FUNDAMENTAL AND APPLIED TOXICOLOGY 40, 205–210 (1997)
ARTICLE NO. FA972383

Rat Olfactory Mucosa Displays a High Activity in Metabolizing Methyl *tert*-butyl Ether and Other Gasoline Ethers

Jun-Yan Hong,[1] Yong-Yu Wang, Flordeliza Y. Bondoc, Chung S. Yang, Maojung Lee, and Wei-Qun Huang

Laboratory for Cancer Research, Department of Chemical Biology, College of Pharmacy, Rutgers University, Piscataway, New Jersey 08854

Received January 23, 1997; accepted September 16, 1997

Rat Olfactory Mucosa Displays a High Activity in Metabolizing Methyl *tert*-butyl Ether and Other Gasoline Ethers. Hong, J-Y., Wang, Y-Y., Bondoc, F. Y., Yang, C. S., Lee, M., and Huang, W-Q. (1997) *Fundam. Appl. Toxicol.* 40, 205–210.

Methyl *tert*-butyl ether (MTBE) is a widely used gasoline oxygenate. Two other ethers, ethyl *tert*-butyl ether (ETBE) and *tert*-amyl methyl ether (TAME), are also used in reformulated gasoline. Inhalation is a major route for human exposure to MTBE and other gasoline ethers. The possible adverse effects of MTBE in humans are a public concern and some of the reported symptoms attributed to MTBE exposure appear to be related to olfactory sensation. In the present study, we have demonstrated that the olfactory mucosa of the male Sprague–Dawley rat possesses the highest microsomal activities, among the tissues examined, in metabolizing MTBE, ETBE, and TAME. The metabolic activity of the olfactory mucosa was 46-fold higher than that of the liver in metabolizing MTBE, and 37- and 25-fold higher, respectively, in metabolizing ETBE and TAME. No detectable activities were found in the microsomes prepared from the lungs, kidneys, and olfactory bulbs of the brain. The observations that the metabolic activity was localized exclusively in the microsomal fraction, depended on the presence of NADPH, and was inhibitable by carbon monoxide are consistent with our recent report on MTBE metabolism in human and mouse livers (Hong *et al.*, 1997) and further confirm that cytochrome P450 enzymes play a critical role in the metabolism of MTBE, ETBE, and TAME. The apparent K_m and V_{max} values for the metabolism of MTBE, ETBE, and TAME in rat olfactory microsomes were very similar, ranging from 87 to 125 μM and 9.8 to 11.7 nmol/min/mg protein, respectively. Addition of TAME (0.1 to 0.5 mM) into the incubation mixture caused a concentration-dependent inhibition of the metabolism of MTBE and ETBE. Coumarin (50 μM) inhibited the metabolism of these ethers by approximately 87%. Further comparative studies with human nasal tissues on the metabolism of these ethers are needed in order to assess the human relevance of our present findings.

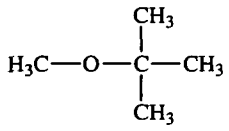
Addition of oxygenates to gasoline improves combustion by increasing the octane number and thereby reduces tailpipe emission of carbon monoxide (CO) and other pollutants. Currently, methyl *tert*-butyl ether (MTBE) is the most widely used gasoline oxygenate. Approximately 20% of the gasoline sold in the United States contains 2 to 15% MTBE by volume (Costantini, 1993). Two other gasoline ethers, ethyl *tert*-butyl ether (ETBE) and *tert*-amyl methyl ether (TAME), are also used in reformulated gasoline. ETBE can be made from renewable sources and TAME has a lower vapor pressure than MTBE, which results in lower evaporative emissions. Therefore, it has been proposed that they replace MTBE in the future (Health Effects Institute, 1996). The use of MTBE and other ethers as gasoline oxygenates is expected to increase over the next decade (Costantini, 1993). However, the possible adverse effects of MTBE exposure in humans are currently a public concern.

