REFERENCE OUTLINE

TOXICOLOGICAL REVIEW OF TERTIARY AMYL METHYL ETHER (CAS No. 994-05-8)

1. INTRODUCTION

NRC (National Research Council) (1983) Risk assessment in the Federal Government: Managing the process. Committee on the Institutional Means for Assessment of Risks to Public Health. Commission on Life Sciences. Washington, DC: National Academy Press.

U.S. EPA. (1986a) Guidelines for the health risk assessment of chemical mixtures. Fed Regist 51(185):34014-34025. Available online at http://www.epa.gov/iris/backgr-d.htm.

U.S. EPA. (1986b) Guidelines for mutagenicity risk assessment. Fed Regist 51(185):34006-34012. Available online at http://www.epa.gov/iris/backgr-d.htm.

U.S. EPA. (1988) Recommendations for and documentation of biological values for use in risk assessment. Prepared by the Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC; EPA 600/6-87/008. Available from: National Technical Information Service, Springfield, VA; PB88-179874/AS.

U.S. EPA. (1989) Interim methods for development of inhalation reference doses. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Research Triangle Park, NC; EPA/600/8-88/066F. Available online at http://www.epa.gov/iris/backgr-d.htm.

U.S. EPA. (1991) Guidelines for developmental toxicity risk assessment. Fed Regist 56(234):63798-63826.

U.S. EPA. (1994a) Interim policy for particle size and limit concentration issues in inhalation toxicity. Fed Regist 59(206):53799. Available online at http://www.epa.gov/iris/backgr-d.htm.

U.S. EPA. (1994b) Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. EPA/600/8-90/066F. Available online at http://www.epa.gov/iris/backgr-d.htm.

U.S. EPA. (1995) Use of the benchmark dose approach in health risk assessment. U.S. Environmental Protection Agency. EPA/630/R-94/007. Available online at http://www.epa.gov/iris/backgr-d.htm.

U.S. EPA. (1996) Guidelines for reproductive toxicity risk assessment. Fed Regist 61(212):56274-56322. Available online at http://www.epa.gov/iris/backgr-d.htm.

U.S. EPA. (1998a) Science policy council handbook: peer review. Prepared by the Office of Science Policy, Office of Research and Development, Washington, DC. EPA/100/B-98/001. Available online at http://www.epa.gov/clariton/clhtml/pubtitleOther.html.

U.S. EPA. (1998b) Guidelines for neurotoxicity risk assessment. Fed Regist 63(93):26926-26954. Available online at http://www.epa.gov/iris/backgr-d.htm.

U.S. EPA. (2000a) Science policy council handbook: peer review. 2nd edition. Prepared by the Office of Science Policy, Office of Research and Development, Washington, DC. EPA/100/B-00/001. Available online at http://www.epa.gov/iris/backgr-d.htm.

U.S. EPA. (2000b) Science policy council handbook: risk characterization. Prepared by the Office of Science Policy, Office of Research and Development, Washington, DC. EPA/100/B-00/002. Available online at http://www.epa.gov/iris/backgr-d.htm.

U.S. EPA. (2000c) Benchmark dose technical support document [external review draft]. EPA/630/R-00/001. Available online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=42601.

U.S. EPA. (2000d) Supplementary guidance for conducting health risk assessment of chemical mixtures. EPA/630/R-00/002. Available online at http://www.epa.gov/iris/backgr-d.htm.

U.S. EPA. (2002) A review of the reference dose concentration and reference concentration processes. Risk Assessment Forum, Washington, DC; EPA/630/P-02/0002F. Available online at http://www.epa.gov/iris/backgr-d.htm.

U.S. EPA (2005a) Guidelines for carcinogen risk assessment. Risk assessment forum. Washington, DC: U.S. Environmental Protection Agency. EPA/630/P-03/001B.

U.S. EPA (2005b) Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens. Risk assessment forum. Washington, DC: U.S. Environmental Protection Agency. EPA/630/R-03/003F.

U.S. EPA. (2005c) Peer review handbook. 3rd edition. Review draft. Science Policy Council, Washington, DC. Available online at http://intranet.epa.gov/ospintra/scipol/prhndbk05.doc.

2. CHEMICAL AND PHYSICAL INFORMATION

Arp, PH; Schmidt TC. (2004) Air-water transfer of MTBE, its degradation products, and alternative fuel oxygenates: The role of temperature. Environ Sci Technol 38:5405-5412.

The gasoline oxygenate methyl tert-butyl ether (MTBE) has become one of the world's mostwidespread groundwater and surface water contaminants. As a result, there has been increasing interest in the environmental behavior of MTBE and its degradation products, mainly tert-butyl formate (TBF) and tert-butyl alcohol (TBA). In contrast, the environmental behavior of the proposed alternatives to MTBE, namely ethyl tert-butyl ether (ETBE),

tert-amyl methyl ether (TAME), and diisopropyl ether (DIPE) has hardly been studied yet, although some of them are already in substantial use in various countries. A key parameter for the assessment of the fate, transport, and possible remediation of these contaminants is the air-water partitioning constant (KiH). The KiH is highly temperature dependent, and it is therefore necessary to obtain reliable experimental values at relevant temperatures. Hence, the KiH of MTBE, ETBE, TAME, and DIPE, along with the degradation products, TBF and methyl acetate, were determined from 5 degrees C-40 degrees C. The alternatives to MTBE generally had a higher KiH, which implies that, upon emission into the environment, the alternatives partition more readily into the air phase than MTBE. This may favor their use, as it is in the air phase where dilution and degradation are the most effective. The degradation products of MTBE, with the exception of TBF, have much lower KiH values at all temperatures. Hence, the degradation products will have a stronger affinity for the water phase. The temperature dependency of the kinetics of air-watertransfer is discussed using a boundary layer model. Only for TBA but not for the ethers a significant effect of temperature was found.

Daubert, TE; Danner, RP. (1995) Physical and thermodynamic properties of pure chemicals: Data compilation. Butane, 2-methoxy-2-methyl. Washington, DC: Taylor & Francis.

Wallington, TJ; Potts, AR; Andino, JM; et al. (1993) Kinetics of the reaction of OH radicals with t-amyl methyl ether revisited. Int J Chem Kinet 25:265-272.

The kinetics of the reaction of OH radicals with t-amyl methyl ether (TAME) have been reinvestigated using both absolute (flash photolysis resonance fluorescence) and relative rate techniques. Relative rate experiments were conducted at 295 K in 99 kPa (740 torr) of synthetic air using ethyl t-butyl ether, cyclohexane, and di-isopropyl ether as reference compounds. Absolute rate experiments were performed over the temperature range 240-400 K at a total pressure of 4.7 kPa (35 torr) of argon. Rate constant determinations from both techniques are in good agreement and can be represented by k sub(1) = (6.32 plus or minus 0.72) x 10 super(-12) exp((-40 plus or minus 70)/T) cm super(3) molecule super(-1) s super(-1). (1) OH + CH sub(3)OC(CH sub(3)) sub(2)C sub(2)H sub(5) (TAME) arrow right products Quoted errors represent 2 sigma from the latest squares analysis and do not include any estimate of systematic errors. We show that results from the previous kinetic study of reaction (1) are in error due to the presence of a reactive impurity. Results are discussed in terms of the atmospheric chemistry of TAME.

Lide, DR, ed. (2005) CRC handbook of chemistry and physics. Boca Raton, FL: CRC Press Inc.; pp. 3-332.

O'Neil, MJ. (2001) Merck index: an encyclopedia of chemicals, drugs, and biologicals. 13th ed. Budavari S, ed. Rahway NJ: Merck & Co., Inc.; pp. 102.

NLM. (National Library of Medicine) (2007) ChemID Plus Advanced. National Library of Medicine, Bethesda, MD. Available online at http://chem.sis.nlm.nih.gov/chemidplus/.

3. TOXICOKINETICS

Amberg, A; Bernauer, U; Scheutzow, D; et al. (1999) Biotransformation of ^{12}C- and ^{13}C-tert-amyl methyl ether and tert-amyl alcohol. Chem Res Toxicol 12:958-964.

tert-Amyl methyl ether (TAME) is intended for use as a gasoline additive to increase oxygen content. Increased oxygen content in gasoline reduces tailpipe emissions of hydrocarbons and carbon monoxide from cars. Due to possible widespread use of TAME, the toxicity of TAME is under investigation. We studied the biotransformation of TAME in rats and one human volunteer after inhalation of (12)C- or (13)C-labeled TAME. In addition, the biotransformation of [(13)C]-tert-amyl alcohol was studied in rats after gavage. Urinary metabolites were identified

by GC/MS and (13)C NMR. Rats (two males and two females) were individually exposed to 2000 ppm [(12)C]- or [(13)C]TAME for 6 h, and urine was collected for 48 h. Free and glucuronidated 2-methyl-2,3-butanediol and a glucuronide of tert-amyl alcohol were identified by (13)C NMR, GC/MS, and LC/MS/MS as major urinary metabolites on the basis of the relative intensities of the (13)C NMR signals. The presence of several minor metabolites was also indicated by (13)C NMR; they were identified as tert-amyl alcohol, 2-hydroxy-2-methylbutyric acid, and 3-hydroxy-3-methylbutyric acid. One human volunteer was exposed to an initial concentration of 27 000 ppm [(13)C]TAME by inhalation for 4 min from a 2 L gas sampling bag, and metabolites of TAME excreted in urine were analyzed by (13)C NMR. All TAME metabolites identified in rats were also present in the human urine samples. To study tert-amyl alcohol biotransformation, male rats (n = 3) were treated with 250 mg/kg [(13)C]-tert-amyl alcohol dissolved in corn oil by gavage, and urine was collected for 48 h. (13)C NMR of the urine samples showed the presence of metabolites identical to those in the urine of [(13)C]TAME-treated rats. Our results suggest that TAME is extensively metabolized by rats and humans to tert-amyl alcohol which may be further oxidized to diols and carboxylic acids. These reactions are likely mediated by cytochrome P450-dependent oxidations.

