes. Arch Toxicol 1990;64:157–60.
21 International Agency for Research on Cancer (IARC). Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol. Vol. 88. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Lyon: IARC; 2006.
22 Baan R, Grosse Y, Straif K, Secretan B, El Ghissassi F, Bouvard V *et al*. A review of human carcinogens — Part F: Chemical agents and related occupations. Lancet Oncol 2009;10:1143–4.
23 International Agency for Research on Cancer (IARC). [cited 2011] Available from: http://monographs.iarc.fr/ENG/Preamble/index.php
24 National Toxicology Program. [cited 2011] Available from: http://ntp.niehs.nih.gov/?objectid=47B37760-F1F6-975E-7C15022B9C93B5A6
25 US Environmental Protection Agency. Guidelines for carcinogen risk assessment. EPA/630/P-03/001F. Washington, DC: US Environmental Protection Agency; 2005.