Inhalation constitutes a major route for environmental exposure to MTBE and other gasoline ethers. When refueling cars, the concentrations of MTBE range from less than 1 to 4 ppm within the breathing zone and from 0.01 to 0.1 ppm inside the cars (Clayton Environmental Consultants, 1991; Hartle, 1993; Lioy *et al.*, 1994). Studies in rats and humans have shown that inhaled MTBE is eliminated primarily through the lungs in exhaled air and through the kidneys in urine (Health Effects Institute, 1996). Oxidative demethylation of MTBE results in the formation of *tert*-butyl alcohol (TBA), a major circulating metabolite and an exposure marker of MTBE (Costantini, 1993). Deethylation of ETBE also results in the formation of TBA, and demethylation of TAME leads to the formation of *tert*-amyl alcohol (TAA). The chemical structures of these ethers and their metabolites are shown in Fig. 1. Metabolism of MTBE in the rat liver microsomes and the involvement of cytochrome P450 enzymes (P450) were previously reported by our group (Brady *et al.*, 1990). We have recently demonstrated that human livers are active in metabolizing MTBE to TBA and P450 enzymes play a critical role in the metabolism of MTBE in human livers (Hong *et al.*, 1997).

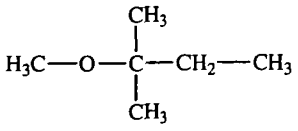
The nasal tissues are exposed to the highest levels of airborne environmental chemicals. Mammalian nasal mucosa is known to be active in metabolizing xenobiotics and the activity levels are comparable to, or in some cases, even

[1] To whom correspondence should be addressed. Fax: 732-445-0687. E-mail: jyhong@rci.rutgers.edu.

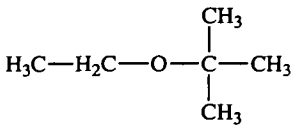
0272-0590/97 $25.00



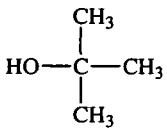
methyl *tert*-butyl ether (MTBE)



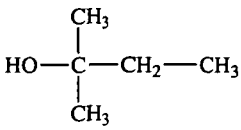
tert-amyl methyl ether (TAME)



ethyl *tert*-butyl ether (ETBE)



tert-butyl alcohol (TBA)



tert-amyl alcohol (TAA)

FIG. 1. Chemical structures of MTBE, ETBE, and TAME, as well as their metabolites TBA and TAA.

much higher than those of the liver when based on the per unit of microsomal protein. Our previous studies have shown that both rabbit and rat nasal mucosa are highly active in the metabolic activation of several carcinogenic nitrosamines, including a tobacco-specific carcinogen (Hong *et al.*, 1991, 1992). Efficient metabolism *in situ* resulting from this high level of xenobiotic-metabolizing activity in the nasal mucosa could be particularly important for the inhaled chemicals, such as MTBE, in determining their fate and elicited biological responses (Dahl, 1989; Morris, 1994).

In the present study, we investigated the metabolism of MTBE and two other gasoline ethers, ETBE and TAME, by rat olfactory mucosa. For comparison, the metabolic activities toward these gasoline ethers in other rat tissues, including the liver, were also determined.

MATERIALS AND METHODS

Chemicals. MTBE, ETBE, TAME, TBA, and TAA were purchased from Aldrich Chemical Co. (Milwaukee, WI). The purity of these chemicals was >99%, except for TAME, which was 97% pure. Glucose 6-phosphate, glucose-6-phosphate dehydrogenase, and $NADP^+$ were from Sigma Chemical Co. (St. Louis, MO). All other chemicals used were of reagent grade and were obtained from standard suppliers.

Animals and microsomes. Male Sprague–Dawley rats at 9 weeks of age were obtained from Taconic Farms (Germantown, NY) and were acclimated for 1 week at the animal facility of Rutgers University. They received lab chow (Lab Diet 5012, The Richmond Standard, PMI Feeds, Inc., St. Louis, MO) and tap water *ad libitum* and were maintained in air-conditioned quarters with 12-h light–dark cycles. The animals were killed by decapitation and the nasal mucosas, including olfactory and respiratory epithelium, were collected and immediately stored at −80°C according to a procedure previously described (Hong *et al.*, 1991). The livers, lungs, kidneys, and olfactory bulbs of these untreated animals were also immediately removed after termination and stored at −80°C prior to further processing.