Amberg, A; Rosner, E; Dekant, W. (2000) Biotransformation and kinetics of excretion of tert-amyl-methyl ether in humans and rats after inhalation exposure. Toxicol Sci 55:274-283.

tert-Amyl methyl ether (TAME) may be widely used as an additive to gasoline in the future. The presence of this ether in gasoline reduces the tail pipe emission of pollutants. Therefore, widespread human exposure to TAME may occur. To contribute to the characterization of potential adverse effects of TAME, its biotransformation was compared in humans and rats after inhalation exposure. Human volunteers (three males and three females) and rats (five males and five females) were exposed to 4 (3.8 +/- 0.2) and 40 (38.4 +/- 1.7) ppm TAME for 4 h in a dynamic exposure system. Urine samples were collected for 72 h in 6-h intervals and blood samples were taken at regular intervals for 48 h in humans. In urine, the TAME metabolites tert-amyl alcohol (t-amyl alcohol), 2-methyl-2, 3-butane diol, 2-hydroxy-2-methylbutyric acid, and 3-hydroxy-3-methylbutyric acid were quantified. TAME and t-amyl alcohol were determined in blood samples. After the end of the exposure period, blood concentrations of TAME were 4.4 +/- 1.7 microM in humans and 9.6 +/- 1.4 microM in rats after 40 ppm TAME, and 0.6 +/- 0.1 microM in humans and 1.4 +/- 0.8 microM in rats after 4 ppm. TAME was rapidly cleared from blood in both rats and humans. The blood concentrations of t-amyl alcohol were 9.2 +/- 1.8 microM in humans and 8.1 +/- 1.5 microM in rats after 40 ppm TAME, and 1.0 +/- 0.3 microM in humans and 1.8 +/- 0.2 microM in rats after 4 ppm TAME. t-Amyl alcohol was also rapidly cleared from blood. In urine of humans, 2-methyl-2,3-butane diol, 2-hydroxy-2-methylbutyric acid, and 3-hydroxy-3-methylbutyric acid were recovered as major excretory products in urine. In rats, 2-methyl-2,3-butane diol and its glucuronide were major TAME metabolites. t-Amyl alcohol and its glucuronide were minor TAME metabolites in both species. All metabolites of TAME excreted with urine in rats were rapidly eliminated, with elimination half-lives of less than 6 h. Metabolite excretion in humans was slower and elimination half-lives of the different metabolites were between 6 and 40 h in humans. The obtained data indicate differences in TAME biotransformation and excretion between rats and humans. In rats, TAME metabolites are rapidly excreted. In humans, metabolic pathways are different and metabolite excretion is slower. Recovery of TAME metabolites in urine was higher in humans as compared to rats, suggesting more intensive biotransformation of TAME in humans.

Dekant, W; Bernauer, U; Rosner, E; et al. (2001) Biotransformation of MTBE, ETBE, and TAME after inhalation or ingestion in rats and humans. Res Rep Health Eff Inst 29-71.

The biotransformation of methyl tert-butyl ether (MTBE), ethyl tert-butyl ether (ETBE), and tert-amyl methyl ether (TAME) was studied in humans and in rats after inhalation of 4 and 40 ppm of MTBE, ETBE, and TAME, respectively, for 4 hours, and the biotransformation of MTBE and TAME was studied after ingestion exposure in humans to 5 and 15 mg in water. tert-Butyl alcohol (TBA), a TBA conjugate, 2-methyl-1,2-propanediol, and 2-hydroxyisobutyrate were found to be metabolites of MTBE and ETBE. tert-Amyl alcohol (TAA), free and glucuronidated 2-methyl-2,3-butanediol (a glucuronide of TAA), 2-hydroxy-2-methyl butyrate, and 3-hydroxy-3-methyl butyrate were found to be metabolites of TAME. After inhalation, MTBE, ETBE, and TAME were rapidly taken up by both rats and humans; after termination of exposure, clearance from blood of the ethers by exhalation and biotransformation to urinary metabolites occurred with half-times of less than 7 hours in rats and humans.

Biotransformation of MTBE and ETBE was similar in humans and rats after inhalation exposure. 2-Hydroxyisobutyrate was recovered as a major product in urine. All metabolites of MTBE and ETBE excreted with urine were eliminated with half-times of less than 20 hours. Biotransformation of TAME was qualitatively similar in rats and humans, but the metabolic pathways were different. In humans, 2-methyl-2,3-butanediol, 2-hydroxy-2-methyl butyrate, and 3-hydroxy-3methyl butyrate were recovered as major urinary products. In rats, however, 2-methyl-2,3-butanediol and its glucuronide were major TAME metabolites recovered in urine. After ingestion of MTBE and TAME, both compounds were rapidly absorbed from the gastrointestinal tract. Hepatic first-pass metabolism of these ethers was not observed, and a significant part of the administered dose was transferred into blood and cleared by exhalation. Metabolic pathways for MTBE and TAME and kinetics of excretion were identical after ingestion and inhalation exposures. Results of studies presented here suggest (1) that excretion of MTBE, ETBE, and TAME in rats and humans is rapid, (2) that biotransformation and excretion of MTBE and ETBE are identical in rats, and (3) that biotransformation and excretion of TAME is quantitatively different in rats and humans.

Dekant, W; Bernauer, U; Rosner, E; et al. (2001) Toxicokinetics of ethers used as fuel oxygenates. Toxicol Lett 124:37-45.

The toxicokinetics and biotransformation of methyl-tert.butyl ether (MTBE), ethyl-tert.butyl ether (ETBE) and tert.amyl-methyl ether (TAME) in rats and humans are summarized. These ethers are used as gasoline additives in large amounts, and thus, a considerable potential for human exposure exists. After inhalation exposure MTBE, ETBE and TAME are rapidly taken up by both rats and humans; after termination of exposure, clearance by exhalation and biotransformation to urinary metabolites is rapid in rats. In humans, clearance by exhalation is slower in comparison to rats. Biotransformation of MTBE and ETBE is both qualitatively and quantitatively similar in humans and rats after inhalation exposure under identical conditions. The extent of biotransformation of TAME is also quantitatively similar in rats and humans; the metabolic pathways, however, are different. The results suggest that reactive and potentially toxic metabolites are not formed during biotransformation of these ethers and that toxic effects of these compounds initiated by covalent binding to cellular macromolecules are unlikely.

Hong, JY; Wang, YY; Bondoc, FY; et al. (1997) Rat olfactory mucosa displays a high activity in metabolizing methyl tert-butyl ether and other gasoline ethers. 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 Vmax values for the metabolism of MTBE, ETBE, and TAME in rat olfactory microsomes were very similar, ranging from 87 to 125 microM 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 microM) 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.

Hong, JY; Yang, CS; Lee, M; et al. (1997) Role of cytochromes P450 in the metabolism of methyl tert-butyl ether in human livers. Arch Toxicol 71:266-269.

Methyl tert-butyl ether (MTBE) is widely used as a gasoline oxygenate for more complete combustion in order to reduce the air pollution caused by motor vehicle exhaust. The possible adverse effects of MTBE on human health is a major public concern. However, information on the metabolism of MTBE in human tissues is lacking. The present study demonstrates that human liver is active in metabolizing MTBE to tert-butyl alcohol (TBA), a major circulating metabolite and a marker for exposure to MTBE. The activity is localized in the microsomal fraction (125 +/- 11 pmol TBA/ min per mg protein, n = 8) but not in the cytosol. This activity level in human liver microsomes is approximately one-half of the value in rat and mouse liver microsomes. Formation of TBA in human liver microsomes is NADPH-dependent, and is significantly inhibited by carbon monoxide (CO), an inhibitor of cytochrome P450 (CYP) enzymes, suggesting that CYP enzymes play a critical role in the metabolism of MTBE in human livers. Both CYP2A6 and 2E1 are known to be constitutively expressed in human livers. To examine their involvement in MTBE metabolism, human CYP2A6 and 2E1 cDNAs were individually co-expressed with human cytochrome P450 reductase by a baculovirus expression system and the expressed enzymes were used for MTBE metabolism. The turnover number for CYP2A6 and 2E1 was 6.1 and 0.7 nmol TBA/min per nmol P450, respectively. The heterologously expressed human CYP2A6 was also more active than 2E1 in the metabolism of two other gasoline ethers, ethyl tert-butyl ether (ETBE) and tert-amyl methyl ether (TAME). Although the contributions of other human CYP forms to MTBE metabolism remain to be determined, these results strongly suggest that CYP enzymes play an important role in the metabolism of MTBE in human livers.

Hong, JY; Wang, YY; Bondoc, FY; et al. (1999) Metabolism of methyl tert-butyl ether and other gasoline ethers by human liver microsomes and heterologously expressed human cytochromes P450: identification of CYP2A6 as a major catalyst. Toxicol Appl Pharmacol 160:43-48.

To reduce the production of carbon monoxide and other pollutants in motor vehicle exhaust, methyl tert-butyl ether (MTBE), ethyl tert-butyl ether (ETBE), and tert-amyl methyl ether (TAME) are added to gasoline as oxygenates for more complete combustion. Previously, we demonstrated that human liver is active in metabolizing MTBE to tert-butyl alcohol (TBA) and that cytochrome P450 (CYP) enzymes play a critical role in the metabolism of MTBE. The present study demonstrates that human liver is also active in the oxidative metabolism of ETBE and TAME. A large interindividual variation in metabolizing these gasoline ethers was observed in 15 human liver microsomal samples. The microsomal activities in metabolizing MTBE, ETBE, and TAME were highly correlated among each other (r, 0.91-0. 96), suggesting that these ethers are metabolized by the same enzyme(s). Correlation analysis of the ether-metabolizing activities with individual CYP enzyme activities in the liver microsomes showed that the highest degree of correlation was with human CYP2A6 (r, 0. 90-0.95), which is constitutively expressed in human livers and known to be polymorphic. CYP2A6 displayed the highest turnover number in metabolizing gasoline ethers among a battery of human CYP enzymes expressed in human B-lymphoblastoid cells. Kinetic studies on MTBE metabolism with three human liver microsomes exhibited apparent Km values that ranged from 28 to 89 microM and the V(max) values from 215 to 783 pmol/min/mg, with similar catalytic efficiency values (7.7 to 8.8 microl/min/mg protein). Metabolism of MTBE, ETBE, and TAME by human liver microsomes was inhibited by coumarin, a known substrate of human CYP2A6, in a concentration-dependent manner. Monoclonal antibody against human CYP2A6 caused a significant inhibition (75% to 95%) of the metabolism of MTBE, ETBE, and TAME in human liver microsomes. Taken together, these results clearly indicate that in human liver, CYP2A6 is the major enzyme responsible for the metabolism of MTBE, ETBE, and TAME.

Hong, JY; Wang, YY; Bondoc, FY; et al. (1999) Metabolism of methyl tert-butyl ether and other gasoline ethers in mouse liver microsomes lacking cytochrome P450 2E1. Toxicol Lett 105:83-88.

To reduce the production of pollutants in motor vehicle exhaust, methyl tert-butyl ether (MTBE) and other ethers such as ethyl tert-butyl ether (ETBE) and tert-amyl methyl ether (TAME) are added to gasoline as oxygenates for more complete combustion. Metabolism of these gasoline ethers is catalyzed by cytochrome P450 (P450) enzymes.

P450 2E1, which metabolizes diethyl ether, was suggested to be an enzyme involved. The present study used 2E1 knock-out mice (2E1-/-) to assess the contribution of 2E1 to the metabolism of MTBE, ETBE and TAME. Liver microsomes prepared from the 2E1 knock-out mice lacked 2E1 activity (assayed as N-nitrosodimethylamine demethylation), but were still active in metabolizing all three gasoline ethers. The levels of ether-metabolizing activity (nmol/min per mg) in the liver microsomes from 7 week old female 2E1 knock-out mice were 0.54+/-0.17 for MTBE, 0.51+/-0.24 for ETBE and 1.14+/-0.25 for TAME at a 1 mM substrate concentration. These activity levels were not significantly different from those of the sex- and age-matched C57BL/6N and 129/Sv mice, which are the parental lineage strains of the 2E1 knock-out mice and are both 2E1+/+. Our results clearly demonstrate that 2E1 plays a negligible role in the metabolism of MTBE, ETBE and TAME in mouse livers.

Hong, JY; Wang, YY; Mohr, SN; et al. (2001) Human cytochrome P450 isozymes in metabolism and health effects of gasoline ethers. Res Rep Health Eff Inst 7-27; discussion 95-109.

To reduce the production of carbon monoxide and other pollutants in motor vehicle exhaust, methyl tert-butyl ether (MTBE*), ethyl tert-butyl ether (ETBE), and tert-amyl methyl ether (TAME) are added to gasoline as oxygenates for more complete combustion. Among them, MTBE is the most widely used. The possible adverse effect of MTBE in humans is a public concern, but the human enzymes responsible for metabolism of these gasoline ethers and the causes or factors for increased sensitivity to MTBE in certain individuals are totally unknown. This information is important to understanding the health effects of MTBE in humans and to assessing the human relevance of pharmacokinetics and toxicity data obtained from animals. In the present study, we demonstrated that human liver is active in metabolizing MTBE to tert-butyl alcohol (TBA), a major circulating metabolite and an exposure marker of MTBE. The activity is localized in the microsomal fraction but not in the cytosol. Formation of TBA in human liver microsomes is NADPH-dependent and is significantly inhibited by carbon monoxide, which inhibits cytochrome P450 (CYP) enzymes. These results provide strong evidence that CYP enzymes play a critical role in the metabolism of MTBE in human livers. Human liver is also active in the oxidative metabolism of 2 other gasoline ethers, ETBE and TAME. We observed a large interindividual variation in metabolizing these gasoline ethers in 15 microsomal samples prepared from normal human livers. The activity level (pmol metabolite/min/mg) ranged from 204 to 2,890 for MTBE; 179 to 3,134 for ETBE; and 271 to 8,532 for TAME. The microsomal activities in metabolizing MTBE, ETBE, and TAME correlated highly with each other (r = 0.91 to 0.96), suggesting that these ethers are metabolized by the same enzyme(s). Correlation analysis of the ether-metabolizing activities with individual CYP enzyme activities in the human liver microsomes showed that the highest degree of correlation was with CYP isoform 2A6 (CYP2A6)+ (r = 0.94 for MTBE, 0.95 for ETBE, and 0.90 for TAME), which is constitutively expressed in human livers and known to be polymorphic. CYP2A6 displayed the highest turnover number in metabolizing gasoline ethers among a battery of human CYP enzymes expressed in human B-lymphoblastoid cells. CYP2A6 coexpressed with human CYP reductase by a baculovirus expression system was also more active than CYP isoform 2E1 (CYP2E1) in the metabolism of MTBE, ETBE, and TAME. Kinetic studies on MTBE metabolism with human liver microsomes (n = 3) exhibited an apparent Michaelis constant (Km) of 28 to 89 microM and a maximum rate of metabolism (Vmax) of 215 to 783 pmol/min/mg. Metabolism of MTBE, ETBE, and TAME by human liver microsomes was inhibited by coumarin, a known substrate of human CYP2A6, in a concentration-dependent manner. Monoclonal antibody against human CYP2A6 caused a significant inhibition (75% to 95%) of the metabolism of MTBE, ETBE, and TAME in human liver microsomes. Taken together, these results clearly indicate that, in human liver, CYP2A6 is a major enzyme responsible for metabolism of MTBE, ETBE, and TAME. Although CYP2E1 metabolizes diethyl ether and was previously suggested to be involved

Kaneko, TP; Wang Y; Sato, A. (2000) Partition coefficients for gasoline additives and their metabolites. J Occup Health 42:86-87.

Le Gal, A; Dreano, Y; Gervasi, PG; et al. (2001) Human cytochrome P450 2A6 is the major enzyme involved in the metabolism of three alkoxyethers used as oxyfuels. Toxicol Lett 124:47-58.

Methyl t-butyl ether (MTBE), ethyl t-butyl ether (ETBE), and t-amyl methyl ether (TAME) are three alkoxyethers added to gasoline to improve combustion and thereby to reduce the level of carbon monoxide and aromatic hydrocarbons in automobile exhaust. Oxidative demethylation of MTBE and TAME and deethylation of ETBE by CYP enzymes results in the formation of tertiary alcohols and aldehydes, both potentially toxic. The metabolism of these three alkoxyethers was studied in a panel of 12 human liver microsomes. The relatively low apparent Km(1) was 0.25+/-0.17 (mean+/-SD), 0.11+/-0.08 and 0.10+/-0.07 mM and the high apparent Km(2) was 2.9+/-1.8, 5.0+/-2.7 and 1.7+/-1.0 mM for MTBE, ETBE and TAME, respectively. Kinetic data, correlation studies, chemical inhibition and metabolism by heterologously expressed human CYPs support the assertion that the major enzyme involved in MTBE, ETBE and TAME metabolisms is CYP2A6, with a minor contribution of CYP3A4 at low substrate concentration.

Nihlen, A; Lof, A; Johanson, G. (1995) Liquid/air partition coefficients of methyl and ethyl t-butyl ethers, t-amyl methyl ether, and t-butyl alcohol. J Expo Anal Environ Epidemiol 5:573-582.

Partition coefficients are essential to a description of the uptake and distribution of volatile substances in humans and in the development of physiologically based pharmacokinetic models. Liquid/air partition coefficients (lambda) of three ethers, methyl t-butyl ether (MTBE), ethyl t-butyl ether (ETBE), and t-amyl methyl ether (TAME) were determined in vitro by head space-gas chromatography. These ethers, and especially MTBE, are used in unleaded gasoline to enhance the oxygen and octane content, and to reduce the output of carbon monoxide during combustion. Partition coefficients of t-butyl alcohol (TBA), a metabolite of MTBE, were determined also. The liquids tested were fresh human blood, water (physiological saline), and olive oil. The (lambda)blood/air values were: 17.7 (95% confidence interval 17.0-18.4) for MTBE; 11.7 (11.3-12.1) for ETBE; and 17.9 (17.3-18.5) for TAME. Corresponding (lambda)water/air values were 15.2 (14.9-15.5), 8.39 (8.19-8.59), and 11.9 (11.7-12.1). The ethers have a higher affinity for oil, the values for (lambda)oil/air being 120 (114-125), 190 (183-197), and 337 (320-354), respectively. As expected, the (lambda)blood/air and (lambda)water/air for TBA were much higher than for the ethers, 462 (440-484) and 603 (590-617), respectively. The (lambda)oil/air was 168 (161-174) for TBA. The interindividual variability of the (lambda)blood/air (10 subjects) was calculated as the coefficient of variation, and estimated as: 14% for MTBE, 20% for ETBE, 20% for TAME, and 30% for TBA. No significant difference was seen in the (lambda)blood/air between the sexes.

Nihlen, A; Lof, A; Johanson, G. (1997) Liquid/air partition coefficients of methyl and ethyl t-butyl ethers, t-amyl methyl ether, and t-butyl alcohol. J Clean Technol Environ Toxicol Occup Med 6:205-213.