Tissues were homogenized in ice-cold Tris–HCl buffer (pH 7.4, containing 1.15% KCl) with an Ultra-Turrax polytron (Janke and Kunkel Co., Germany). Microsomes and cytosolic fractions were prepared by differential centrifugation (Hong and Yang, 1985). The protein content was determined by the method of Lowry *et al.* (Lowry *et al.*, 1951).

Metabolism of gasoline ethers. Incubations for the metabolism of MTBE, ETBE, and TAME were performed according to a previously described protocol (Brady *et al.*, 1990). The incubation mixture (0.4 ml final volume) contained 50 mM Tris–HCl (pH 7.4), 10 mM $MgCl_2$, 150 mM KCl, an NADPH-generating system (0.4 mM $NADP^+$, 10 mM glucose 6-phosphate, 0.2 unit glucose-6-phosphate dehydrogenase), and microsomal or cytosolic proteins. Unless specified, the substrate concentration was 0.25 mM. Incubations were carried out in sealed headspace vials and the reaction was initiated by injecting the NADPH-generating system into the solution. After a 30 min incubation at 37°C, the reaction was terminated with 50 μl each of 25% $ZnSO_4$ and saturated $Ba(OH)_2$. The metabolism rates were linear under our assay conditions. For the blanks, $ZnSO_4$ and $Ba(OH)_2$ were added into the incubation mixture prior to the initiation of reaction with the NADPH-generating system.

For kinetic studies, the metabolizing activities were determined at different substrate concentrations and the determinations were always repeated at least two times to ensure data reproducibility. Kinetic parameters were determined by nonlinear regression analysis using the Enzymekinetics V1.11 software (Trinity Software, Plymouth, NH).

Headspace gas chromatography (GC) analysis. TBA, from the metabolism of MTBE and ETBE, and TAA, from the metabolism of TAME, were determined by a headspace GC method as previously described (Brady *et al.*, 1990) with slight modifications. A Perkin–Elmer model 8500 gas chromatograph was used with a Carbopack B/5% Carbowax 20M stainless steel column (1/8" × 6', Supelco, Inc., Bellefonte, PA) and a HS-101 headspace autoinjector. The carrier gas was helium and the flow rate was 20 ml/min. The injector and flame ionization detector were at 160°C, while the oven temperature was 60°C. Under these analytical conditions, the retention time (min) of these chemicals on headspace GC were: MTBE, 5.2; ETBE, 10.3; TAME, 14.6; TBA, 6.77; and TAA, 19.4.

RESULTS

A representative chromatograph for the metabolic profile of MTBE in the microsomes prepared from the rat liver and olfactory mucosa is shown in Fig. 2. Although the metabolic profile is the same, the activity in metabolizing MTBE to TBA is dramatically higher in the olfactory mucosa microsomes than that in the liver microsomes under the same incubation conditions. Further analysis with more samples and using ETBE and TAME as substrates showed that rat olfactory mucosa microsomes possess the highest activities in metabolizing all three ethers. The microsomal activity level of the olfactory mucosa was 46-fold higher than the liver in metabolizing MTBE, and 37- and 25-fold higher, respectively, in metabolizing ETBE and TAME (Table 1). The activity level of the olfactory mucosa microsomes was approximately 5- to 9-fold higher than that of the respiratory mucosa microsomes, depending on which substrate was used. No detectable activities were found in the microsomes prepared from the lungs, kidneys, and olfactory bulbs of these untreated rats (Table 1).