BIOSIS COPYRIGHT: BIOL ABS. Partition coefficients are essential to a description of the uptake and distribution Of volatile substances in humans and in the development of physiologically based pharmacokinetic models. Liquid/air partition coefficients (lambda) of three ethers, methyl t-butyl ether (MTBE), ethyl t-butyl ether (ETBE), and t-amyl methyl ether (TAME) were determined in vitro by head space-gas chromatography. These ethers, and especially MTBE, are used in unleaded gasoline to enhance the oxygen and octane conte and to reduce the output of carbon monoxide during combustion. Partition coefficients of t-butyl alcohol (TBA), a metabolite of MTBE, were determined also. The liquids tested were fresh human blood, water (physiological saline), and olive oil. The deltablood/air values were: 17.7 (95% confidence interval 17.0-18.4) for MTBE; 11.7 (11.3-12.1) for ETBE; and 17.9 (17.3-18.6) for TAME. Corresponding lambdawater/air values were 15.2 (14.9-15.5), 8.39 (8.19-8.59), and 11.9 (11.7-12.1).

Sumner, SC; Asgharian, B; Moore, TA; et al. (2003a) Characterization of metabolites and disposition of tertiary amyl methyl ether in male F344 rats following inhalation exposure. J Appl Toxicol 23:411-417.

Tertiary amyl methyl ether (TAME) is a fuel additive used to reduce carbon monoxide in automobile emissions. Because of the potential for human exposure, this study was conducted to develop methods for the characterization

and quantitation of metabolites in expired air and excreta of rats exposed to a mixture of [13C]- and [14C]TAME ([2,3,4-13C]- and [2-14C]2-methoxy-2-methylbutane). The distribution of TAME in rats was determined following inhalation exposure, and TAME-derived metabolites were characterized in expired air and urine. Male rats were exposed for 6 h via nose-only inhalation to 2500 ppm [14C/13C]TAME, and expired air, urine and feces were collected for up to 7 days. Over 95% of the total recovered radioactivity was excreted by 48 h after exposure. Recovered radioactivity was expired as organic volatiles (44%) and 14CO2 (3%) and excreted in urine (51%) and feces (1%). Both TAME and its metabolite tertiary amyl alcohol (TAA) accounted for > or =90% of the radiolabel in expired air 0-8 h following exposure termination. Three major urinary metabolites of TAME were identified: (1) a direct glucuronide conjugate of TAA; (2) a product of oxidation at the methylene carbon of TAA (2,3-dihydroxy-2-methylbutane); (3) a glucuronide conjugate of metabolite 2. Metabolite 1 accounted for most of the TAME-derived metabolites excreted 0-8 h following exposure termination. Further metabolic products of TAA (metabolites 2 and 3) accounted for most of the excreted TAME-derived metabolites at later time points.

Sumner, SC; Janszen, DB; Asgharian, B; et al. (2003b) Blood pharmacokinetics of tertiary amyl methyl ether in male and female F344 rats and CD-1 mice after nose-only inhalation exposure. J Appl Toxicol 23:419-425.

Interest in understanding the biological behavior of aliphatic ethers has increased owing to their use as gasoline additives. The purpose of this study was to investigate the blood pharmacokinetics of the oxygenate tertiary amyl methyl ether (TAME), its major metabolite tertiary amyl alcohol (TAA) and acetone in rats and mice following inhalation exposure to TAME. Species differences in the area under the curve (AUC) for TAME were significant at each exposure concentration. For rats, the blood TAME AUC increased in proportion with an increase in exposure concentration. For mice, an increase in exposure concentration (100-500 ppm) resulted in a disproportional increase in the TAME AUC. Mice had greater (two- to threefold) blood concentrations of TAA compared with rats following exposure to 2500 or 500 ppm TAME. Mice had a disproportional increase in the TAA AUC with an increase in exposure concentration (100-500 ppm). This difference could result from saturation of a process (e.g. oxidation, glucuronide conjugation) that is involved in the further metabolism of TAA. For each species, gender and exposure concentration, acetone increased during exposure and returned to control values by 16 h following exposure. The source of acetone could be both as a metabolite of TAA or an effect on endogenous metabolism produced by exposure to TAME.

Sumner, SC; Janszen, DB; Asgharian, B; et al. (2003c) Species and gender differences in the metabolism and distribution of tertiary amyl methyl ether in male and female rats and mice after inhalation exposure or gavage administration. J Appl Toxicol 23:427-436.

Tertiary amyl methyl ether (TAME) is a gasoline fuel additive used to reduce emissions. Understanding the metabolism and distribution of TAME is needed to assess potential human health issues. The effect of dose level, duration of exposure and route of administration on the metabolism and distribution of TAME were investigated in male and female F344 rats and CD-1 mice following inhalation or gavage administration. By 48 h after exposure, >96% of the administered radioactivity was expired in air (16-71%) or eliminated in urine and feces (28-72%). Following inhalation exposure, mice had a two- to threefold greater relative uptake of [14C]TAME compared with rats. Metabolites were excreted in urine of rats and mice that are formed by glucuronide conjugation of tertiary amyl alcohol (TAA), oxidation of TAA to 2,3-dihydroxy-2-methylbutane and glucuronide conjugation of 2,3-dihydroxy-2-methylbutane. A saturation in the uptake and metabolism of TAME with increased exposure concentration was indicated by a decreased relative uptake of total [14C]TAME equivalents and an increase in the percentage expired as volatiles. A saturation of P-450 oxidation of TAA was indicated by a disproportional decrease of 2,3-dihydroxy-2-methylbutane and its glucuronide conjugate with increased exposure concentration.

Vainiotalo, S; Riihimaki, V; Pekari, K; et al. (2007) Toxicokinetics of methyl tert-butyl ether (MTBE) and tert-amyl methyl ether (TAME) in humans, and implications to their biological monitoring. J Occup Environ Hyg 4:739-750. (Note: In Retrieval)

Healthy male volunteers were exposed via inhalation to gasoline oxygenates methyl tert-butyl ether (MTBE) or tert-amyl methyl ether (TAME). The 4-hr exposures were carried out in a dynamic chamber at 25 and 75 ppm for MTBE and at 15 and 50 ppm for TAME. The overall mean pulmonary retention of MTBE was 43 +/- 2.6%; the corresponding mean for TAME was 51 +/- 3.9%. Approximately 52% of the absorbed dose of MTBE was exhaled within 44 hr following the exposure; for TAME, the corresponding figure was 30%. MTBE and TAME in blood and exhaled air reached their highest concentrations at the end of exposure, whereas the concentrations of the metabolites tert-butanol (TBA) and tert-amyl alcohol (TAA) concentrations were highest 0.5-1 hr after the exposure and then declined slowly. Two consecutive half-times were observed for the disappearance of MTBE and TAME from blood and exhaled air. The half-times for MTBE in blood were about 1.7 and 3.8 hr and those for TAME 1.2 and 4.9 hr. For TAA, a single half-time of about 6 hr best described the disappearance from blood and exhaled air; for TBA, the disappearance was slow and seemed to follow zero-order kinetics for 24 hr. In urine, maximal concentrations of MTBE and TAME were observed toward the end of exposure or slightly (</= 1 hr) after the exposure and showed half-times of about 4 hr and 8 hr, respectively. Urinary concentrations of TAA followed first-order kinetics with a half-time of about 8 hr, whereas the disappearance of TBA was slower and showed zero-order kinetics at concentrations above approx. 10 mu mol/L. Approximately 0.2% of the inhaled dose of MTBE and 0.1% of the dose of TAME was excreted unchanged in urine, whereas the urinary excretion of free TBA and TAA was 1.2% and 0.3% within 48 hr. The blood/air and oil/blood partition coefficients, determined in vitro, were 20 and 14 for MTBE and 20 and 37 for TAME. By intrapolation from the two experimental exposure concentrations, biomonitoring action limits corresponding to an 8-hr time-weighted average (TWA) exposure of 50 ppm was estimated to be 20 mu mol/L for post-shift urinary MTBE, 1 mu mol/L for exhaled air MTBE in a post-shift sample, and 30 mu mol/L for urinary TBA in a next-morning specimen. For TAME and TAA, concentrations corresponding to an 8-hr TWA exposure at 20 ppm were estimated to be 6 mu mol/L (TAME in post-shift urine), 0.2 mu mol/L (TAME in post-shift exhaled air), and 3 mu mol/L (TAA in next morning urine).

3.5. PHYSIOLOGICALLY-BASED TOXICOKINETIC MODELS

Collins, AS; Sumner, SC; Borghoff, SJ; et al. (1999) A physiological model for tert-amyl methyl ether and tert-amyl alcohol: hypothesis testing of model structures. Toxicol Sci 49:15-28.

The oxygenate tert-amyl methyl ether (TAME) is a gasoline fuel additive used to reduce carbon monoxide in automobile emissions. To evaluate the relative health risk of TAME as a gasoline additive, information is needed on its pharmacokinetics and toxicity. The objective of this study was to use a physiologically-based pharmacokinetic (PBPK) model to describe the disposition of TAME and its major metabolite, tert-amyl alcohol (TAA), in male Fischer-344 rats. The model compartments for TAME and TAA were flow-limited. The TAME physiological model had 6 compartments: lung, liver, rapidly perfused tissues, slowly perfused tissues, fat, and kidney. The TAA model had 3 compartments: lung, liver, and total-body water. The 2 models were linked through metabolism of TAME to TAA in the liver. Model simulations were compared with data on blood concentrations of TAME and TAA taken from male Fischer-344 rats during and after a 6-hour inhalation exposure to 2500, 500, or 100 ppm TAME. The PBPK model predicted TAME pharmacokinetics when 2 saturable pathways for TAME oxidation were included. The TAA model, which included pathways for oxidation and glucuronide conjugation of TAA, underpredicted the experimental data collected at later times postexposure. To account for biological processes occurring during this time, three hypotheses were developed: nonspecific binding of TAA, diffusion-limited transport of TAA, and enterohepatic circulation of TAA glucuronide. These hypotheses were tested using three different model structures. Visual inspection and statistical evaluation involving maximum likelihood techniques indicated that the model incorporating nonspecific binding of TAA provided the best fit to the data. A correct model structure, based upon experimental data, statistical analyses, and biological interpretation, will allow a more accurate extrapolation to humans and, consequently, a greater understanding of human risk from exposure to TAME.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS - EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

Oral

No studies were located regarding health effects in humans following oral exposure.

Inhalation

No studies were located regarding health effects in humans following inhalation exposure.

4.2. LESS-THAN-LIFETIME AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

4.2.1. Oral Exposure

4.2.1.1. Acute Studies

Amoco Corporation. (1991a) Acute oral toxicity study of tert-amyl methyl ether (TAME) in rats, with cover letter dated 02/22/95. Study performed by ITT Research Institute (Study No. 1650). TSCA Section 8D submission. OTS0557663.

Daughtrey, WC; Bird, MG. (1995) Genotoxicity and twenty-eight-day subchronic toxicity studies on tertiary amyl methyl ether. J Appl Toxicol 15:313-319

Exxon Chemical Americas. (1985) Acute oral LD50 study of MRD-85-548 (tertiary amyl methyl ether) in the rat, with cover letter dated 02/10/95. Study performed by Bio/dynamics Inc. (Project No. 254802). TSCA Section 8D submission. OTS0557625.

Exxon Chemical Americas. (1989a) Acute oral toxicity test in the rat of MRD-89-374 (tertiary amyl methyl ether), with cover letter dated 02/10/95. Study performed by Exxon Biomedical Sciences Inc. (Project Number 237402). TSCA Section 8D submission. OTS0557624.

4.2.1.2. Short-Term Studies

Daughtrey, WC; Bird, MG. (1995) Genotoxicity and twenty-eight-day subchronic toxicity studies on tertiary amyl methyl ether. J Appl Toxicol 15:313-319.

Tertiary amyl methyl ether (TAME) is an oxygenate with a potential role as a component in reformulated gasolines. The genotoxic potential of TAME was assessed in an Ames assay and a mouse micronucleus assay. The Ames assay was carried out using five standard salmonella strains and doses ranging from 100 to 10,000 micrograms per plate. Tertiary amyl methyl ether was not mutagenic in any of the strains, either with or without metabolic activation. In the micronucleus assay, mice were given a single intraperitoneal injection of TAME at doses of 0.15, 0.375 or 0.75 g kg-1. Bone marrow samples were collected and evaluated for micronucleus formation at 24, 48 and 72 h after dosing. No elevation in micronucleus frequency was observed at any dose or at any of the collection times. Thus, TAME was not clastogenic to mouse bone marrow under the conditions of this study. Preliminary test data indicated that the acute oral LD50 for TAME in Sprague-Dawley rats was ca. 2.1 g kg-1. In the 28-day subchronic study, Sprague-Dawley rats of both sexes were dosed orally with vehicle, 0.125, 0.5 or 1.0 g kg-1 day-1 TAME in corn oil at a dose volume of 2 ml/kg-1. Dosing continued 7 days a week for a period of 28 days. Deaths of two out of 10 animals in the high-dose group (1 g kg-1 day-1) appeared to be compound related. Food consumption and body

weights were reduced in the high-dose male group relative to controls; otherwise, clinical observations were minimal.

Exxon Chemical Americas. (1989b) 28-Day subchronic oral toxicity of MRD-89-374 (94.5% tertiary-amyl methyl ether) in the rat, with cover letter dated 02/10/95. Study performed by Exxon Biomedical Sciences Inc. (Project Number 237470). TSCA Section 8D submission. OTS0557621.

4.2.1.3. Longer-Term Studies

No longer-term oral studies were located.

4.2.1.4. Chronic and Cancer Studies

Belpoggi, F; Soffritti, M; Minardi, F; et al. (2002) Results of long-term carcinogenicity bioassays on tert-amyl-methyl-ether (TAME) and di-isopropyl-ether (DIPE) in rats. Ann NY Acad Sci 982:70-86.

Tert-amyl-methyl ether (TAME) was administered by gavage in extra virgin olive oil solution at concentrations of 750, 250, or 0 mg/kg bw to groups of 100 male and 100 female Sprague-Dawley rats 8 weeks old at the start of the experiment. Di-isopropyl ether (DIPE) was administered in the same manner at the doses of 1000, 250, or 0 mg/kg body weight to groups of 100 male and 100 female Sprague-Dawley rats. TAME and DIPE were each delivered in 1-mL solution 4 days a week for 78 weeks. Control animals received 1 mL of extra virgin olive oil without TAME or DIPE. At the end of the treatment period, all animals were kept under observation until spontaneous death. Under these test conditions, TAME and DIPE were found to be potential carcinogenic agents for various organs and tissues.

4.2.2. Inhalation Exposure

4.2.2.1. Acute Studies

Amoco Corporation. (1991b) Acute inhalation toxicity study of tert-amyl methyl ether in rats, with cover letter dated 02/22/95. Study performed by ITT Research Institute (Study No. 1652). TSCA Section 8D submission. OTS0557658.

4.2.2.2. Short-Term Studies

Amoco Corporation. (1992) Four-week inhalation toxicity study of tert-amyl methyl ether (TAME) in rats, with cover letter dated 02/22/95. Study performed by ITT Research Institute (Study No. 1653). TSCA Section 8D submission. OTS0557657.

White, RD; Daughtrey, WC; Wells, MS. (1995) Health effects of inhaled tertiary amyl methyl ether and ethyl tertiary butyl ether. Toxicol Lett 82/83:719-724.

A study was conducted examining health effects associated with tertiary-amyl-methyl-ether (994058) (TAME) and ethyl-tertiary-butyl-ether (637923) (ETBE). Rats were exposed 6 hours/day, 5 days/week, for 4 weeks to up to 4,000 parts per million (ppm) TAME or ETBE. A 25% mortality rate was seen in animals exposed to the highest concentration of TAME. No deaths were seen in the ETBE exposed groups. Evidence of central nervous system

depression was seen in animals exposed to higher concentrations of both vapors but were more pronounced in TAME exposed rats. Central nervous system function returned to normal 15 minutes after exposure in ETBE exposed rats. Only high and mid dose TAME exposed rats demonstrated alterations in functional observational battery parameters. These returned to normal 18 hours after exposure. Only males exposed to 4,000ppm TAME demonstrated significantly decreased body weights. Relative liver weights increased in both TAME and ETBE rats exposed to 4,000ppm. No other histopathological alterations were identified. TAME exposed animals had minimal alterations in clinical chemistry and hematological parameters; no alterations in these parameters were seen in ETBE exposed rats. The authors conclude that 500ppm is a no observed adverse effect level for both TAME and ETBE in this study.

4.2.2.3. Longer-Term Studies

American Petroleum Institute. (1997) A 13-week inhalation toxicity/neurotoxicity study of tert-amyl methyl ether (TAME) in the rat and mouse via whole-body exposures with a 4-week recovery period, with cover letter dated 9/2/97. Study performed by Huntington Life Sciences (Study No. 95-6105). TSCA Section 4 submission. OTS0558892.

Wolf, DC; Medinsky, MA; Bond, JA; et al. 1998. Cell Proliferation and alpha-2µ-globulin nephropathy in F344 rat kidney following inhalation of ethyl tertiary butyl ether or tertiary amyl methyl ether [abstract]. Toxicol Pathol 26:173.

Biosis copyright: biol abs. rrm. Meeting abstract meeting poster rat alpha-2u-globulin nephropathy kidney ethyl tertiary butyl ester adverse effects gasoline additive inhalation tertiary amyl methyl ester toxicology urinary system toxicity urologic disease cell proliferation excretory system

4.2.2.4. Chronic Studies

No chronic inhalation studies were located.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES - ORAL AND INHALATION

American Petroleum Institute. (1996) Letter from American Petroleum Institute to USEPA Re: Toxicity studies on tertiary amyl methyl ether with attachments, dated 04/25/1996. Includes concentration range-finding study for the developmental toxicity evaluation of inhaled TAME in CD rats (final report), and concentration range-finding study for the developmental toxicity evaluation of inhaled TAME in CD-1 mice (final report). TSCA FYI submission. OTS0001271.

American Petroleum Institute. (1998) Final report, two-generation reproductive toxicity evaluation of inhaled tertiary amyl methyl ether (TAME) vapor in CD (Sprague-Dawley) rats, with cover letter dated 3/23/1998. Study performed by Chemical Industry Institute of Toxicology and Research Triangle Institute. TSCA Section 4 submission. OTS0559331.

Berger, T; Horner, CM. (2003) In vivo exposure of female rats to toxicants may affect oocyte quality. Reprod Toxicol 17:273-281.

A potential endpoint for female reproductive toxicants is fertilizability of the oocytes. This endpoint has not been adequately examined for mammalian females. The objective of these studies was to evaluate fertilizability of rat oocytes following in vivo exposure to known male reproductive toxicants that exert effects via pathways that do not

include endocrine disruption and to 4-vinylcyclohexene diepoxide, known to interfere with early follicular development. Oocytes were obtained from females following exposure and quality assessed by in vitro fertilization rate. One study evaluated fertilizability following 2 weeks exposure of females to inhaled tetrachloroethylene (2h/day, 5 days/week). The remaining studies evaluated fertilizability immediately following 2 weeks exposure via drinking water to tetrachloroethylene, trichloroethylene, the fuel oxidants methyl tertiary butyl ether (MTBE), ethyl tertiary butyl ether (ETBE), tertiary amyl methyl ether (TAME), and a metabolite of the first two ethers 2-methyl-1,2-propanediol (2M2P), and to 4-vinylcyclohexene diepoxide. The percentage of oocytes fertilized was reduced following inhalation exposure to tetrachloroethylene, or consumption of trichloroethylene or TAME. Fertilizability was not altered by exposures to the other reproductive toxicants or to the other fuel oxidants. Consistent with the reduced oocyte fertilizability following exposure to trichloroethylene, oocytes from exposed females had a reduced ability to bind sperm plasma membrane proteins. Female reproductive capability assessed by the endpoint, oocyte fertilizability, was reduced by exposure to trichloroethylene and inhaled tetrachloroethylene.