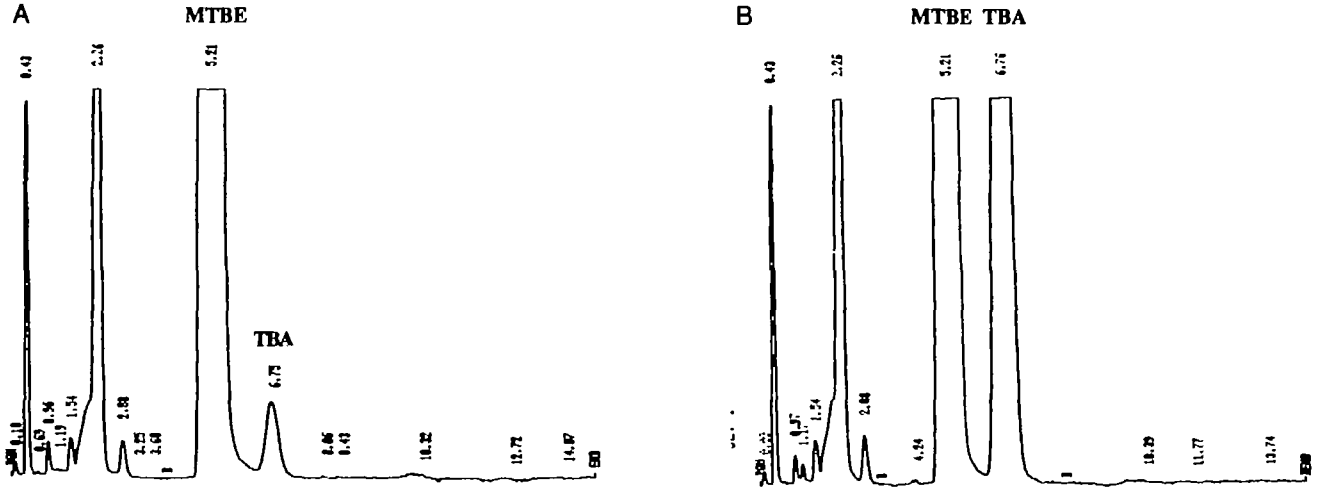


FIG. 2. Headspace GC chromatogram of MTBE metabolism in the rat liver (A) and olfactory mucosa (B) microsomes. Microsomes (600 μg protein) were incubated with 1 mM MTBE at 37°C for 30 min. Formation of TBA was determined by headspace GC as described under Materials and Methods. The sensitivity of the detector (absorbance units full scale) was the same for both chromatograms.

For all the rat tissues examined, there was no detectable TBA or TAA formation when the cytosolic fractions were used for the incubation with MTBE, ETBE, and TAME, even though a larger amount of proteins (2.5-fold of the microsomes) were used in the incubation (data not shown). We further determined the effects of NADPH, a cofactor required for P450-catalyzed reactions, and carbon monoxide, an inhibitor of P450, on the metabolism of these gasoline ethers in the rat olfactory microsomes. When the incubation was carried out in the absence of an NADPH-generating system, the activities in metabolizing MTBE, ETBE, and TAME became undetectable (Table 2). Bubbling the microsomal incubation mixture with 95% CO for 3 min prior to initiation of the reaction caused 85 to 87% reductions in the formation of TBA or TAA (Table 2). These results are consistent with our recent studies on human and mouse livers (Hong *et al.*, 1997), providing further evidence to support the role of P450 enzymes in the metabolism of these gasoline ethers.

Kinetic studies on the metabolism of MTBE, ETBE, and TAME in the rat olfactory microsomes were conducted and the results are summarized in Fig. 3. At substrate concentrations ranging from 0.01 to 2.5 mM, Michaelis–Menton kinetics were observed in the formation of TBA from MTBE or ETBE and the formation of TAA from TAME. The apparent K_m and V_{max} values for the metabolism of MTBE, ETBE,

TABLE 1
Metabolism of MTBE, ETBE, and TAME in the Microsomes Prepared from Various Rat Tissues

		Activity (nmol metabolite/min/mg protein)		
	n	MTBE	ETBE	TAME
Nasal mucosa				
Olfactory	6	9.28 ± 1.33	8.78 ± 0.55	13.15 ± 0.64
Respiratory	6	1.84 ± 0.26	0.95 ± 0.18	2.08 ± 0.37
Liver	6	0.20 ± 0.01	0.24 ± 0.01	0.52 ± 0.03
Lung	5	n.d.	n.d.	n.d.
Kidney	5	n.d.	n.d.	n.d.
Olfactory bulb	3	n.d.	n.d.	n.d.