Tyl, RW; Myers, CB; Marr; MC; et al. (2003) Two-generation reproductive toxicity study of inhaled tertiary amyl methyl ether (TAME) vapor in CD rats. J Appl Toxicol 23:397-410.

Under Office of Prevention, Pesticides and Toxic Substances draft guidelines, CD weanling F0 rats (30 of each gender per group) inhaled tertiary amyl methyl ether vapor at 0, 250, 1500 or 3000 ppm 5 days a week and 6 h a day for 10 weeks, with vaginal cytology evaluated for weeks 8-10. The F0 animals then produced F1 offspring, with exposure 7 days a week from mating through to lactation. During the F1 prebreed exposure period, vaginal patency, preputial separation (PPS) and vaginal cytology were evaluated. The F1 animals were mated, with F2 anogenital distance measured on postnatal day zero. At F2 weaning 30 of each gender per group were selected for postwean retention, with no exposures, through vaginal patency and PPS. Body weights, feed consumption and clinical signs were recorded throughout the study. Adult F0 and F1 systemic toxicity was present at 1500 and 3000 ppm. Minor adult male reproductive toxicity was present at 3000 ppm. There were no adult effects on vaginal cyclicity, estrous cycle length, mating, fertility, pregnancy, gestational length or ovarian and uterine weights. There were no treatment-related gross or histopathologic findings in parental male or female systemic or reproductive organs. The F1 and F2 offspring toxicity was present at 1500 and 3000 ppm. The no-observable-adverse-effect level for adult systemic and offspring toxicity was 250 ppm and 1500 ppm for male reproductive toxicity (females at >3000 ppm).

Welsch, FB; Elswick, R; Arden James, MC; et al. (2003) Developmental toxicity evaluation of inhaled tertiary amyl methyl ether in rats and mice. J Appl Toxicol 23:387-395.

This evaluation was part of a much more comprehensive testing program to characterize the mammalian toxicity potential of the gasoline oxygenator additive tertiary amyl methyl ether (TAME), and was initiated upon a regulatory agency mandate. A developmental toxicity hazard identification study was conducted by TAME vapor inhalation exposure in two pregnant rodent species. Timed-pregnant CD(Sprague-Dawley) rats and CD-1 mice, 25 animals per group, inhaled TAME vapors containing 0, 250, 1500 or 3500 ppm for 6 h a day on gestational days 6-16 (mice) or 6-19 (rats). The developmental toxicity hazard potential was evaluated following the study design draft guidelines and end points proposed by the United States Environmental Protection Agency. Based on maternal body weight changes during pregnancy, the no-observable-adverse-effect level (NOAEL) was 250 ppm for maternal toxicity in rats and 1500 ppm for developmental toxicity in rats using the criterion of near-term fetal body weights. In mice, more profound developmental toxicity was present than in rats, at both 1500 and 3500 ppm. At the highest concentration, mouse litters revealed more late fetal deaths, significantly reduced fetal body weights per litter and increased incidences of cleft palate (classified as an external malformation), as well as enlarged lateral ventricles of the cerebrum (a visceral variation). At 1500 ppm, mouse fetuses also exhibited an increased incidence of cleft palate and the dam body weights were reduced. Therefore, the NOAEL for the mouse maternal and developmental toxicity was 250 ppm under the conditions of this study.

4.4. OTHER ENDPOINT-SPECIFIC STUDIES [*e.g., in vivo neurological, immunological studies*]

4.4.1. Odor and Taste Thresholds

Texaco Inc. (1993) Odor and taste threshold studies performed with tertiary amyl methyl ether (TAME), with cover letter dated 02/22/95. Study performed by TRC Enviromental Corporation for American Petroleum Institute (API Publication No. 4591). TSCA Section 8D submission. OTS0557645.

4.4.2. Studies of gasoline-TAME mixtures

Immunotoxicity

Twerdok, L; Peachee, VL; White, KL. (2005) Comparative Inhalation Immunotoxicity of Gasoline and Gasoline Plus Oxygenate Additives in Rats. Toxicol Sci 84(1-S):1096-6080. (Note: In Retrieval)

Comparative immunotoxicity testing was conducted on evaporative emissions of gasoline alone, and gasoline plus ether or alcohol oxygenates in the Sprague-Dawley rat. The inhalation exposures simulate, at much higher exposures and durations, human exposures during self-serve refueling of automobiles. Seven vapor condensates of gasoline alone, or gasoline plus an ether or alcohol oxygenate were evaluated for effects on the humoral component of the immune system using antibody-forming cell (AFC) response to the T-dependent antigen, sheep erythrocyte. Female Sprague-Dawley rats were exposed to the test agents (2000, 10000 and 20000 mg/m3) by inhalation for 4 weeks (6 hrs/day, 5 days/week). Vapor condensates of gasoline alone, gasoline plus MTBE, tert-butyl alcohol (TBA), or tertiary amyl methyl ether (TAME) did not affect humoral response in the plaque assay. Vapor condensates of gasoline plus ethanol or diisopropyl ether (DIPE) did result in statistically significant decreases in the AFC response when evaluated as either AFC/106 spleen cells or AFC/spleen at the highest dose level. Gasoline plus ethyl tertiary butyl ether (ETBE) produced a statistically significant dose-dependent decrease in AFC response at the mid- and high-doses. Recently, there has been speculation in the literature that some immune effects are related, or secondary to concurrent neurotoxicity. Comparative neurotoxicity studies (functional observational battery and motor activity) at the same dose levels with these three test materials were negative, suggesting no relationship between immune and nervous system effects. The neat oxygenates (MTBE, TBA, TAME, ethanol, ETBE, DIPE) tested in these gasoline blends share a number of common metabolites (isopropyl alcohol; acetaldehyde, acetate). Although these studies were not definitive, the negative effects for gasoline alone, gasoline/MTBE, gasoline/TBA and gasoline/TAME suggest that neither gasoline components nor the common metabolites of the neat oxygenates are responsible for the positive effects observed for these mixtures.

Human Exposure

Saarinen, L; Hakkola, M; Pekari, K; et al. (1998) Exposure of gasoline road-tanker drivers to methyl tert-butyl ether and methyl tert-amyl ether. Int Arch Occup Environ Health 71:143-147.

Organic oxygenates, namely, methyl tert-butyl ether (MTBE) and methyl tert-amyl ether (MTAE), are added to gasoline to reduce carbon monoxide in exhausts and to enhance the octane number. The aim of this study was to investigate road-tanker drivers' exposure to oxygenate vapors during road-tanker loading and unloading as well as to evaluate the measurements of these ethers and their metabolites in the urine as a means of assessing the uptake of the ethers. A total of 11 drivers in different parts of Finland were trained to monitor their exposure with personal samplers, to report their working conditions, and to collect their whole-day urine samples. Charcoal tubes of the air samples were analyzed for MTBE, MTAE, benzene, toluene, and aliphatic hydrocarbons. For biological monitoring purposes the two main oxygenates, tertiary ethers MTBE and MTAE, as well as their main metabolites, tertiary alcohols tert-butanol (TBA) and tert-amyl alcohol (TAA), were determined in urine specimens. On average the drivers were exposed to vapors for short periods (21 +/- 14 min) three times during a work shift. The mean concentrations of MTBE and MTAE (mean +/- SD) were 8.1 +/- 8.4 and 0.3 +/- 0.4 mg/m3. The total MTBE uptake

during the shift was calculated to be an average of 106 +/- 65 mumol. The mean concentrations of MTBE, TBA, MTAE and TAA detected in the first urine after the work shift were 113 +/- 76, 461 +/- 337, 16 +/- 21, and 40 +/- 38 nmol/l, and those found the next morning, 16 h later, were 18 +/- 12, 322 +/- 213, 9 +/- 10, and 20 +/- 27 nmol/l. The good relationship (r = 0.84) found between MTBE exposure and postshift excretion suggests that urinary MTBE can be used for biological monitoring of exposure, but at the present low level of exposure the corresponding metabolite TBA is not equally reliable. The determination of MTAE and its metabolite TAA in urine is sensitive enough to detect the low degree of exposure to MTAE, but in this study the data were too scarce to allow calculation of the correlations due to very low levels of MTAE exposure.

Vainiotalo, S; Pekari, K; Aitio, A. (1998) Exposure to methyl tert-butyl ether and tert-amyl methyl ether from gasoline during tank lorry loading and its measurement using biological monitoring. Int Arch Occup Environ Health 71:391-396.

OBJECTIVE AND METHODS: The exposure of Finnish tank lorry drivers to methyl tert-butyl ether (MTBE) and tert-amyl methyl ether (TAME) during loading of gasoline was studied using biological and breathing-zone sampling. During the field measurements in October 1994 and August 1995 the gasolines (95, 98, 99 RON) contained MTBE to 5.2-11.8% and TAME to 0-6%. RESULTS: The geometric mean (GM) breathing-zone concentration of MTBE was 4.3 mg/m3 (n = 15) in October and 6.4 mg/m3 (n = 20) in August. The GM concentration of TAME, measured only in August, was 0.98 mg/m3. The mean loading/sampling times were 37 and 35 min, the mean wind speeds were 0.8 and 0.6 m/s, and the mean air temperatures were -4.9 degrees and + 14.1 degrees C, respectively. Blood samples collected on average at 20 min after gasoline loading/exposure showed an MTBE concentration of 143 nmol/l (GM, n = 14) in October and 213 nmol/l (GM, n = 20) in August. Pearson's coefficient of correlation (r) between the MTBE breathing-zone concentrations and MTBE in blood was 0.86 (P = 0.0001) in October and 0.81 (P = 0.00001) in August. No correlation was found between MTBE in air and the metabolite tert-butanol (TBA) in blood. MTBE, but not TBA, in urine samples collected on average at 2.5 h after exposure showed a correlation with MTBE in air. The concentrations of TAME and its metabolite tert-amyl alcohol were below the quantitation limits (< 7 and < 100 nmol/l, respectively) in most blood and urine samples. CONCLUSIONS: The breathing-zone measurements showed low levels of exposure to the two oxygenates, the concentrations being well below the current hygienic standards for MTBE (250-360 mg/m3 for 15 min and 90-180 mg/m3 for 8 h). The linear correlations obtained for MTBE suggest that MTBE in blood or urine can be adopted as a valid biological exposure index.