Note. Microsomes were incubated with 1 mM substrate in a final volume of 400 μl at 37°C for 30 min. The microsomal protein used was 600 μg for liver, lung, kidney, and olfactory bulb, and 100 μg for olfactory or respiratory mucosa microsomes. Values are shown as mean ± SE. n.d., not detectable. Tissues from three to five rats were pooled to make one sample. *n*, number of samples.

TABLE 2
Effects of NADPH and CO on the Metabolism of MTBE, ETBE, and TAME in Rat Olfactory Mucosa Microsomes

	Activity (nmol/min/mg protein)		
	Control	Without NADPH	With CO pretreatment
MTBE	7.1	n.d.	0.9 (87)
ETBE	9.5	n.d.	1.2 (88)
TAME	11.4	n.d.	1.8 (85)

Note. Rat olfactory mucosa microsomes were pooled from six samples (each sample was pooled from five rats). Microsomes (50 μg) were incubated with 0.25 mM substrate at 37°C for 30 min. To determine the effects of NADPH, the NADPH-generating system was omitted from the incubation. The effect of CO was determined by bubbling the microsomal mixture with 95% CO for 3 min before the addition of substrate. The activity values are the average of two separate determinations. The percentage of inhibition is shown in parentheses. n.d., not detectable.

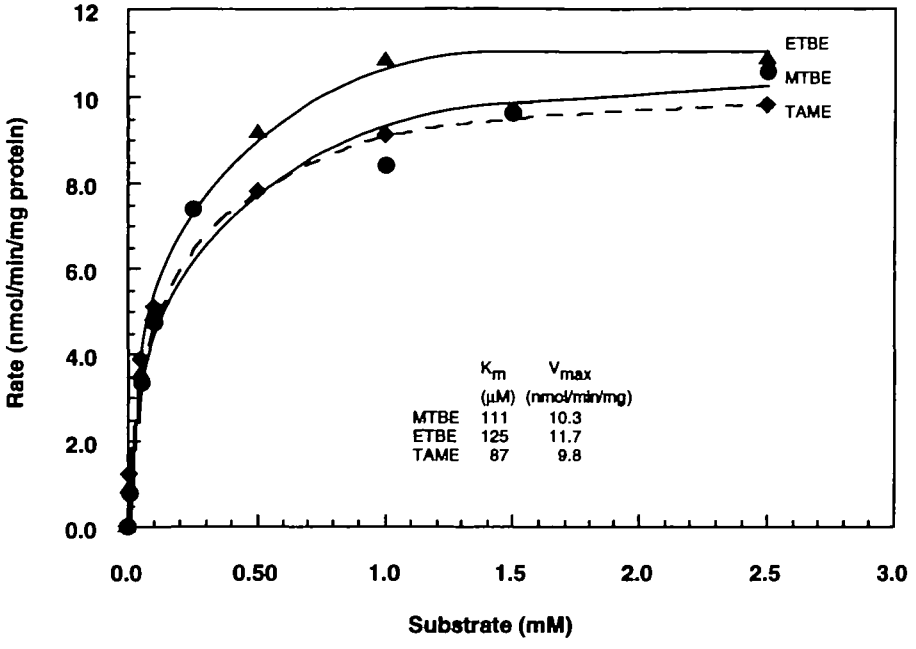


FIG. 3. Kinetics of the metabolism of MTBE, ETBE, and TAME in rat olfactory mucosa microsomes. Microsomes (50 μg protein) were incubated with the substrates at different concentrations and the formation of TBA or TAA was determined by headspace GC.

and TAME were very close, ranging from 87 to 125 μM and 9.8 to 11.7 nmol/min/mg protein, respectively.