Vainiotalo, S; Peltonen, Y; Ruonakangas, A; et al. (1999) Customer exposure to MTBE, TAME, C6 alkyl methyl ethers, and benzene during gasoline refueling. Environ Health Perspect 107:133-140.

We studied customer exposure during refueling by collecting air samples from customers' breathing zone. The measurements were carried out during 4 days in summer 1996 at two Finnish self-service gasoline stations with "stage I" vapor recovery systems. The 95-RON (research octane number) gasoline contained approximately 2.7% methyl tert-butyl ether (MTBE), approximately 8.5% tert-amyl methyl ether (TAME), approximately 3.2% C6 alkyl methyl ethers (C6 AMEs), and 0.75% benzene. The individual exposure concentrations showed a wide log-normal distribution, with low exposures being the most frequent. In over 90% of the samples, the concentration of MTBE was higher (range <0.02-51 mg/m3) than that of TAME. The MTBE values were well below the short-term (15 min) threshold limits set for occupational exposure (250-360 mg/m3). At station A, the geometric mean concentrations in individual samples were 3.9 mg/m3 MTBE and 2. 2 mg/m3 TAME. The corresponding values at station B were 2.4 and 1.7 mg/m3, respectively. The average refueling (sampling) time was 63 sec at station A and 74 sec at station B. No statistically significant difference was observed in customer exposures between the two service stations. The overall geometric means (n = 167) for an adjusted 1-min refueling time were 3.3 mg/m3 MTBE and 1.9 mg/m3 TAME. Each day an integrated breathing zone sample was also collected, corresponding to an arithmetic mean of 20-21 refuelings. The overall arithmetic mean concentrations in the integrated samples (n = 8) were 0.90 mg/m3 for benzene and 0.56 mg/m3 for C6 AMEs calculated as a group. Mean MTBE concentrations in ambient air (a stationary point in the middle of the pump island) were 0.16 mg/m3 for station A and 0.07 mg/m3 for station B. The mean ambient concentrations of TAME, C6 AMEs, and benzene were 0.031 mg/m3, approximately 0.005 mg/m3,

and approximately 0.01 mg/m3, respectively, at both stations. The mean wind speed was 1.4 m/sec and mean air temperature was 21 degreesC. Of the gasoline refueled during the study, 75% was 95 grade and 25% was 98/99 grade, with an oxygenate (MTBE) content of 12.2%.

Vainiotalo, S; Ruonakangas, A. (1999) Tank truck driver exposure to vapors from oxygenated or reformulated gasolines during loading and unloading. Am Ind Hyg Assoc J 60:518-525.

Tank truck drivers' exposure to gasoline vapors was studied by collecting breathing zone samples during loading and unloading of gasoline. The field studies were conducted at three dispatches and at seven service stations in Finland. The gasolines included in the study (95, 98, 99 research octane number, RON) were of reformulated or oxygenated grade containing about 2% (w/w) oxygen and 0.5-1.5% (v/v) benzene. The sampling times ranged from 16 to 57 min (mean 35 min), and time-weighted average concentrations for a 30-min period were calculated. Using the time-adjusted values, geometric mean concentrations (GM) were calculated for three periods of dispatch measurements (n = 15,20,7) and a period of unloading measurements at service stations (n = 7). The GM for methyl tert-butyl ether ranged from 0.95 to 7.3 mg/m3 and that for tert-amyl methyl ether from 0.30 to 1.1 mg/m3. The GM concentrations of hexane, benzene, and toluene were in the range of 0.25-2.3 mg/m3, 0.15-0.28 mg/m3, and 0.73-1.7 mg/m3, respectively. Multiple regression analysis yielded an r2 value of 0.98 for the daily mean concentration of toluene and correspondingly 0.94 for benzene when daily wind speed (0.1-3.7 m/sec) and daily air temperature (-7.4(-)+17.2 degrees C) were used as independent variables. The average number of gasoline loads per tank truck was 2.5, corresponding to 23,000 L of gasoline.

Vainiotalo, S; Kuusimaki, L; Pekari, K. (2006) Exposure to MTBE, TAME and aromatic hydrocarbons during gasoline pump maintenance, repair and inspection. J Occup Health 48:347-357.

The exposure of gasoline pump repairers and inspectors to gasoline was studied at service stations and repair shops in Finland in April-June 2004. The average air temperature ranged from 7 degrees C to 16 degrees C and wind speed from 2.5 to 7 m/s. The gasoline blends contained mixtures of methyl tert-butyl ether (MTBE) and tert-amyl methyl ether (TAME), the total content of oxygenates being 11-12%. The content of benzene was <1%. Breathing zone air was collected during the work task using passive monitors. The mean sampling period was 4.5 h. The mean TWA-8 h concentrations for MTBE, TAME, hexane, benzene, toluene, ethylbenzene and xylene were 4.5, 1.3, 0.55, 0.23, 2.2, 0.26 and 1.1 mg/m3, respectively. None of the individual benzene concentrations exceeded the binding limit value for benzene (3.25 mg/m3). The sum concentration of MTBE and TAME in urine was between 8.9 and 530 nmol/l in individual post-shift samples. The individual sum concentrations of the metabolites tert-butyl alcohol and tert-amyl alcohol collected the following morning after the exposure ranged from 81 to 916 nmol/l. All individual results were below corresponding biological action levels. Exposure to aromatic hydrocarbons was estimated from post-shift urine samples, with benzene showing the highest concentration (range 4.4 and 35 nmol/l in non-smokers). The exposure levels were similar to those measured in previous studies during unloading of tanker lorries and railway wagons. The results indicated a slightly higher exposure for inspectors, who calibrated fuel pump gauges at the service stations, than for pump repairers. No significant skin exposure occurred during the study.

4.5. MECHANISTIC DATA AND OTHER STUDIES IN SUPPORT OF THE MODE OF ACTION [*e.g., in vitro and ex vivo studies using isolated target tissues/organs or cells, metabolite studies, genotoxicity, SAR, etc.*]

Genotoxicity

American Petroleum Institute. (1996a) CHO/HGPRT mutation assay of tertiary amyl methyl ether with cover letter dated 04/25/1996. Study performed by Microbiological Associates, Inc. (Study No. G95CA89.782). TSCA FYI submission. OTS0001273.

American Petroleum Institute. (1996b) Final report, chromosome aberrations in Chinese hamster ovary (CHO) cells with tertiary amyl methyl ether (TAME), with cover letter dated 6/21/96. Study performed by Microbiological Associates, Inc. (Study No. G95CA89.330). TSCA Section 4 submission. OTS0558865.

Daughtrey, WC; Bird, MG. (1995) Genotoxicity and twenty-eight-day subchronic toxicity studies on tertiary amyl methyl ether. J Appl Toxicol 15:313-319

Exxon Chemical Americas. (1989c) Microbial mutagenesis in Salmonella mammalian microsome plate incorporation assay of MRD-89-374 (tertiary amyl methyl ether), with cover letter dated 02/10/95. Study performed by Exxon Biomedical Sciences Inc. (Project Number 237425). TSCA Section 8D submission. OTS0557623.

Exxon Chemical Americas. (1989d) In vivo mammalian bone marrow micronucleus assay of MRD-89-374 (tertiary amyl methyl ether), with cover letter dated 02/10/95. Study performed by Exxon Biomedical Sciences Inc. (Project Number 237430). TSCA Section 8D submission. OTS0557622.

Mechanisms

Martin, JV; Bilgin, NM; Iba, MM. (2002) Influence of oxygenated fuel additives and their metabolites on the binding of a convulsant ligand of the gamma-aminobutyric acid(A) (GABA(A)) receptor in rat brain membrane preparations. Toxicol Lett 129:219-226.

As a foundation for evaluating potential mechanisms of the neurological effects (e.g. headache, nausea, dizziness) of some octane boosters, we studied the gamma-aminobutyric acid(A) (GABA(A)) receptor in a series of binding assays in membranes from rat brain. The GABA(A) receptor was probed using the radioligand [3H]t-butylbicycloorthobenzoate ([3H]TBOB) which binds to the convulsant recognition site of the receptor. The results demonstrated that the short-chain t-ethers and their t-alcohol metabolites inhibit binding at the convulsant site of the GABA(A) receptor. The potency of the inhibition tended to correlate with carbon chain length. For agents having an equal number of carbon atoms, potency of inhibition of [3H]TBOB binding was greater in magnitude for the alcohols than for the ethers. The descending rank order of potency for the ethers and alcohols were as follows, t-amyl alcohol (TAA); t-amyl-methyl ether (TAME); ethyl-t-butyl ether (ETBE)>t-butyl alcohol (TBA)>methyl-t-butyl ether (MTBE)>ethanol. In additional saturation binding assays, MTBE reduced apparent density of convulsant binding (B(max)).

Martin, JV; Iyer, SV; McIlroy, PJ; et al. (2004) Influence of oxygenated fuel additives and their metabolites on gamma-aminobutyric acidA (GABAA) receptor function in rat brain synaptoneurosomes. Toxicol Lett 147:209-217.