Because of the structure similarity, MTBE, ETBE, and TAME are probably metabolized by the same P450 enzyme(s). We, therefore, examined the effects of different concentrations of TAME on the metabolism of MTBE and ETBE in the rat olfactory mucosa microsomes. When TAME (0.1 to 0.5 mM) was added into the incubation mixture, a concentration-dependent inhibition was observed in the metabolism of MTBE and ETBE (Table 3). On the other hand, the metabolism of TAME was also inhibited by the presence of MTBE or ETBE in the incubation mixture. At 0.25 mM TAME, the presence of MTBE or ETBE in the incubation mixture caused a 24 or 32% decrease (7.0 and 6.2 vs 9.2 nmol/min/mg) in the formation of TAA (Table 3). The metabolism of MTBE, ETBE, and TAME in the olfactory mucosa microsomes was also significantly inhibited to a similar extent by addition of coumarin. At 50 μM, coumarin caused approximately an 87% inhibition in the metabolism of these ethers (data not shown).

DISCUSSION

The present study clearly demonstrates that rat nasal mucosa, in particular the olfactory mucosa, possesses a high activity towards the oxidative metabolism of MTBE, ETBE, and TAME. The results that the metabolizing activities for these gasoline ethers were localized exclusively in the microsomal fraction, depended on the existence of NADPH, and were inhibitable by CO are consistent with our recent report on the metabolism of MTBE in human and mouse livers (Hong *et al.*, 1997) and further confirm that P450 enzymes catalyze the metabolism of MTBE, ETBE, and TAME.

MTBE, ETBE, and TAME are similar in structure and

TABLE 3
Effects of TAME on the Metabolism of MTBE and ETBE in Rat Olfactory Mucosa Microsomes

	Activity (nmol/min/mg protein)			
	MTBE	TAME	ETBE	TAME
TAME (mM)				
0	6.7		9.2	
0.10	2.8	4.2	4.8	3.7
	(58)		(48)	
0.25	2.0	7.0	3.7	6.2
	(71)		(60)	
0.50	1.2	8.5	2.2	8.5
	(82)		(76)	
TAME alone (mM)				
0.25		9.2		9.2

Note. The microsomes were pooled from six samples (each sample was pooled from five rats). Microsomal protein (50 μg) was incubated with the substrates in a final volume of 400 μl at 37°C for 30 min. The substrate concentrations for MTBE and ETBE were 0.25 mM. Metabolism of MTBE and ETBE was assayed as TBA formation, and metabolism of TAME was assayed as TAA formation. The activity values are the average of duplicate determinations. The percentage of inhibition is shown in parentheses.

physical–chemical properties. Several lines of evidence appear to support our hypothesis that in rat olfactory mucosa, the metabolism of these ethers is catalyzed by the same P450 enzyme(s). These include: (1) The apparent K_m values for oxidative dealkylation of MTBE, ETBE, and TAME are nearly the same; (2) the inhibition of MTBE and ETBE metabolism by TAME appears to be competitive in nature, and the metabolism of TAME was also inhibited by MTBE and ETBE; (3) the metabolism of these ethers were all inhibited to the same extent by coumarin. In addition, our recent study showed that heterologously expressed human P450 2A6 was efficient in catalyzing the metabolism of MTBE, ETBE, and TAME (Hong *et al.,* 1997). Whereas the exposure, metabolism, and health effects of MTBE have been under extensive investigation, corresponding information for ETBE and TAME is lacking. If further studies demonstrate that the metabolism of ETBE and TAME is indeed catalyzed by the same P450 enzymes metabolizing MTBE, the obtained MTBE data could be useful in predicting or interpreting the pharmacokinetics and health effects of ETBE and TAME.