Experimental and occupational inhalational exposure to oxygenate fuel additives in reformulated gasoline has been reported to induce neurological symptoms (e.g., headache, nausea, dizziness). We reported previously that the ether additives (methyl-t-butyl ether (MTBE), t-amyl-methyl ether (TAME) and ethyl-t-butyl ether (ETBE)) and their metabolites (t-amyl alcohol (TAA), t-butyl alcohol (TBA) and ethanol) alter the binding of [3H]t-butylbicycloorthobenzoate ([3H]TBOB), a ligand for the gamma-aminobutyric acidA (GABAA) receptor in rat brain membrane preparations. To more directly assess the effects of the ethers and their alcohol precursors on GABAA receptor function, the uptake of 36Cl- was measured in synaptoneurosomes, a preparation of closed membrane sacs comprised of pre- and postsynaptic membranes from adult rat cerebral cortex. Each of the compounds caused a concentration-dependent enhancement of muscimol-stimulated uptake of 36CI-, which

diminished with further increasing concentrations. The potency of the enhancement by the compounds was in the rank order: MTBE = TAME > TAA = ETBE > TBA > ethanol. The half-maximally effective concentration (EC50) for the facilitation of muscimol-stimulated 36Cl- uptake ranged from 0.06 to 3 mM, and that for the higher-dose inhibitory effect (IC50) ranged from 3 to 50 mM. The facilitatory concentrations of the compounds are in the range of the blood concentrations reported in experimental animals after exposures known to induce CNS effects such as ataxia. The results suggest a potential role of the GABAA receptor in some of the reported neurotoxic effects of gasoline additives.

Zhang, YP; Macina, OT; Rosenkranz, HS; et al. (1997) Prediction of the metabolism and toxicological profiles of gasoline oxygenates. Inhal Toxicol 9:237-254.

An analysis of the metabolism and toxicity of gasoline oxygenates by expert systems was performed. Methyl-tert-butyl-ether (1634044) (MTBE), ethyl-tert-butyl-ether (637923) (ETBE), tert-amyl-methyl-ether (994058) (TAME), and diisopropyl-ether (108203) (DIPE) were evaluated using the Computer Automated Structure Evaluation (CASE) and Multiple Computer Automated Structure Evaluation (MULTICASE) programs to determine if they might present a human health hazard. CASE was a computer algorithm which in response to inputed data about a specific molecular structure utilizing statistically significant molecular fragments classified the structure as being biologically active or inactive. CASE also derived quantitative structure activity relationships (QSARs) that could be used to predict the potency or extent of biological activity. MULTICASE used a set of statistically significant molecular descriptors to identify a particular descriptor that had the highest probability of being responsible for the observed biological activity of a specific compound. MULTICASE also attempted to derive a QSAR within a group of compounds containing a specific biophore which could then be used to identify molecular features that controlled the biological activity. The data sets inputed into the CASE programs included information on possible descriptors that might explain symptoms such as headache, nausea and sensory irritation that were reported by persons exposed to gasoline oxygenates and molecular features that might be predictive of mutagenicity, developmental toxicity, or carcinogenicity. Neither MTBE, ETBE, TAME, nor DIPE was predicted by the CASE/MULTICASE programs to be sensory or eye irritants, contact sensitizers, or to present a risk of mutagenicity, developmental toxicity, or carcinogenicity. The putative metabolites of ETBE were evaluated by a computer algorithm known as META to determine if any of these might be toxic, then evaluated by the CASE/MULTICASE programs. Twenty seven putative ETBE metabolites were evaluated by the procedure. A number of the metabolites were predicted to be sensory irritants, contact sensitizers, mutagens, carcinogens, or developmental toxicants. The authors conclude that this analysis shows that MTBE, ETBE, TAME, and DIPE are not likely to be toxic. Some putative metabolites of ETBE, however, are likely to be toxic.

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS

4.7. EVALUATION OF CARCINOGENICITY

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

No studies regarding possible childhood susceptibility were located.

4.8.2. Possible Gender Differences

Sumner, SC; Janszen, DB; Asgharian, B; et al. (2003c) Species and gender differences in the metabolism and distribution of tertiary amyl methyl ether in male and female rats and mice after inhalation exposure or gavage administration. J Appl Toxicol 23:427-436.

Tertiary amyl methyl ether (TAME) is a gasoline fuel additive used to reduce emissions. Understanding the metabolism and distribution of TAME is needed to assess potential human health issues. The effect of dose level, duration of exposure and route of administration on the metabolism and distribution of TAME were investigated in male and female F344 rats and CD-1 mice following inhalation or gavage administration. By 48 h after exposure, >96% of the administered radioactivity was expired in air (16-71%) or eliminated in urine and feces (28-72%). Following inhalation exposure, mice had a two- to threefold greater relative uptake of [14C]TAME compared with rats. Metabolites were excreted in urine of rats and mice that are formed by glucuronide conjugation of tertiary amyl alcohol (TAA), oxidation of TAA to 2,3-dihydroxy-2-methylbutane and glucuronide conjugation of 2,3-dihydroxy-2-methylbutane. A saturation in the uptake and metabolism of TAME with increased exposure concentration was indicated by a decreased relative uptake of total [14C]TAME equivalents and an increase in the percentage expired as volatiles. A saturation of P-450 oxidation of TAA was indicated by a disproportional decrease of 2,3-dihydroxy-2-methylbutane and its glucuronide conjugate with increased exposure concentration.

Secondary Sources to be Reviewed

ACGIH (American Conference of Governmental Industrial Hygienists). (2001) Documentation of the threshold limit values and biological exposure indices Vol:7th Ed. ACGIH, Cincinnati, OH.

Ahmed, FE. (2001) Toxicology and human health effects following exposure to oxygenated or reformulated gasoline. Toxicol Lett 123:89-113.

In order to replace antiknock leaded derivatives in gasoline, legislations were enacted in the United States and other countries to find safer additives and to reduce CO, O3, and volatile organic compounds (VOCs) in non-attainment areas. Oxygenates commonly used include various alcohols and aliphatic ethers. Methyl tert-butyl ether (MTBE) is the most widely used and studied ether oxygenate and is added to gasoline at concentrations up to 15% by volume. Inhalation of fumes while fueling automobiles is the main source of human exposure to MTBE. Humans are also exposed when drinking water contaminated with MTBE. Epidemiological, clinical, animal, metabolic and kinetic studies have been carried out to address human health risks resulting from exposure to MTBE. MTBE is an animal carcinogen, but its human carcinogenic potential remains unclear. Because MTBE functions as a non-traditional genotoxicant, several mechanisms were suggested to explain its mode of action, such as, functioning as a cytotoxic as opposed to a mitogenic agent; involvement of hormonal mechanisms; or operating as a promoter instead of being a complete carcinogen. Some studies suggested that carcinogenicity of MTBE might be due to its two main metabolites, formaldehyde or tributanol. A role for DNA repair in MTBE carcinogenesis was recently unveiled, which explains some, but not all effects. The totality of the evidence shows that, for the majority of the non-occupationally exposed human population, MTBE is unlikely to produce lasting adverse health effects, and may in some cases improve health by reducing the composition of emitted harmful VOCs and other substances. A small segment of the population (e.g. asthmatic children, the elderly, and those with immunodeficiency) may be at increased risk for toxicity. However, no studies have been conducted to investigate this hypothesis. Concern over ground and surface water contamination caused by persistent MTBE has lead the Environmental Protection Agency (EPA) to proposed reducing or eliminating its use as a gasoline additive. The major potential alternatives to MTBE are other forms of ethers such as ethyl tert-butyl ether (ETBE) or tert-amyl methyl ether (TAME), and alcohols such as ethanol. More definitive studies are needed to understand the mechanism(s) by which aliphatic ethers may pose health and environmental impacts. The switch from MTBE to ethanol is not without problems. Ethanol costs more to produce, poses challenges to the gasoline distribution system, extends the spread of hydrocarbons through ground water in gasoline plumes, and in the short-term is unlikely to be available in sufficient quantity. Moreover, its metabolite acetaldehyde is a possible carcinogen that undergoes a photochemical reaction in the atmosphere to produce the respiratory irritant peroxylacetate nitrate (PAN). Congress is addressing whether the Clean Air Act Amendments (CAA) provisions concerning reformulated gasoline (RFG) should be modified to allow refineries to discontinue or lessen the use of oxygenates.

Caprino, L; Togna, GI. (1998) Potential health effects of gasoline and its constituents: A review

of current literature (1990-1997) on toxicological data. Environ Health Perspect 106:115-125.

BIOSIS COPYRIGHT: BIOL ABS. We reviewed toxicological studies, both experimental and epidemiological, that appeared in international literature in the period 1990-1997 and included both leaded and unleaded gasolines as well as their components and additives. The aim of this overview was to select, arrange, and present references of scientific papers published during the period under consideration and to summarize the data in order to give a comprehensive picture of the results of toxicological studies performed in laboratory animals (including carcinogenic, teratogenic, or embryotoxic activity), mutagenicity and genotoxic aspects in mammalian and bacterial systems, and epidemiological results obtained in humans in relation to gasoline exposure. This paper draws attention to the inherent difficulties in assessing with precision any potential adverse effects on health, that is, the risk of possible damage to man and his environment from gasoline.

Mehlman, MA. (2001) Ethers. Section 8.0, *t*-Amyl Methyl Ether. In: Bingham, E.; Cohrssen, B.; Powell, C.H.; eds. Patty's Toxicology. Fifth Edition. Volume 5. New York, NY: John Wiley & Sons, Inc.: p. 895.

Literature Searches

Literature searches for studies relevant to development of the IRIS Toxicological Review were conducted in September 2007 for tert amyl methyl ether (TAME, CASRN 994-05-8), tert amyl ethyl ether (TAEE, CASRN 919-94-8) and tert amyl alcohol (TAA, CASRN 75-85-4) in MEDLINE, TOXLINE special, BIOSIS update (>1998), DART/ETIC, TSCATS/TSCATS2, RTECS, CCRIS, HSDB, GENETOX, and Current Contents (past 6 months). Searches were not date-limited, except as noted above. The searches also encompassed the resources of the EPA and other relevant national and international governmental and quasi-governmental agencies, as listed in Attachment B (Chemical Information Search Resources) to the FY06 IRIS SOPs.