The present finding is consistent with our previous findings and the reports by others that rat nasal mucosa is highly active in metabolizing many environmental chemicals, and the olfactory mucosa is usually more active than the respiratory mucosa (Bond, 1983; Bond and Li, 1983; Brittebo and Ahlman, 1984; Hadley and Dahl, 1983; Hong *et al.,* 1991, 1992). Several P450 enzymes have been demonstrated to be present in rat nasal mucosa (Ding and Coon, 1993). At present, the particular P450 forms responsible for the metabolism of MTBE, ETBE, and TAME are not known. Significant inhibition of the metabolism of these ethers by coumarin suggests that the P450 enzymes responsible for the ether metabolism are probably also involved in coumarin metabolism. In human livers, coumarin hydroxylation is mainly catalyzed by P450 2A6, which has been demonstrated by us to be active in metabolizing MTBE, ETBE, and TAME (Hong *et al.,* 1997). Preliminary results showed that baculovirus-expressed rat P450 2A3, a predominant P450 form expressed in rat olfactory mucosa and an ortholog of human P450 2A6 (Liu *et al.,* 1996), is active in the metabolism of MTBE, ETBE, and TAME (Hong and Ding, unpublished results). The roles of P450 2A3 and other P450 enzymes in the metabolism of these ethers in rat nasal mucosa remain to be determined.

The presence of a high level of P450 activities in rat olfactory mucosa is intriguing. Little is known about the physiological and toxicological significance of these nasal P450 enzymes. An even more important question is how relevant to the human situation is this high level of P450 activity in rat olfactory mucosa. In the case of MTBE, the reported symptoms attributed to MTBE exposure include headache, nausea or vomiting, burning sensation of the nose or mouth, coughing, dizziness, disorientation, and eye irritation (Middaugh, 1992, 1993). Some of them may be related to olfactory sensation. In addition to TBA and TAA, formaldehyde (from MTBE and TAME) and acetaldehyde (from ETBE) are produced in the oxidative metabolism of these ethers. Both of these aldehydes have been reported to be nasal carcinogens in rodents (Swenberg *et al.,* 1980; Woutersen *et al.,* 1986). However, the kinetics of their production in the metabolism of gasoline ethers must be considered in addressing the relevant cancer risk of ether inhalation. To assess the importance of nasal metabolism in the health effects of these gasoline ethers in humans, it is critical to conduct a comparative study on the metabolism of MTBE, ETBE, and TAME in human nasal tissues.

ACKNOWLEDGMENTS

We thank Dr. Z-J. Liu for assistance in part of the study, Dr. H. E. Lowndes and Mr. A. Stein for advice in collecting the rat olfactory bulbs, Dr. T. J. Smith and Ms. D. Wong for manuscript preparation, and Dr. Xinxin Ding (The Wadsworth Center, New York State Department of Health) for helpful discussions. This work was supported in part by grants from the Health Effects Institute (96-2), the New Jersey State Commission on Cancer Research (693-016), NIH (ES03938), and NIEHS Center Grant ES05022.

REFERENCES

Bond, J. A. (1983). Bioactivation and biotransformation of 1-nitrapyrene in liver, lung and nasal tissue of rats. *Mutat. Res.* **124,** 315–324.

Bond, J. A., and Li, A. P. (1983). Rat nasal tissue activation of benzo(a)pryene and 2-aminoanthracene to mutagens in *Salmonella typhimurium. Environ. Mutagen.* **5,** 311–318.

Brady, J. F., Xiao, F., Ning, S. M., and Yang, C. S. (1990). Metabolism of methyl *tertiary*-butyl ether by rat hepatic microsomes. *Arch. Toxicol.* **64,** 157–160.

Brittebo, E. B., and Ahlman, M. (1984). Metabolism of a nasal carcinogen, phenacetin, in the mucosa of the upper respiratory tract. *Chem.–Biol. Interact.* **50,** 233–245.

Clayton Environmental Consultants. (1991). Gasoline vapor exposure assessment for the American Petroleum Institute (API). Clayton Project Number 31774.00. American Petroleum Institute, Washington, DC.

Costantini, M. G. (1993). Health effects of oxygenated fuels. *Environ. Health Perspect.* **101**(Suppl. 6), 151–160.

Dahl, A. R. (1989). The cyanide-metabolizing enzyme rhodanese in rat nasal respiratory and olfactory mucosa. *Toxicol. Lett.* **45,** 199–205.

Ding, X., and Coon, M. J. (1993). Extrahepatic microsomal forms: Olfactory cytochrome P450, In *Cytochrome P450, Handbook of Experimental Pharmacology* (J. B. Schenkman and H. Greim, Eds.), pp. 351–361, Springer-Verlag, New York.

Hadley, W. M., and Dahl, A. R. (1983). Cytochrome P450-dependent monooxygenase activity in nasal membrane of six species. *Drug Metab. Dispos.* **11,** 275–276.

Hartle, R. (1993). Exposure to methyl *tert*-butyl ether and benzene among service station attendants and operators. *Environ. Health Perspect.* **101**(Suppl. 6), 23–26.

Health Effects Institute. (1996). The potential health effects of oxygenates added to gasoline: A review of the current literature. Health Effects Institute, Boston, MA.

Hong, J.-Y., Ding, X., Smith, T. J., Coon, M. J., and Yang, C. S. (1992). Metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco-specific carcinogen, by rabbit nasal microsomes and cytochrome P450s NMa and NMb. *Carcinogenesis (London)* **13,** 2141–2144.

Hong, J.-Y., Smith, T. J., Lee, M.-J., Li, W., Ma, B.-L., Ning, S. M., Brady, J. F., Thomas, P. E., and Yang, C. S. (1991). Metabolism of carcinogenic nitrosamines by rat nasal mucosa and the effect of diallyl sulfide. *Cancer Res.* **51,** 1509–1514.

Hong, J.-Y., and Yang, C. S. (1985). The nature of microsomal *N*-nitrosodimethylamine demethylase and its role in carcinogen activation. *Carcinogenesis (London)* **6,** 1805–1809.

Hong, J.-Y., Yang, C. S., Lee, M., Wang, Y.-Y., Huang, W.-q., Tan, Y., Patten, C. J., and Bondoc, F. Y. (1997). Role of cytochromes P450 in the metabolism of methyl *tert*-butyl ether in human livers. *Arch. Toxicol.* **71,** 266–269.

Lioy, P. J., Weisel, C. P., Jo, K.-W., Pellizzari, E., and Raymer, J. H. (1994). Microenvironmental and personal measurements of methyl-*tertiary* butyl ether associated with automobile use activities. *J. Exp. Anal. Environ. Epidemiol.* **4,** 427–441.

Liu, C., Zhuo, X., Gonzalez, F. J., and Ding, X. (1996). Baculovirus-mediated expression and characterization of rat CYP2A3 and human CYP2A6: Role in metabolic activation of nasal toxicants. *Mol. Pharmacol.* **50,** 781–788.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275.

Middaugh, J. (1992). Evaluation of health effects from exposure to oxygenated fuel in Fairbanks, Alaska. *State Alaska Epidemiol. Bull.* **26,** 1.

Middaugh, J. (1993). Potential illness due to exposure to oxygenated fuel in Anchorage, Alaska. *State Alaska Epidemiol. Bull.* **1,** 1.

Morris, J. B. (1994). In vivo measurements of uptake. *Inhal. Toxicol.* **6**(suppl), 99–111.

Swenberg, J. A., Kerns, W. D., Mitchell, R. I., Gralla, E. J., and Pavkov, K. L. (1980). Induction of squamous cell carcinomas of the rat nasal cavity by inhalation exposure to formaldehyde vapor. *Cancer Res.* **40,** 3398–3402.

Woutersen, R. A., Appleman, L. M., Van Garderen-Hoetmer, A., and Feron, V. J. (1986). Inhalation toxicity of acetaldehyde in rats. III. Carcinogenicity study. *Toxicology* **41,** 213–231.